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Organic, conventional and sustainable palm oil (RSPO): Formation of 2- and 3-MCPD esters and glycidyl esters and influence of aqueous washing on their reduction

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A R T I C L E I N F O

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A B S T R A C T

Palm oil is a type of vegetable oil which presents a variety of applications including food, energy, and international trading. However, one of the major concerns associated with palm oil uses as a food ingredient is the significant amount of processing contaminants, such as monochloropropanediol esters (MCPDE) and glycidyl esters (GE) which are formed during the refining process. These contaminants may pose a health risk to consumers due to their carcinogenicity. Thus, mitigation strategies have been studied to reduce these substances in palm oil. In this study, we investigated the effect of the application of an aqueous washing step, as a mitigation strategy, prior to deodorization in three different palm oil cultivation systems: organic, conventional and certified palm oil by the Roundtable on Sustainable Palm Oil (RSPO). In addition, we evaluated the quality parameters after the application of the washing step. For the organic, conventional and RSPO palm oil samples, the aqueous washing step reduced approximately 41%, 34% and 36% of the 3-MCPDE, respectively. The levels of 2-MCPDE for the organic, conventional and RSPO palm oil presented a reduction of 55%, 41% and 32%, respectively. The GE levels are considerably low for all the deodorized palm oils, and presented no statistically significant difference ($p > 0.05$). Besides, the quality parameters such as free fatty acids, color, and OSI met the recommended limits. Therefore, the aqueous washing could be used as a supplementary strategy to reduce these contaminants from palm oil.

1. Introduction

The esterified forms of monochloropropanediol (MCPDE) and glycidol (GE) are a group of chemical contaminants formed during the processing of vegetable oils, especially in the refining steps that use high temperatures. MCPDE is formed by acylglycerol and chloride, and has two positional isomers known as 3-monochloropropane-1,2-diol esters (3-MCPDE) and 2-monochloropropane-1,3-diol esters (2-MCPDE). Glycidol is an organic compound structurally formed by a glycerol molecule containing epoxide and alcohol functional groups (Arisseto, Marcolino, Vicente, & Sampaio, 2013; EFSA, 2016; Destaillats et al., 2012a).

The free forms of these substances (3-MCPD, 2-MCPD e glycidol) have shown toxicity and carcinogenicity in animal models. The International Agency for Research on Cancer (IARC) classifies 3-MCPD as a possible human carcinogen (group 2B) and glycidol as a probable human carcinogen (group 2A) (IARC, 2000, 2012). In recent years, large amounts of 3-MCPDE, 2-MCPDE and GE have been found in processed foods and edible oils. The presence of 3-MCPDE and GE in the diet is a potential concern as these esters are efficiently hydrolyzed by enzymes digestive tract by releasing their free forms, 3-MCPD and glycidol which are potentially toxic (Abraham et al., 2013).

Palm oil is the edible oil 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 fertilizers containing chloride salts as well as the use of HCl-activated clays during the bleaching step of the

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refining process (Craft & Destaillats, 2014; Silva et al., 2014). Palm oil has been extensively applied in the food industry due to its various benefits such as high productivity, stability at high temperatures and during storage, excellent sensory attributes such as neutral odor and flavor, and creamy texture (Norhaizan et al., 2013; Sampaio et al., 2017).

Given the potential health risk that these contaminants may pose to the consumers, recently, the European regulatory body has set limits regarding the levels of these contaminants in edible oils and foodstuff that food industries must comply (European Commission, 2020). These limits have been urging the food sector to develop methods to mitigate these contaminants in vegetable oils specially palm oil.

One of the main strategies to reduce the formation of these contaminants in refined vegetable oils is the removal of their precursors before deodorization (Crews et al., 2013; Arisseto, Silva, Tivanello, Sampaio, & Vicente, 2018). The introduction of a washing step of the crude oil before the refining process could be a suitable alternative, since it could promote the removal of some chlorine "donor" substances, resulting in the prevention of 3-MCPDE and 2-MCPDE formation (Stadler, 2015; Silva et al., 2019).

