



## Washing bleached palm oil to reduce monochloropropanediols and glycidyl esters

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### ABSTRACT

The formation of toxic compounds, potentially carcinogenic, during food processing has been considered an important food safety issue. Among them, particular attention has been given to 3-monochloropropane-1,2-diol esters (3-MCPDE), 2-monochloropropane-1,3-diol esters (2-MCPDE) and glycidyl esters (GE), which can be formed during vegetable oil refining, especially palm oil. These substances may pose a health risk to humans due to their toxicity and carcinogenicity. The aim of this study was to investigate the effect of washing bleached palm oil (BPO) with different solvents, and evaluate the reduction of 3-MCPDE, 2-MCPDE and GE as well as assess the quality parameters of the final product. For this purpose, we used two types of washing with different solvents. A single washing was carried out in one step and a double washing in two steps using a solvent gradient. Single washing had a limited reduction in the levels of 3-MCPDE and 2-MCPDE and resulted in an increased level of GE, whereas double washing slightly reduced 3-MCPDE and 2-MCPDE and resulted in a significant reduction of GE levels. The reduction achieved in this study was up to 17.1% for 3-MCPDE, 56.4% for 2-MCPDE and 76.9% for GE levels. The reduction of 3-MCPDE and 2-MCPDE might be due to the removal of part of the ethanol-soluble chlorinated precursors from the oil which suggests that highly lipophilic forms of these substances are present in BPO. The substantial reduction on GE levels might be associated with the removal of the precursors present in the oil such as diacylglycerols. Thus, the washing treatment could be used as a supplementary strategy to reduce processing contaminants from palm oil, especially GEs.

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## Introduction

3-Monochloropropane-1,2-diol esters (3-MCPDE) and 2-monochloropropane-1,3-diol esters (2-MCPDE) are a group of food processing contaminants formed from acylglycerols and chlorides during the refining process of edible oils. Glycidyl esters (GE) are compounds also originating during the refining of vegetable oils but structurally formed by an acylglycerol containing the epoxide functional group (Seefelder et al. 2011; Arisseto et al. 2013).

The presence of 3-MCPDE, 2-MCPDE and GE in the diet is a potential health concern since these esters are hydrolysed by enzymes in the gastrointestinal tract, releasing their free forms, which have been considered toxic (Abraham et al. 2013). According to the International Agency for Research on Cancer (IARC), 3-MCPD is classified

as a possible human carcinogen (group 2B) and glycidol (the free form of GE) as a probable human carcinogen (group 2A) (IARC, 2000, 2012). For free 2-MCPD and its esters, there is no risk assessment published in the literature to date, which is due to the lack of data on this contaminant (EFSA 2016).

These substances are formed during the deodorisation step of the refining process of edible oils. In this process, high temperatures (180–270°C) are employed to remove free fatty acids and/or other undesirable compounds, which is a critical factor for the formation of these contaminants (Dijkstra and Segers 2007; Pudiel et al. 2011; Sampaio et al. 2013). Besides temperature, other factors have been identified to play an important role in the contamination of refined oils, such as the presence of potential precursors.

In the case of 3-MCPDE and 2-MCPDE, the occurrence of a chlorine 'donor' in the crude oil is a crucial issue. Among vegetable edible oils, palm oil is the one that has shown the highest concentrations of these contaminants compared to other oils. This may be due to the high amounts of chlorinated substances originated from the endogenous metabolism of the plant, the use of fertilisers containing chloride salts as well as the use of HCl-activated clays during the bleaching step of the refining process (Craft and Destailats 2014; Silva et al. 2014). For GE, the presence of diacylglycerols (DAG) and monoacylglycerols (MAG) favours their formation by an intramolecular rearrangement that results in fatty acid elimination (Destailats et al. 2012). In addition, cyclic acyloxonium ions can be formed at deodorisation conditions and intermediate the formation of GEs (Weißhaar and Perz 2010).

One of the strategies to minimise the formation of these contaminants in refined vegetable oils is the reduction of their precursors prior to deodorisation. Washing palm oil before deodorisation could be a possible alternative since it could remove some of the precursors from the oil, mainly chlorine 'donor' substances, consequently preventing the formation of 3-MCPDE and 2-MCPDE (Stadler 2015). Washing crude palm oil and fruit pulps has been reported in the literature, and a reduction of 3-MCPDE ranging from 20% to 90% has been observed (Matthäus et al. 2011; Ramli et al. 2015).

