

METHOD FOR THE DETERMINATION OF TOTAL CHLORIDE CONTENT IN EDIBLE OILS

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ABSTRACT

Edible oils are important component of food products and have to meet with food safety requirements. However, a group of compounds called chloropropanols has been detected in edible oils which have compromised its safety. The precursor for these compounds is chloride. The chlorinated compounds can be in the form of organic or inorganic contaminants. The growing importance of chemical measurements for this entity has greatly pressured the method development to improve the quality of analytical results and to guarantee quality to the end users. In this work, a method for the determination of total chloride (TC) in edible oils was validated. The analysis of samples was performed by a combination of combustion and titration process using a Total Chloride Analyser (TCA). The results showed good linearity in the range of 0.5 to 20.0 $\mu\text{g mL}^{-1}$, with the correlation coefficient (R^2) of more than 0.999. The average recoveries of TC evaluated at three spike levels were 80% to 105% with relative standard deviations (RSD) of less than 10%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.03 and 0.10 $\mu\text{g mL}^{-1}$, respectively. The results indicated that this method could be used for routine analysis of TC in edible oils.

Keywords: chloropropanols, combustion, edible oils, titration, total chloride.

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INTRODUCTION

Edible oils are important ingredients in most food products. However, the presence of chloropropanols has been a major food safety issues in the processed edible oils. The chloropropanols detected are 3-monochloropropane-1,2-diol esters (3-MCPDE) and 2-monochloropropane-1,3-diol esters (2-MCPDE). They are a group of food processed contaminants formed from acylglycerols and chlorides during the refining process of edible oils. Glycidyl esters (GE) are mainly formed during the

deodourisation step in the refining process of edible oils and therefore occur in almost all refined edible oils (Smidrkal *et al.*, 2016).

Studies have shown that 3-MCPDE is formed in oils during deodourisation, which is performed at temperatures up to 270°C. Chemically, 3-monochloropropane-1,2-diol (3-MCPD) is a glycerol chlorohydrin formed when one hydroxyl group in a glycerol molecule is replaced by a chlorine atom. The single positional isomer, 2-chloropropane-1,3-diol (2-MCPD) and the two enantiomers of 3-MCPD are formed when -OH is replaced by -Cl at the *sn*-1 or *sn*-3 positions on the glycerol backbone (Figure 1) (Hamlet and Sadd, 2002).

The presence of 2-MCPDE, 3-MCPDE and GE in the diet is a potential health concern since these esters are hydrolysed by enzymes in the gastrointestinal tract and transform into their free forms, which have been considered toxic (Hamlet *et al.*, 2002). The free forms of 2-MCPDE, 3-MCPDE

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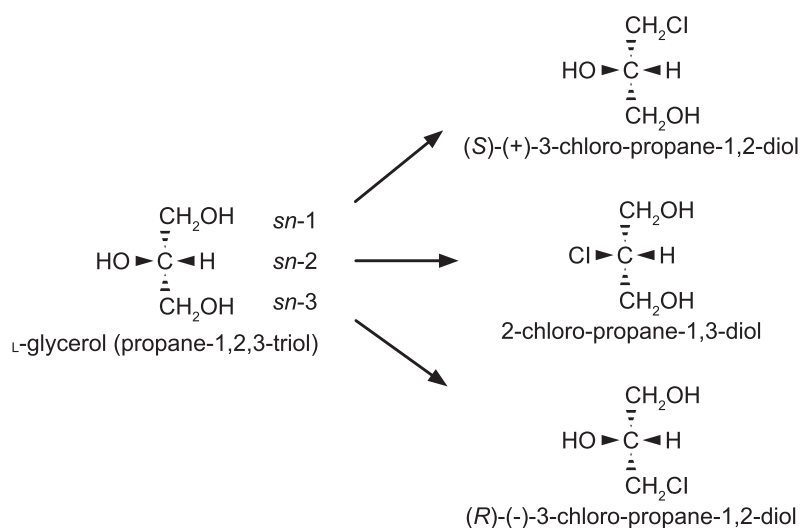


Figure 1. Formation of 2-MCPD and 3-MCPD with the presence of chloride atom.

and GE are free 2-MCPD, free 3-MCPD and glycidol, respectively. According to the International Agency for Research on Cancer (IARC), free 3-MCPD is classified as a possible human carcinogen (Group 2B) and glycidol as a probable human carcinogen (Group 2A) (Abraham *et al.*, 2013; IARC, 2012). For the free 2-MCPD and its esters, there is no risk assessment published in the literature to date, due to the lack of data on this contaminant (IARC, 2012).