Palm oil has may be obtained from three different cultivation systems: conventional palm oil, organic palm oil and the Roundtable on Sustainable Palm Oil (RSPO). The conventional palm oil is cultivated by applying fertilizers and pesticides which may result in soil deterioration and accumulation of residues generated by these chemicals (Lairon, 2011). Organic palm oil is obtained without the use of chemical inputs and/or fertilizers, emphasizing the use of locally adapted systems instead of synthetic materials (Nascimento et al., 2013). The RSPO system involves a set of actions related to compliance with local laws and regulations, economic viability, better agricultural practices, environmental responsibility and management of new plantations (RSPO, 2013).

To date, the levels of 3-MCPDE, 2-MCPDE and GE in palm oil from different cultivation systems (conventional, organic and RSPO) and mitigation strategies to reduce the levels of these substances have not been object of any previous investigation. Effective mitigation strategies must be developed in order to reduce the exposure of the consumers to these contaminants as well as to support the food sector to comply with the regulations that has been laid down.

This study aims to evaluate the levels of the contaminants 3-MCPDE, 2-MCPDE, and GE in three different palm oil cultivation systems (organic, conventional, and RSPO), as well as assess the addition of an aqueous washing step to crude palm oil obtained from different cultivation systems on the formation of the contaminants (3-MCPDE, 2-MCPDE, and GE). Moreover, the quality parameters were evaluated in order to understand the impact of the washing step on the refined palm oil quality.

2. Material and methods

2.1. Crude palm oil

The crude palm oil samples, obtained from different cultivation systems (organic, conventional and RSPO), were kindly supplied by a local company.

2.2. Standards

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

2.3. Solvents and reagents

Methanol was acquired from J. T. Baker. Hexane, heptane (purity ≥ 99%), tetrahydrofuran (THF, anhydrous, purity ≥ 99.99%), toluene (purity 99.9%), sodium bromide (NaBr, purity ≥ 99.5%), sodium bicarbonate, acetone and phenylboronic acid (PBA, purity ≥ 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfuric acid (H_2SO_4), sodium sulfate, sodium hydroxide and sodium thiosulfate were obtained from Merck (Darmstadt, Germany). Citric acid, ethanol 99.5%, phenolphthalein, tetradecane, BTSFA were purchased from Sigma-Aldrich Co. Ultrapure water was obtained from a Milli-Q Plus system (Millipore, Bedford, MA, USA). The activated bleaching earth (180 FF Supreme Tonsil) was kindly donated by Clariant®, Brazil.

2.4. Aqueous washing

The aqueous washing step of the different palm oil samples (organic, conventional, and RSPO) was adapted from a method previously described by Silva et al. (2019) in which the authors obtained a significant reduction in the levels of 3-MCPD when washing bleached palm oil with water and ethanol in high amounts. Moreover, the choice of using this proportion of water for the washing step in this study was based on preliminary results that showed a substantial reduction on the levels of chlorides in the CPO. Samples of organic, conventional and RSPO palm oil without the washing step were also analysed and named as control samples. This process was performed in duplicate for each type of oil.

2.5. Physical refining

The CPO samples washed and unwashed were submitted to a bleaching and deodorization steps. These processes were performed in duplicate for each type of oil (organic, conventional, and RSPO).

2.5.1. Bleaching process

The bleaching process was performed according to the method previously describe by Silva et al. (2013). In each trial that was used 500 g of oil (with and without aqueous washing) and 1% (w/w) of activated bleaching earth (Tonsil Supreme 180 FF, Clariant®) was added.

2.5.2. Deodorization

Deodorization experiments were performed in a lab-scale equipment described by Silva et al. (2019), and for each trial 200 g of oil was used. Deodorization parameters were fixed to all experiments and set as 260 °C of deodorization temperature, 2–7 mbar of pressure, 2% v/w steam injection, and 120 min of residence time. The process parameters were monitored every 30 min to assure their stability.