To our best knowledge, the washing of bleached palm oil (BPO) to reduce the formation of 3-MCPDE, 2-MCPDE and GE has not been investigated to date. As during the refining process other sources of chlorine could be introduced to the oil prior to deodorisation (Silva et al. 2014), this strategy represents a realistic scenario of the practices of palm oil refining in industries that could be a way out to mitigate these contaminants. Therefore, the present study aims to evaluate the effect of washing BPO with different solvents on the reduction of 3-MCPDE, 2-MCPDE and GE as well as assess the quality parameters of the final product.

## Materials and methods

### Bleached palm oil

The BPO used in the experiments was supplied by a local company.

### Standards

The following analytical standards were purchased from Toronto Research Chemicals Inc.: rac 1,2-bis-palmitoyl-3-chloropropanediol (PP-3-MCPD, purity 98%), rac 1,2-bis-palmitoyl-3-chloropropanediol-d5 (PP-3-MCPD-d5, purity 98%), 1,3-dipalmitoyl-2-chloropropanediol (PP-2-MCPD, purity 98%), glycidyl palmitate (P-Gly, purity 98%) and glycidyl palmitate-d5 (P-Gly-d5, chemical purity 97% and isotopic purity 97.6%). Lipid standards (monoglyceride, diglyceride and triglyceride mixtures) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

### Solvents and reagents

Methanol was acquired from J. T. Baker. Heptane (purity  $\geq 99\%$ ), tetrahydrofuran (THF, anhydrous, purity  $\geq 99.99\%$ ), toluene (purity 99.9%), sodium bromide (NaBr, purity  $\geq 99.5\%$ ), sodium bicarbonate, acetone and phenylboronic acid (PBA, purity  $\geq 97\%$ ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulphuric acid ( $H_2SO_4$ ), sodium sulphate, sodium hydroxide and sodium thiosulphate were obtained from Merck (Darmstadt, Germany). Ethanol 99.5%, phenolphthalein, acetic acid, isooctane, potassium iodide, soluble starch and sodium chloride were purchased from Synth (Diadema, SP, Brazil). Ultrapure water was obtained from a Milli-Q Plus system purchased from Millipore (Bedford, MA, USA).

### Selection of washing solvents

The selection of the most suitable solvents to wash BPO was based on their miscibility in the oil and the ability to be separated from the oil after agitation. The following solvents were tested: water, acetone, isopropanol, ethanol 99.5% and ethanol

75%. The ratio oil/solvent was 2:1. For water, 99.5% ethanol and ethanol 75% the separation was carried out at 40°C and for acetone and isopropanol at 0°C.

### **Sample treatment**

#### **Single washing**

BPO (1000 g) was placed in a reactor and heated at 70°C to allow a thoroughly melting. Then, the oil was cooled to 55°C and 500 mL of the washing solutions (water, ethanol 99.5% and ethanol 75%) was added. The mixture was shaken at 250 rpm during 15 min and allowed to stand for phase separation at 40°C to avoid crystallisation. After partition, the samples were dried under vacuum to remove remaining ethanol/water and an aliquot of 500 g of the oil phase was submitted to deodorisation. A non-washed deodorised BPO was used as control.

#### **Double washing**

This process consisted in firstly washing the BPO with ethanol 99.5% as previously described, followed by a second washing with different solvents (ethanol 40% aqueous solution, ethanol 10% aqueous solution and water) after phase separation. Final partition and deodorisation were carried out under the same conditions employed for samples obtained by single washing. In this process, two separations were carried out but the remaining solvent in the oil was dried only after the second separation. A non-washed deodorised BPO was used as control.

#### **Deodorisation**

The deodorisation equipment consisted of a three-necked flask placed in a heating mantle. The stripping steam was introduced through one of the necks of the flask. The other two necks were the inlet for the vacuum pump and a thermocouple, respectively. The distillation vapour from the flask was condensed into a glass condenser. A mass of 500 g of BPO was introduced inside the three-necked flask, and the parameters set for deodorisation were: temperature 250°C; time 2 h; pressure 2–4 mbar; stripping steam 3% v/w.

### **Analytical methods**

#### **Determination of diacylglycerols (DAG) and monoacylglycerols (MAG)**

DAG and MAG determination was carried out in BPO by high performance size exclusion chromatography (HPSEC) adapted from the method Cd 22-91 (AOCS, 2014a). This analysis used a size exclusion column and refractive index detector (RID). Samples were dissolved in THF and eluted in the same solvent at a flow rate of 1 mL/min. Quantification was achieved by normalisation followed by transformation of the area percentage into g/100 g.