The presence of a chloride 'donour' in crude oil is critical in the formation of 3-MCPDE and 2-MCPDE. This may be due to high levels of chlorinated substances generated by the plant's endogenous metabolism, the use of chloride salts in fertilisers, and the use of hydrochloric acid activated clays during the bleaching step of the refining process (Craft and Destailats, 2014; EFSA, 2016).

One of the strategies to minimise the formation of these contaminants in refined edible oils is the reduction of their precursors before deodourisation. Washing palm oil before deodourisation may be a viable option since it removes some of the precursors from the oil, mostly chloride 'donour' compounds, preventing the formation of 2-MCPDE and 3-MCPDE (Ramli *et al.*, 2020). In the literature, it was reported that washing crude palm oil (CPO) and fruit pulps reduced 3-MCPDE levels in refined oil by up to 90% (Ramli *et al.*, 2015; Silva *et al.*, 2014; Stadler, 2015).

Determination of chloride content is often conducted through titrimetric method due to its inherent reproducibility, accuracy and low costing absolute method of analysis. Nevertheless, detection of chloride in palm oil is not straight forward due to the complexity of the sample, comprising both polar and non-polar chlorides, as well as the possibility of analyte loss during the analytical procedures before the analysis.

There are numerous publications on the determination of chloride in soil, water and plant tissues. Analysis of chloride in these matrices are usually conducted by decomposition of the samples or salt extraction and followed by analysis using ion chromatography (IC), precipitation titration, potentiometric titration, and inductively coupled plasma mass spectrometry (ICP-MS) apparatus with special equipment (Krzyszaniak *et al.*, 2016). Chloride content in soil is determined in a soil-water extract. For plant tissue, it is either washed with lime or extracted with diluted nitric acid to determine chloride (Mesko *et al.*, 2016). Ion chromatography is well established as a routine technique for the determination of chloride in environmental samples, such as natural water, tap water, drinking water and soil. In fact, one of the earliest applications of IC is determination of chloride in soils (Mishra *et al.*, 2018).

The methods of precipitation and potentiometric titration use silver nitrate standard solution to determine the concentration of chloride by calculating the consumption of silver nitrate in the standard solution. The distinction between the two methods is the end point of titration. In potentiometric titration method, the end point is determined by the indicator electrode that records the change of the potential as a function of the amount (usually the volume) of the added titrant of exactly known concentration. In the precipitation titration method, the end point is determined by the formation of orange colour precipitate. Potentiometric titration is shown to be more sensitive than precipitation titration (Dianne, 2021).

The oldest method to determine chloride is the classical gravimetric method whereby chloride is measured as silver chloride. The Mohr and Volhard methods, which were established in the late 1800s, are classical volumetric chloride methods that have

been extensively used for soil and plant analysis. The Association of Official Analytical Chemists (AOAC) has official methods for the gravimetric and potentiometric determination of chloride in plant tissue, and for the volumetric determination of chloride in mineral water (Tian *et al.*, 2020). All of these methods are accurate. Nevertheless, any methods involving final weighing or manual titration is time consuming. Rapid measurements of chloride content have been reported for plant materials using an automatic chloride titrator with accuracy and precision comparable to manual potentiometric titration (Liao and Chou, 2006). A sensitive chloride colorimetric method developed by Zall *et al.* (1956) for determining chloride in water provides the basis for the development of an automated chloride colorimetric method.

A number of procedures for determination of chloride in oil matrix have been published and recognised as official methods for the American Society for Testing and Materials (ASTM) [ASTM D3230, ASTM D6470-6 and ASTM D4929-7], US Environmental Protection Agency (EPA) [Method; EPA 9075.8] and the Universal Oil Products (UOP) [Method UOP 779]. The ASTM D3230 and ASTM D6470 Methods are indirect quantification of chloride through the measurement of salt content. The ASTM D4929 and UOP 779 describe the quantification of organic chlorides while EPA 9075 defines the total chloride (TC) in petroleum products.