2.6. Analytical methods

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

3-MCPDE, 2-MCPDE, and GE were analysed by gas chromatography coupled to a mass spectrometer detector MSD 5975C (Agilent Technologies 7890A, United States) according to the official method Cd 29a-13 (AOCS, 2013). Therefore, 3 and 2- MCPDE as well as GE are expressed as free 2 and 3- MCPD and free glycidol.

2.6.2. Chloride content

The method used for the analysis of chloride content was adapted from a previous method optimized by Silveira, Caland, and Tubino (2014). The water obtained from the washed samples was filtered through a 0.45 µm chromatographic filter and analysed by ion chromatography using a Metrosep A Supp 05 (4.0 × 100 mm × 5.0 µm). A flow rate of 0.7 mL/min and conductimetric detector (Metrohm - 761 SD Compact IC, Switzerland) was used for the analysis. A Na_2CO_3 (3.2 $mmol.L^{-1}$) and $NaHCO_3$ (1.0 $mmol.L^{-1}$) aqueous solution was used as the eluent.

2.6.3. Free fat acids (FFA)

FFA were determined by titration with sodium hydroxide, according to the official method Ca 5a-40 (AOCS, 2008a,b), and expressed as oleic acid. Approximately, 2 g of sample was weighed and dissolved in 50 mL of neutralized ethanol. The sample was then titrated with sodium hydroxide until the phenolphthalein indicator changed color.

2.6.4. Acylglycerols composition

The content of DAG and MAG in palm oil samples were analysed according to the official method Cd 11b-91 (AOCS, 1997). The sample was injected into a gas chromatograph (GC Agilent 7890A, Japan) with OnColumn injection, FID detection.

2.6.5. Minerals

Minerals, such as P (Phosphorus), Fe (Iron), Ca (Calcium) and Mg (Magnesium) were quantified using the Inductively Coupled Plasma (ICP) technique (Agilent Technologies, Japan) according to the official method Ca 20-99 (AOCS, 2009).

2.6.6. Oxidative stability index (OSI)

The oxidative stability index of crude and refined palm oil samples was measured using a Rancimat® equipment (Metrohm 168 743 Rancimat, Metrohm, Riverview, FL, USA), based on the official method Cd12b-92 (AOCS, 1998). The oxidative stability of the oil was expressed as the induction time (h) and is defined as the time required for a rapid change in the rate of oxidation.

2.6.7. Deterioration of bleachability index (DOBI)

The DOBI was analysed in a UV–vis spectrophotometer. The value is given by the spectrophotometric absorbance ratio at 446 nm (maximum of nonoxidized carotene) and the absorbance at 268 nm (maximum oxidized carotene) (MPOB, 2005). Approximately, 0.1 mg of fully melted and homogenized oil was weighed and diluted in 25 mL isooctane. The DOBI was calculated using Eq. (1).

$$DOBI = \frac{Absorbance\ \ at\ \ 446\ \ nm}{Absorbance\ \ at\ \ 268\ \ nm} \tag{1}$$

2.6.8. Color

Color was determined according to the official method Cc 13e-92 (AOCS, 2008a,b), using a Lovibond Tintometric Color Scale at 70 °C. Color of the refined samples was performed using 5 ¼ (133.4 mm) glass cells. For the CPO samples, the color was determined using a 1″ (25.4 mm) glass cell. The scales range from 0 to 70 red (R) and 0 to 70 yellow (Y). The result was expressed as R and Y values.

2.6.9. Determination of pH

An aliquot of 600 g of oil was mixed with 30% w/w of water during 20 min at 55 °C. After partition, the water was removed and the pH analyses were carried out using a pH meter (LAB1000, Brazil).

2.7. Statistical analysis

Mean results were evaluated for significance using one-way analysis of variance (one-way ANOVA) and Tukey's test, employing the software STATISTICA® (Statsoft, version 7.0). The chosen level of significance was 5%. The term significant is used to indicate differences for which $p \leq 0.05$.