#### **Determination of 3-MCPDE, 2-MCPDE and GE**

The 3-MCPDE, 2-MCPDE and GE were analysed by gas chromatography coupled to a MSD 5975C mass spectrometer (Agilent Technologies, model 7890A, New Castle, DE, USA), according to the official method Cd 29a-13 (AOCS, 2013). This method consisted of a simultaneous determination of 3-MCPDE, 2-MCPDE and GE, in which the GE were converted to 3-monobromopropanediol (3-MBPD) in an acid medium by the addition of sodium bromide. After that, 3-MCPDE, 2-MCPDE and the esters of 3-MBPD were hydrolysed in the presence of an acid methanolic solution releasing their free forms which were derivatised with phenylboronic acid prior to injection.

#### **Free fatty acids**

The analysis of free fatty acids was carried out by titration with sodium hydroxide according to the official method Ca 5a-40 (AOCS, 1998a). Approximately, 1 g of sample was dissolved in 50 mL of neutralised ethanol. The sample was then titrated with sodium hydroxide solution.

#### **Peroxide value**

The determination of the peroxide value was according to the official method Cd 8b-90 (AOCS, 2014b). A mixture of acetic acid–isooctane was added to the sample. After reaction with a saturated potassium iodide solution, the mixture was titrated with a sodium thiosulphate solution. This method determines all compounds (peroxides or other products of lipid oxidation) in terms of milliequivalents of peroxide per kg of sample.

### Oxidative stability

Oxidative stability of deodorised palm oil was measured using a Rancimat (Metrohm 168 743 Rancimat, Metrohm, Riverview, FL, USA), based on the method Cd 12b-92 (AOCS, 1998b). Briefly, 1 g of oil sample was placed into the Rancimat reaction vessel and heated at 120°C with an air flow rate of 20 L/h. The volatile compounds released into the distilled water during the oxidation process resulted in the increase of water conductivity. The conductivity of water during the process was plotted against time (h). The oxidative stability of oil was expressed as the induction time and is defined as the time (h) required for a rapid change in the rate of oxidation.

### Colour

Colour was determined according to the official method Cc 13e-92 (AOCS, 2017), using a Lovibond Tintometer Colour Scale at 70°C. Colour measurement was performed using 5" 1/4 (133.4 mm) glass cells. The scales range from 0 to 70 red (R) and 0 to 70 yellow (Y). The result was expressed as R values.

### Statistical analysis

Mean results were evaluated for significance using one-way analysis of variance (ANOVA) and Tukey's test, employing XLSTAT software (Addinsoft, France). The chosen level of significance was 0.05. The term significant was used to indicate differences for which  $p \leq 0.05$ .

## Results and discussion

### Selection of washing solvents

An ideal solvent to wash palm oil, aiming to remove chlorinated compounds, should have low miscibility in the oil so distinct phases can be formed and separated without the necessity of evaporation. Consequently, the solvent could wash out a higher content of chlorinated compounds from the oil. Moreover, it is important that the solvent does not have a high polarity, so that a large amount of non-polar chlorinated organic compounds can be removed. Nevertheless, polar chlorinated compounds seem to be the major source of chloride for the 3-MCPDE and 2-MCPDE formation (Ermacora and Hrnčirik 2014).

**Table 1.** Percentage of recovery of the solvents after partition.

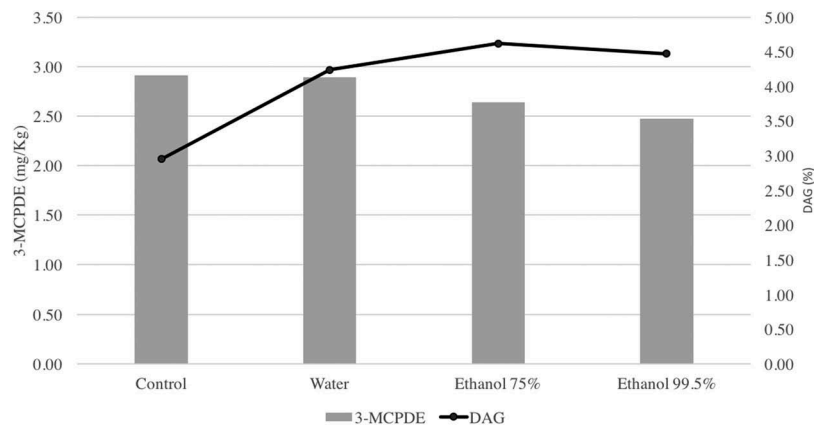
Solvent	Recovery after partition (%)
Acetone	0
Isopropanol	0
99.5% Ethanol	64
75% Ethanol	81
Water	98