It is important to note that all the described official methods are solely for the measurement of chloride content in mineral oils or petroleum derivatives. Currently, there are still no methods available to detect the presence of chloride in edible oils, particularly CPO. Realising the importance to determine the level of chloride content in edible oils, an analytical method needs to be established by taking into account the distinction of chemical compositions and physical properties between mineral and edible oils. The availability of method to measure chloride content in edible oils would benefit the palm oil industry in gauging the CPO feedstock and thus, mitigates the presence of 3-MCPDE in the refined oils.

Therefore, in this article, a modified ASTM D4929 analytical procedure is proposed to determine the TC content in edible oils using a Total Chloride Analyser (TCA).

MATERIALS AND METHODS

Sample Collection

A total of 40 samples of various edible oils were collected. Twenty samples were palm oil products including CPO and refined, bleached and

deodourised palm oil (RBDPO) and were collected from local mill and refinery, respectively. The remaining 20 samples of commercial cooking oils consisted of soybean oil, canola oil, sunflower oil, corn oil, rice bran oil, macadamia oil, walnut oil, black seed oil, grape seed oil and olive oil and were purchased from local supermarket in Malaysia.

Chemicals and Reagents

Milli-Q system (Millipore Corp. Bedford, USA) ultrapure water was used to prepare all reference solutions and reagents. The resistivity of ultrapure water used has to be maintained at 18.2 MΩ cm or higher. The 2,4-nitrochlorobenzene stock standard solution was purchased from Merck (Darmstadt, Germany), while toluene, acetic acid, sodium acetate, potassium nitrate, potassium chloride and hydrochloric acid were obtained from Sigma-Aldrich Chemical, USA. All chemicals and organic solvents were of analytical grade. Carrier gases *i.e.*, argon (99.98% pure) and oxygen (99.70% pure) were purchased from MOX-Linde, Malaysia. All the plastic and glass wares were cleaned by soaking in a combination of diluted nitric acid and distilled water, in the ratio of 1:9 and were rinsed with ultrapure water before use, to avoid any possible contamination.

Chloride Stock Solution

Stock standard solutions of 5000 μg mL⁻¹ chloride was prepared by adding of 2.857 g (X) of 2,4-dinitrochlorobenzene in 100 mL of toluene (Y). The chloride concentration of this stock solution is obtained according to the following Equation (1) (Mitsubishi Chemical Analytech, 2014):

$$\text{Chloride concentration} \left(\mu\text{g mL}^{-1} \right) = \frac{X \text{ (g)}}{Y \text{ (mL)}} \times \frac{MW_{Cl}}{MW_{Cl Std}} \times 10^6 \quad (1)$$

where

MW_{Cl} is molecular weight of chloride (35.45)

$MW_{Cl Std}$ is molecular weight of chloride standard (202.55)

X is weight of chloride standard

Y is volume of toluene

Chloride Working Standard Solution

Working standard solution of 100 μg mL⁻¹ chloride was prepared daily by diluting 5000 μg mL⁻¹ chloride stock standard solution with toluene.

Preparation of Standard Calibration Curve

The calibration curve was established using five different chloride concentrations ranging from

0.5 to 20.0 $\mu\text{g mL}^{-1}$. Standard solutions of 0.5, 2.0, 5.0, 10.0 and 20.0 $\mu\text{g mL}^{-1}$ were prepared by diluting 100 $\mu\text{g mL}^{-1}$ working standard solution with appropriate volumes of toluene. The calibration ranges were selected according to the expected concentrations of the chloride in the edible oils.

Analytical Instruments and Devices

Quantitative analyses of the chloride were carried out using a TCA (NSX2100H, Nittoseiko Analytech Co. Ltd., Japan) equipped with an auto boat controller (ABC-210, Nittoseiko Analytech Co. Ltd., Japan), horizontal furnace (HF-210, Nittoseiko Analytech Co. Ltd., Japan) and coulometer detector (MCD-210, Nittoseiko Analytech Co. Ltd., Japan). *Table 1* shows the typical operating conditions of the TCA for the TC analysis.

Sample Preparation

The oil aliquot was first heated in a water bath at temperature of approximately 70°C for 20 min, to ensure homogeneity prior to sampling. About 50 mg of sample was then weighed and placed in a ceramic boat for combustion and titration process in the TCA. The combustion and titration conditions for the TCA are as shown in *Table 1*.