3. Results and discussion

3.1. Characterization of the raw materials: organic, conventional and RSPO palm oil

In order to determine the quality of the different crude oils (organic, conventional and RSPO), the samples were submitted to a physicochemical characterization which includes the determination of the acylglycerols classes, FFA, minerals content, oxidative stability index, and DOBI value (Table 1). As can be seen, the CPO from different cultivation systems is mainly represented by triacylgycerols (TAG) (93–95%). Nevertheless, considerable amounts of partial acylglycerols, such as DAG (3.5–3.7%) and MAG (0.15–0.17%) were also quantified. The FFA content of all CPO samples were considerably low (1.9–2.6%) compared with the amount generally found in other studies (2.3–6.0%) (Saad et al., 2007).

In over-ripe fruits or during the harvesting, a highly active lipase, probably originated from the yeast cells naturally present in the plant, will be responsible for increasing the FFA and partial acylglycerols content (Gibon, De Greyt, & Kellens, 2007). According to CODEX-STAN 210 (CAC, 2015) and the regulations from the Brazilian Health Regulatory Agency (ANVISA, 2005), the maximum recommended FFA content in CPO is 5.0%. All CPO samples were below the maximum value established by the legislation.

According to Table 1, all the oils showed high values for acylglycerols, while the lower FFA level was observed in the conventional crude palm oil. The content of minerals in CPO may vary according to the extraction process and machinery used (Sambanthamurthi, Sundram, & Tan, 2000) and, in the present study, the higher concentrations were reported for organic crude palm oil. The organic palm oil extraction differs from the RSPO and conventional palm oil since it is performed in a dedicated plant in order to avoid cross contamination of chemical inputs. However, the differences in minerals among the crude oils do not affect substantially the formation of the contaminants, since after bleaching it is not observed substantial differences in the mineral content. It is important to note that trace quantities of Fe reduce the oxidative stability of fat and oils, while P, Ca and Mg can reduce the efficiency of degumming and bleaching steps (Sampaio et al., 2015; Szydłowska-Czerniak, Trokowski, Karlovits, & Szlyk, 2013).

The DOBI value is a good parameter to assess the quality of a CPO in terms of the ease of oil bleaching (Gibon et al., 2007). The obtained DOBI mean values for organic, conventional, and RSPO palm oils were 3.4, 3.8, and 3.9, respectively. These values indicate that the oil samples can be easily bleached. The induction period, which was used to determine the oxidative stability index (OSI) of the CPO samples, were 27.0 h, 24.7 h, and 26. 9 h for organic, conventional, and RSPO palm oils, respectively, indicating their good quality.

3.2. Aqueous washing

The presence of inorganic chlorine in palm oil is related to its cultivation system, through the use of fertilizers and pesticides, containing chloride salts, as well as the oil extraction stage where a great amount of water is used (Blumhorst, Venkitasubramanian, & Collison, 2011). In fact, during palm oil processing some quantity of potable

Table 1
Characterization of organic, conventional and RSPO crude palm oil.

Parameters	Type of oils		
	Organic	Conventional	RSPO
Acylglycerols (%)			
TAG	93.53 ± 0.01	94.54 ± 0.06	93.91 ± 0.15
DAG	3.70 ± 0.44	3.42 ± 0.28	3.57 ± 0.18
MAG	0.17 ± 0.01	0.13 ± 0.01	0.14 ± 0.00
FFA*	2.60 ± 0.06	1.91 ± 0.03	2.38 ± 0.33
Minerals (mg/kg)			
P	20.90 ± 0.50	6.78 ± 0.03	4.56 ± 0.15
Ca	20.10 ± 0.50	3.99 ± 0.03	2.43 ± 0.10
Mg	3.99 ± 0.09	2.48 ± 0.03	1.26 ± 0.11
Fe	11.18 ± 0.08	1.66 ± 0.05	2.54 ± 0.14
DOBI	3.42 ± 0.41	3.77 ± 0.11	3.89 ± 0.05
OSI (h)	27.01 ± 0.00	24.70 ± 0.00	26.86 ± 0.00