As shown in Table 1, ethanol and water were suitable to be used as a washing agent for BPO given the higher recovery percentage after partition. Therefore, these solvents were used in further experiments involving both single and double washings. Although the separation of acetone and isopropanol from the oil was achieved at low temperatures (0°C), as reported by some authors (Kurosawa and Omori 2012), no satisfactory recovery of these solvents was observed in this study after partition at 0°C.

### Effects of single washing on the formation of 3-MCPDE, 2-MCPDE and GE

Figure 1 presents the levels of DAG and 3-MCPDE in BPO washed with different solvents (single washing), after deodorisation. As can be seen, the levels of DAG increased for all the evaluated solvents, suggesting that water and ethanol in association with heat would prompt hydrolysis reactions which are responsible for increasing the levels of partial acylglycerols (Pinto and Lanças 2006). As expected, none of the samples used in this study showed detectable amounts of MAG since this type of acylglycerol occurs in only low concentrations in palm oil (Gibon et al. 2007).

The results do not show a positive correlation between the DAG content and the formation of 3-MCPDE, which is in agreement with results reported in other studies (Matthäus et al. 2011; Ramli et al. 2015). This fact suggests that chlorinated compounds and high temperatures are the only critical variables for 3-MCPDE formation. Washing BPO reduced 3-MCPDE levels by 0.7%, 9.3% and 15.1% when using water, ethanol 75% and ethanol 99.5%, respectively. This reduction had no significance ( $p > 0.05$ ) and it is still limited when compared to results found in other studies which reported a decrease of approximately 30% by washing crude palm oil with ethanol (Matthäus and Pudel 2013), 95% by washing palm fruit pulp before



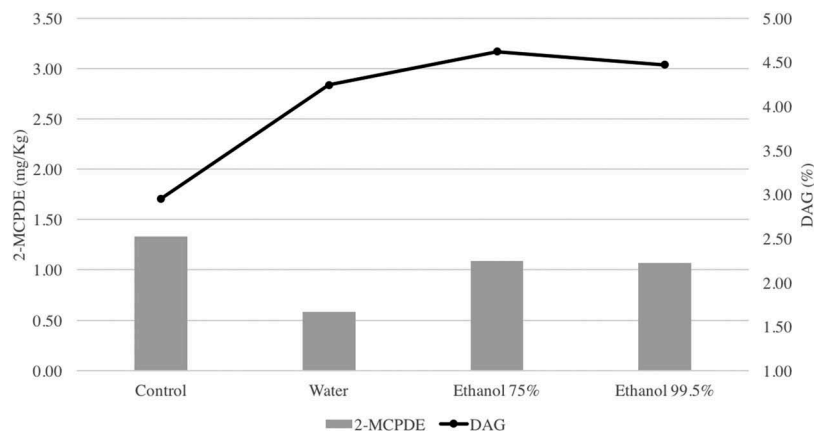
**Figure 1.** Levels of diacylglycerols (DAG) and 3-MCPDE in BPO washed with different solvents (single washing), after deodorisation.

oil extraction with water (Craft et al. 2012), and 20% and 25% by washing the fruit pulps with water and ethanol, respectively (Matthäus et al. 2011).

The minimal reduction for 3-MCPDE achieved in the present study might be associated with the type of palm oil used since BPO was washed instead of crude palm oil or the fruit pulps. Some authors stated that washing and removal of chlorinated compounds in the early stages of palm oil refining could be easier since the compounds that act as chlorine donors are in smaller amounts and more polar in these stages of the processing chain (Nagy et al. 2011). However, targeting the removal of chlorinated substances from BPO was adopted in this study because it represents a realistic scenario of the practices in the palm oil refineries. During the refining process, other sources of chlorine are introduced to the oil prior to deodorisation which is illustrated by the use of

acid activated bleaching earth (ABE) in the dry-degumming step. ABE is usually activated with hydrochloric acid which is a source of chlorine. ABE is preferred over the neutral bleaching earth because it can avoid colour fixation since it works simultaneously as an acidic catalyst, adsorptive agent, ion exchanger and filter aid whereas neutral bleaching earth acts only as an adsorptive agent (Silva et al. 2014).

As shown in Figure 2, washing BPO reduced 2-MCPDE levels by 56.4%, 18.1% and 19.6% when using water, ethanol 75% and ethanol 99.5%, respectively. Washing BPO with water gave a significant reduction ( $p < 0.05$ ) in 2-MCPDE levels. No correlation was observed between DAG and 2-MCPDE content. As can be seen, the reduction of 2-MCPDE levels was more substantial compared to 3-MCPDE. The data obtained in this study do not allow inferring any



**Figure 2.** Levels of DAG and 2-MCPDE in BPO washed with different solvents (single washing), after deodorisation.

hypothesis of these differences on the reduction of the contaminants when washing with water, which should be evaluated in further investigations.

Figure 3 illustrates the levels of GE and DAG in BPO washed with different solvents (single washing), after deodorisation. The levels of GE increased by 21.0%, 77.8% and 129.6% when using water, ethanol 75% and ethanol 99.6%, respectively. Ethanol 99.5% and ethanol 75% gave rise to a significant increase in GE levels compared to the control sample ( $p < 0.05$ ). As observed in Figure 3, the DAG content influenced GE formation positively. The increase in DAG content due to the washing step resulted in higher GE levels, corroborating the hypothesis of DAG contributing to the formation of GE in refined oil (Cheng et al. 2017). A previous study also observed the correlation between DAG in bleached oils and GE in deodorised oil (Li et al. 2016). It has been demonstrated that GE can be formed during deodorisation conditions from DAG by an intramolecular rearrangement resulting in fatty acid elimination (Destailats et al. 2012). Furthermore, at high temperatures, cyclic acyloxonium ions can be formed and may act as a reactive intermediate in the formation of GEs (Weißhaar and Perz 2010).

### Effects of double washing on the formation of 3-MCPDE, 2-MCPDE and GE

Given the limited reduction of 3-MCPDE, 2-MCPDE and the increase in GE levels seen after single washing, we proposed a double washing procedure using ethanol and water in two steps. These solvents were chosen due to their

polarity that could solubilise the chlorinated compounds and their ability to be separated from the oil phase. Furthermore, applying double washing on BPO prior to deodorisation has not been the subject of any previous report. The first step of the double washing was performed with ethanol 99.5% which was used as an attempt to remove the majority of the non-polar chlorinated compounds, while the second step was conducted using aqueous ethanol solutions (40% and 10%) and water. Thus, the polarity gradient present in the medium could remove a higher variety of chlorinated substances in terms of polarity.

For 3-MCPDE, a slight reduction was achieved by double washing (Figure 4). Washing BPO with ethanol 40%, 10% and 0% in the second washing step decreased 3-MCPDE levels by 12.1%, 17.1% and 10.2%, respectively, which was not significant ( $p > 0.05$ ) compared to the control.

For 2-MCPDE, the same pattern was observed, i.e. little reduction on the contaminant content (Figure 5). The levels of 2-MCPDE reduced by 11.2%, 18.1% and 9.8% when washing with ethanol 40%, 10% and 0% in the second washing step, respectively.

Despite the polarity gradient present in double washing treatment, the obtained results suggest that this approach was not effective to reduce chlorinated precursors from BPO, indicating that compounds of low polarity could also be involved in the formation of the contaminants. These results reinforce the idea that removing chlorinated compounds from BPO can be difficult given the high amount of chlorinated compounds with low polarity present in the oil at this stage (Nagy et al. 2011).

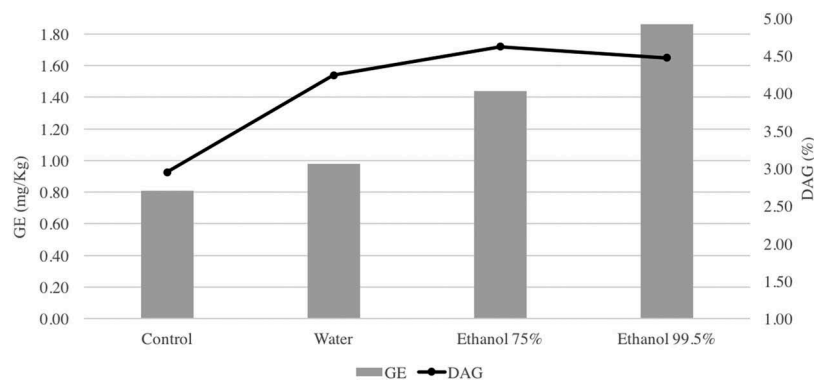


Figure 3. Levels of DAG and GE in BPO washed with different solvents (single washing), after deodorisation.