* Free Fatty Acids (FFA) expressed as oleic acid.

water is necessary to leach out the oil, and it is known that iron (III) chloride is used as a coagulant in water treatment, hence a source of chlorinated compounds. According to Nagy, Sandoz, Craft, and Destaillats (2011), there are several types of chlorides present in palm oil, either inorganic or organic. Inorganic compounds are derived from ferric, ferrous, magnesium and calcium chlorides. On the other hand, organic chlorides are originated from the endogenous metabolism of plants that need these compounds for their growth.

In order to evaluate the removal of chloride polar compounds, which could act as precursors of MCPD esters, an aqueous washing step was proposed in this work. Fig. 1 illustrates the amount of chlorides, as well as the pH values present in the water originated from the wash of organic, conventional, and RSPO crude palm oil. The results revealed that the highest amount of chlorides was found in the water used to wash conventional CPO, which corresponded to 10.9 mg/kg, followed by the organic (5.9 mg/kg) and RSPO (1.4 mg/kg) cultivation systems. In fact, during the growing of conventional oil palm there is an important input of inorganic chloride containing compounds in the form of fertilizers (Matthäus & Pudel, 2013), which can be partially removed by water addition. On the other hand, some producers use the conventional oil palm bunches to improve organic oil palm growth and yield. This practice may contaminate the organic oil palm with inorganic chlorides coming from the bunches, which explains the significant amount of chloride found in the organic palm oil. For RSPO, the small farm holders probably do not use inorganic chloride sources, which justifies the low amount of chlorides. The pH values of the water after washing of conventional, organic, and RSPO palm oils were 4.6, 4.5 and 3.9, respectively, while the pH of the pure water (control) before washing was 5.2. According to Ramli, Siew, Ibrahim, Kuntom, and Abd Razak (2015), the decrease of the water pH is related to the acidity found in the vegetative material of the palm tree fruits bunches, which present an acidic character.

3.3. Physical refining

3.3.1. Bleaching process

The clay added in the bleaching step is used to remove lipid oxidation products, metals, and pigments such as carotenes (Oey, Van der Fels-Klerx, Fogliano, & Van Leeuwen, 2019). The clays are commonly activated by the addition of hydrochloric acid, sulfuric acid or phosphoric acid in order to increase its surface area, avoid color fixation, act as a catalyst, and also as an auxiliary during the filtration (Ramli et al., 2011; Taylor, 2009; Silva et al., 2014). However, residual acids (especially hydrochloric acid) in the bleaching clay may act as an external chloride source, which can lead to the formation of 3-MCPDE (and possibly 2-MCPDE) (Destaillats, Craft, Sandoz, & Nagy, 2012; Matthäus, Pudel, Fehling, Vosmann, & Freudenstein, 2011; Šmidrkal et al., 2016). Taking this into consideration, the clay used in this work (Tonsil Supreme 180 FF) was activated by sulfuric acid.

Table 2 presents the minerals (Ca, Fe, Mg, and P), the FFA content, and the OSI value for the bleached organic, conventional and RSPO palm oils, washed and unwashed. As can be observed, all the minerals were reduced, i.e., adsorbed by the activated clay during the bleaching treatment. Comparing the samples, it can be verified that the water addition did not have a considerable removal effect on the minerals. In fact, as the palm oil extraction process uses water to leach out the oil from the fruits, the remaining phosphorus compounds are mostly bound to calcium, magnesium and iron ions resulting on the formation of the non-hydratable phospholipids, which are easily removed by the addition of a chelating agent such as citric acid. Silva et al. (2013) studied the bleaching process of CPO and observed a removal of 99% of the phosphorus content using activated bleaching earth.