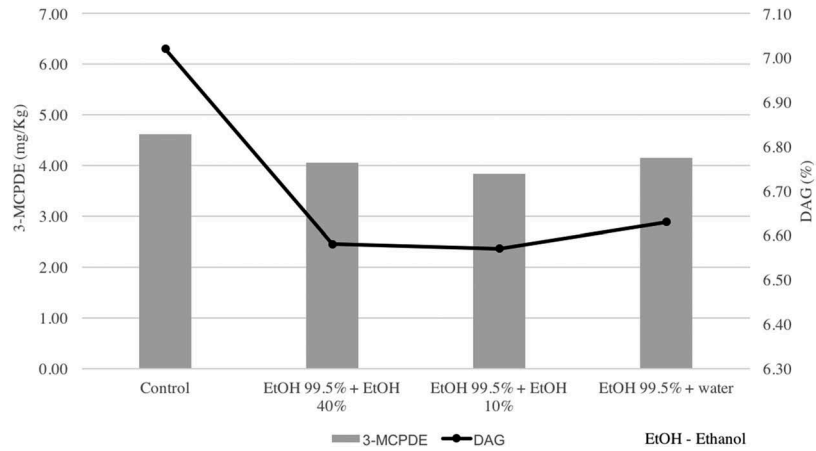


Figure 4. 3-MCPDE and DAG levels in BPO washed with different solvents (double washing), after deodorisation.

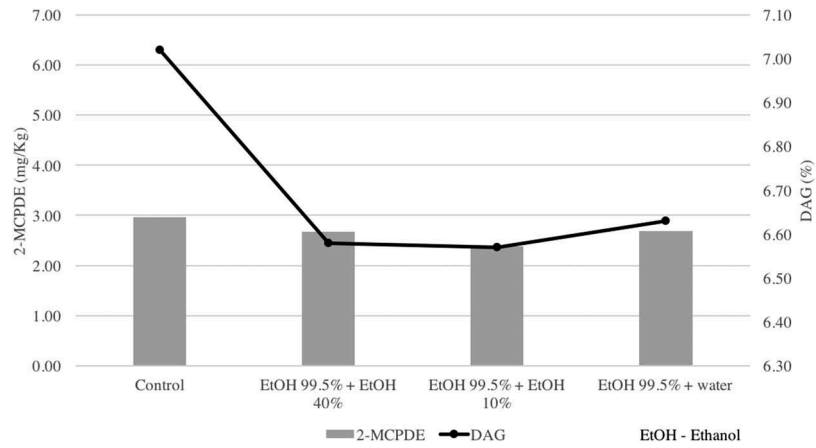


Figure 5. 2-MCPDE and DAG levels in BPO washed with different solvents (double washing), after deodorisation.

Washing BPO in two steps with different solvent polarity had a distinct impact on GE formation compared to the single washing. Figure 6 shows a substantial reduction on DAG content and, consequently, on GE levels. Double washing

with ethanol 40%, 10% and 0% in the second washing step reduced GE levels by 75.7%, 76.9% and 57.4%, respectively. The reduction observed for all treatments was significantly different ( $p < 0.05$ ) compared to control. This could be

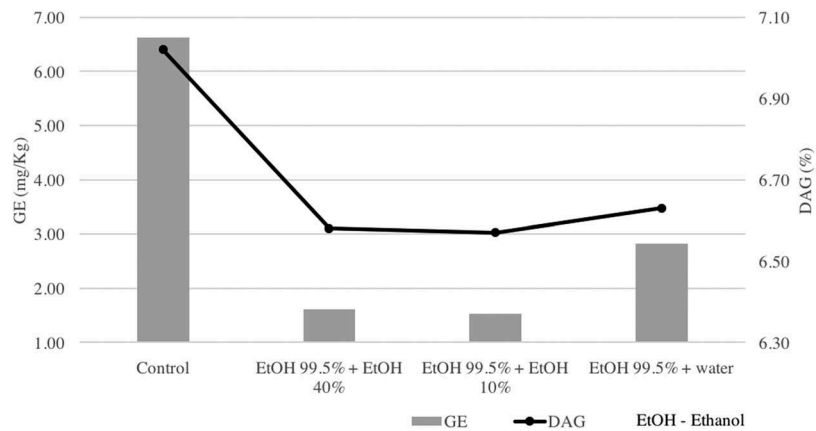


Figure 6. GE and DAG levels in BPO washed with different solvents (double washing), after deodorisation.