The bleached palm oils (organic, conventional, and RSPO) were also evaluated regarding its FFA content and OSI values. Comparing the washed and unwashed oil samples, it can be seen that the FFA content slightly decreased by the addition of the washing step, which is possibly due to the solubilization of the most polar acidic components. The OSI values remained practically unchanged when comparing the washed and unwashed samples of organic, conventional, and RSPO palm oil, with values varying between 26 and 29 h.

3.3.2. Deodorization and contaminants: 3-MCPDE, 2-MCPDE and GE

After the bleaching treatment, the palm oil samples (organic, conventional, and RSPO) followed the deodorization process. According to Sampaio, Ceriani, Silva, Taham, and Meirelles (2011) the deodorization process intends to remove free FFA and odoriferous compounds from the oil by applying steam flow, high temperatures at low pressures. Table 3 shows the content of 3-MCPDE and 2-MCPDE in organic, conventional and RSPO palm oil after the deodorization process. The addition of an aqueous washing step to the crude palm oil aims to reduce potential precursors of these contaminants and, consequently, their formation (Stadler & Lineback, 2009). According to Matthaus, Pudel, Fehling, Vosmann, & Freudestein (2011) during the growing of oil palm, the plant receives an important input of inorganic chloride containing compounds in the form of fertilizers. In fresh oils, these inorganic chlorinated compounds can be relatively easily removed by the addition of a polar solvent, such as water or ethanol.

The levels of 3-MCPDE, after applying a washing step, was reduced

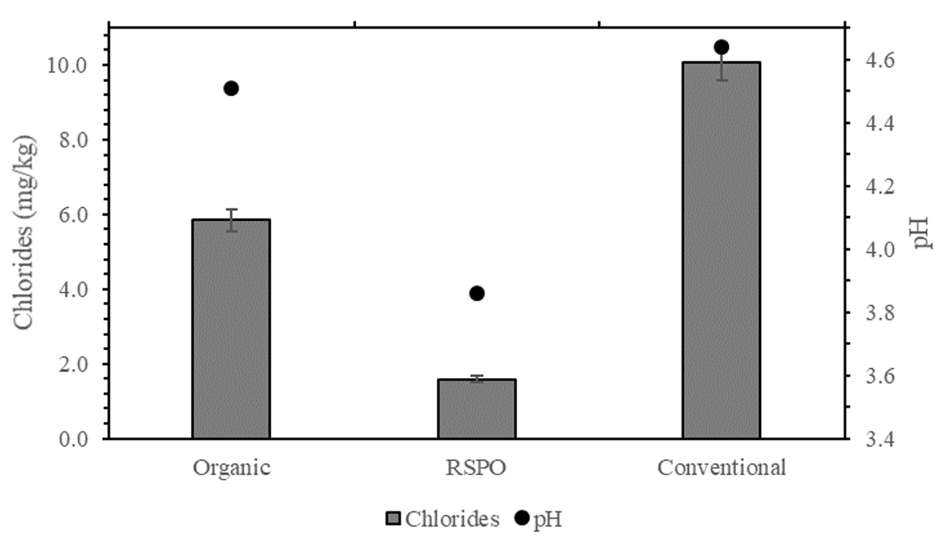

Fig. 1. Content of chloride compounds and pH of water after the washing step of organic, conventional and RSPO crude palm oil.

Table 2
Physicochemical parameters of palm oils samples (organic, conventional and RSPO) after bleaching.

Type of oil		FFA (%)*	OSI (h)	Minerals (mg/kg)			
				Ca	Fe	Mg	P
Organic	Unwashed	2.83 ± 0.02	26.83 ± 0.29	0.68 ± 0.02	3.38 ± 0.11	0.87 ± 0.02	1.04 ± 0.10
	Washed	2.61 ± 0.16	27.29 ± 0.16	1.39 ± 0.08	4.26 ± 0.05	1.11 ± 0.01	0.46 ± 0.03
Conventional	Unwashed	2.57 ± 0.01	28.76 ± 1.80	0.10 ± 0.00	0.89 ± 0.00	0.24 ± 0.02	0.33 ± 0.01
	Washed	2.38 ± 0.02	29.35 ± 0.27	< 0.10	1.81 ± 0.06	0.44 ± 0.01	0.47 ± 0.06
RSPO	Unwashed	2.20 ± 0.03	27.90 ± 0.28	0.46 ± 0.01	2.06 ± 0.06	0.61 ± 0.00	2.17 ± 0.44
	Washed	1.99 ± 0.01	27.22 ± 0.32	0.56 ± 0.04	2.61 ± 0.09	0.66 ± 0.00	1.21 ± 0.01

* Free Fatty Acids (FFA) expressed as oleic acid.

Table 3
Esters of monochloropropanols (3-MCPDE and 2-MCPDE) in deodorized palm oils.

Treatment	Type of oil	3-MCPDE (mg/kg)	2-MCPDE (mg/kg)
Unwashed	Organic	1.29 ± 0.11[a]	0.66 ± 0.02[a]
	Conventional	1.48 ± 0.02[b]	0.70 ± 0.09[b]
	RSPO	3.47 ± 0.06[c]	1.59 ± 0.08[c]
Washed	Organic	0.76 ± 0.02[b]	0.30 ± 0.03[b]
	Conventional	0.97 ± 0.08[c]	0.41 ± 0.05[c]
	RSPO	2.21 ± 0.33[a]	1.07 ± 0.18[a]

3-MCPDE e 2-MCPDE: Different letters in the same column indicate significant differences ($p < 0.05$).

by 41%, 34% and 36%, in organic, conventional and RSPO palm oil, respectively. These results are in agreement with the data reported by other researchers that found a decrease of approximately 30% of 3-MCPDE by washing palm fruit with ethanol (Oey et al., 2019). Washing palm fruit pulps with water and ethanol have been reported in literature, and a reduction of 20 and 25%, respectively, was observed for 3-MCPDE (Matthäus & Pudel, 2013; Matthäus, Pudel, Fehling, Vosmann, & Freudenstein, 2011). The reduction on the levels of 2 and 3-MCPDE observed for the washed compared to the unwashed organic palm oil, RSPO and conventional palm oil was significant different ($p < 0.05$). The levels of 2-MCPDE for the organic, conventional and RSPO palm oil presented a reduction of 55%, 41% and 32%, respectively. Similar results were found by Silva et al. (2019) when evaluating 2-MCPDE in conventional bleached palm oil. These results suggest that washing palm oil prior to deodorization can partially prevent the formation of both 2-MCPDE and 3-MCPDE. However, the aqueous washing can only remove the water-soluble chlorine species. According to Nagy et al. (2011), significant amount of low-polarity chlorinated compounds may be present in palm oil in later stages of the refining process, which cannot be removed by the aqueous washing.

According to the literature, the most crucial parameters for GE formation are DAG quantity and deodorization time and temperature (Cheng, Liu, Wang, & Liu, 2017). However, the greatest difference between the MCPDE and GE reaction formation is the absence of chloride, which may be responsible for the different formation rates (Pudel et al., 2011; Cheng et al., 2017). Table 4 presents the levels of GE in the organic, conventional, and RSPO deodorized palm oil as well as the DAG content after bleaching and deodorization processes. As can be seen, the GE levels are considerably low for all the deodorized palm oils, and presented no statistically significant difference ($p > 0.05$).

Table 4
Glycidyl esters (GE) and DAG content in deodorized palm oils.

Treatment	Type of oil	Bleaching	Deodorization	
		DAG[1] (%)	DAG[2] (%)	GE (mg/kg)
Unwashed	Organic	4.74 ± 0.09	4.61 ± 0.19	0.11 ± 0.03[a]
	Conventional	4.40 ± 0.66	4.60 ± 0.27	0.16 ± 0.01[a]
	RSPO	3.74 ± 0.50	3.89 ± 0.01	0.16 ± 0.08[a]
Washed	Organic	4.34 ± 0.68	4.99 ± 0.81	0.16 ± 0.02[a]
	Conventional	4.42 ± 0.00	4.47 ± 0.20	0.22 ± 0.08[a]
	RSPO	4.54 ± 0.03	4.44 ± 0.19	0.14 ± 0.01[a]

[1] DAG bleached oil.
[2] DAG deodorized oil; GE: The same letters do not indicate significant differences ($p < 0.05$).