explained by the fact that the polarity gradient created by the solvents combination was capable of washing out DAG from the oil outweighing the effect of hydrolysis observed in the single washing. Published data on experimental liquid–liquid equilibrium showed that part of DAG present in edible oils could be partially soluble in ethanol at 45°C and 35°C (Shiozawa et al. 2015; Soares et al. 2018). This could explain the reduction in DAG when double washing was applied in BPO. Therefore, these results suggest that double washing could be applied for GE reduction, given the significant decrease achieved in this study, without leading to an increase in 3-MCPDE and 2-MCPDE formation. For single washing, after removing the solvent from the oil, the recovery of the oil was 97%, 90% and 70%, for water, ethanol 75% and ethanol 99.5%, respectively. For double washing after removing the residual solvent in the oil, the recovery of the oil was 71%, 81% and 86% when using ethanol 40%, 10% and water in the second step of washing, respectively.

Since double washing treatment showed the best results in terms of contaminants reduction, considering the three groups of substances, the impact of this procedure in the quality parameters of the oil was evaluated after the deodorisation, including determinations of free fatty acids, peroxide value, oxidative stability and colour (Table 2).

An increase of free fatty acids in comparison to the control was observed in all samples in which the washing treatment was applied. These results exceeded the limit recommended by CAC which is 0.3% for refined palm oil (CAC, 2015). For peroxide value, the results were below the standard recommended by CAC (Codex Alimentarius Commission (CAC) 2015) which is 10 meq O<sub>2</sub>/kg for refined palm oil. This could indicate that washing treatments did not favour lipid oxidation. However, we observed a decrease in induction time in all samples in which the washing treatments were applied. The highest decrease in induction

time was observed in the sample washed with ethanol 99.5% followed by ethanol 10%. This probably happened due to the presence of solvent into the oil which contributed to oil hydrolysis and consequently, to a reduction of the induction time. All treatments, including the control, exceeded the limits for colour recommended by CAC which is 3.0 for refined palm oil. This could be due to the colour fixation as a result of the presence of high molecular weight compounds derived from carotenoids oxidation reactions.

It is worth mentioning that the quality standards recommended by CAC are intended for voluntary application by commercial partners and not for application in governmental regulations. For all quality parameters tested in this study, washing palm oil with ethanol 99.5% followed by water had the most satisfactory result.

## Conclusion

Ethanol and water were demonstrated to be the most suitable solvents to be used in washing treatment of BPO given their ability to be separated from the oil after partition. Moreover, these solvents could present a feasible application in palm oil refineries. The employment of single and double washing in BPO prior to deodorisation slightly reduced the levels of 3-MCPDE and 2-MCPDE in the final product. This slight reduction might be due to the high content of non-polar chlorinated compounds in the oil, leading to removal of only part of the chlorinated species. Double washing effectively reduced GE levels which are associated with the significant removal of DAG in BPO prior to deodorisation. Thus, controlling and avoiding DAG formation during the washing treatment and the refining process is crucial to reduce GE levels. The washing treatment could be used as a supplementary strategy to reduce processing contaminants from palm oil. In addition, most of

**Table 2.** Quality parameters in deodorised palm oil after double washing treatment.

Sample	Free fatty acids (%)	Peroxide value (meq O <sub>2</sub> /kg)	Induction time (h)	Colour (R)
Control	0.14 ± 0.03	2.93 ± 1.35	14.32 ± 1.82	3.7
99.5% EtOH + 40% EtOH	0.80 ± 0.02	7.82 ± 1.36	11.12 ± 4.15	4.8
99.5% EtOH + 10% EtOH	1.06 ± 0.05	6.23 ± 0.31	3.27 ± 1.20	3.9
99.5% EtOH + water	0.43 ± 0.01	5.89 ± 1.38	13.53 ± 1.73	3.2

EtOH: ethanol.



these treatments did not result in a substantial loss of quality in the refined palm oil.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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