Table 5 shows the FFA content, color, and OSI values of the deodorized palm oils. As can be seen, the FFA content decreased to values between 0.06 and 0.26% in all the evaluated palm oils samples. The highest FFA values were measured in the palm oil samples which were submitted to the aqueous washing step probably as a function of hydrolysis. Nevertheless, the results were below the upper limit recommended which is 0.3% for refined palm oil (CAC, 2015).

The color analysis performed in the deodorized palm oil samples (organic, conventional and RSPO) ranged from 0.9 to 1.65 for the red (R) index and from 4 to 10.0 for the yellow (Y) index. These two indexes together translate the concentration of carotenoids present in the oils. For instance, the Palm Oil Refiners Association of Malaysia (PORAM, 2018) requires levels below 3R for refined bleached deodorized (RBD) palm oil; therefore, all samples met the established standards. As stated by Sampaio et al. (2013), the conventional refining practiced in the industry removes all the carotenoids resulting in a light-colored oil.

Regarding the OSI values, a decrease in the induction period was observed in all samples that were submitted to the washing step. For instance, the organic palm oil samples decreased from 25.9 h to 23.0 h, the conventional palm oil samples presented a decrease from 20.7 h to 19.9 h, while the RSPO palm oil showed a decrease from 21.9 h to 20.1 h.

4. Conclusion

This study is the first report on the occurrence of 3-MCPDE, 2-MCPDE and GE in palm oil obtained from organic and RSPO cultivation system. The levels of these contaminants show significant difference among the cultivation systems evaluated in this study. Organic palm oil showed the lowest levels of contaminants which may be related to the absence of the use of chemical inputs that contributes to the formation of the contaminants during the refining process. Washing palm oil in the refining steps prior to deodorization seems to exert a positive effect on the mitigation of these substances in the oil without substantially affect its quality. Moreover, the washing treatment could be used as a supplementary strategy to reduce these processing contaminants in palm

Table 5
Physicochemical analysis of organic, conventional and RSPO oils after deodorization.

Type of oil		FFA (%)*	COLOR**	OSI (h)
Organic	Unwashed	0.12 ± 0.01	0.90R / 5.10Y	25.91 ± 0.27
	Washed	0.15 ± 0.03	1.00R / 9.70Y	22.96 ± 0.18
Conventional	Unwashed	0.06 ± 0.03	0.80R / 5.00Y	20.66 ± 0.18
	Washed	0.15 ± 0.00	0.70R / 4.10Y	19.92 ± 0.73
RSPO	Unwashed	0.18 ± 0.01	1.60R / 8.60Y	21.90 ± 0.23
	Washed	0.26 ± 0.03	1.60R / 9.00Y	20.10 ± 0.68

* Free Fatty Acids (FFA) expressed as oleic acid.
** Color (Lovibond) with $5''^{1/4}$ cuvette for deodorized.

oil. Further studies on the mitigation and the presence of this chemical contaminants should be undertaken specially using different cultivation systems.

CRediT authorship contribution statement

Jéssika Karolline Santiago: Investigation, Writing - original draft. **Willian Cruzeiro Silva:** Writing - original draft. **Maisa Freitas Capristo:** Investigation. **Marcela Cravo Ferreira:** Formal analysis. **Roseli Aparecida Ferrari:** Investigation. **Eduardo Vicente:** Investigation. **Antônio José A. Meirelles:** Project administration. **Adriana Pavesi Arisseto:** Methodology. **Klicia Araujo Sampaio:** Supervision, Writing - original draft, 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.

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