TABLE 1. OPERATING CONDITIONS OF TCA FOR COMBUSTION PROCEDURE

Parameter	Condition
Reactant gas flow, oxygen	200 mL min ⁻¹
Carrier gas flow, argon	200 mL min ⁻¹
Furnace temperature inlet	1 000°C
Furnace temperature outlet	1 100°C
Sample amount	40-60 mg
Detection mode	Coulometric titration

Validation and Recoveries

The analytical method was validated in-house for the determination of each TC in all edible oils in terms of linearity, accuracy, repeatability, reproducibility, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Linearity or calibration of instruments was estimated by injecting 2,4-dinitrochlorobenzene/toluene (chloride) standard solution at five different concentrations (0.5, 1.0, 5.0, 10.0, 20.0 $\mu\text{g mL}^{-1}$) in triplicate. Linearity acceptance criteria were at a correlation coefficient (R^2) >0.999.

Recovery, Repeatability and Reproducibility

Recovery studies were carried out by spiking 2,4-dinitrochlorobenzene in four selected oil samples, namely CPO, RBDPO, sunflower oil and canola oil at concentrations of 0.5, 1.0 and 5.0 $\mu\text{g mL}^{-1}$ in six replications. The percentage recovery of the analyte was calculated to evaluate the accuracy of the analytical procedure. Recovery (R) was then calculated as Equation (2):

$$\% R = \frac{\text{Concentration in spiked sample} - \text{Concentration in unspiked sample}}{\text{Actual spike concentration}} \times 100 \quad (2)$$

Repeatability was measured in terms of precision in order to determine the variability of independent test results obtained with the same method by the same operator using the same equipment in a series of analyses for similar samples (Nagy *et al.*, 2011). The repeatability (RSD_r) of the method was carried out by conducting recovery experiments using 2,4-dinitrochlorobenzene standard solutions at three different concentration levels (0.5, 1.0, 5.0 $\mu\text{g mL}^{-1}$) in six replicates of CPO, RBDPO, sunflower oil and canola oil which was then analysed using the established method. To evaluate within-laboratory reproducibility (RSD_R), two different analysts performed the recovery experiment with six replicates, for three consecutive days, using the same concentration, samples and method. Repeatability and reproducibility were determined according to the ISO5725-2 Guidelines and expressed by Relative Standard Deviation (RSD).

LOD and LOQ

The reagent blanks (toluene) were prepared following the same procedures for the quantification of chloride in the samples, in order to determine LOD and LOQ of the analytical procedure. LOD is the minimum concentration of analyte that can be detected but not necessarily quantified with an acceptable uncertainty. LOQ is the lowest concentration of an analyte in a sample which can be quantitatively determined with acceptable uncertainty. First, the standard deviation (SD) must be evaluated before the calculation of LOD and LOQ. The Equation (3) for determining the LOD and LOQ is as follows:

$$\text{Standard Deviation (SD)} = \frac{A \times B}{100} \quad (3)$$

where

A is an average count value of chloride in reagent blank (μg)

B is RSD of chloride in reagent blank

Next, the Equation (4) for the calculation of LOD and LOQ is as follows:

$$LOD/LOQ = \frac{C \times SD \text{ (derived from aboved calculation)}}{D \times E} \quad (4)$$

where

C is sigma (σ) (use 3 for LOD calculation and use 10 for LOQ calculation)

D is slope (B) of calibration curve

E is injection volume used for calibration (mL)

Harmonisation of Sample Preparation Procedure

To assess the homogeneity of chloride, the CPO samples were heated at 50°C, 60°C and 70°C and at each temperature, the samples were melted at two different periods; 10 and 20 min. Seven sets of samples of each CPO were placed in the oven at the indicated temperature and time. Once melted, the samples were vortexed for 1 min to ensure the homogeneity of chloride in the samples. Then, the samples were analysed on TC using the above validated method.

Statistical Method

The descriptive statistics (denoted as mean, SD and range) and analysis using the Students't-test were employed using SPSS software version 13.0 (IBM Corporation, New York).

RESULTS AND DISCUSSION

The development of the proposed methodology was performed in two steps: (1) validation and optimisation of the instrument conditions, and (2) application to real samples (edible oils).

Method Performance

Evaluation of linearity, recovery percentage (repeatability and reproducibility), LOD and LOQ are essential to assess the method performance (Zanella *et al.*, 2000). The calibration curve was obtained by analysing five different solutions of known concentrations of chloride between 0.5 and 20.0 $\mu\text{g mL}^{-1}$ for three times.

The data for the calibration curve of chloride standard is shown in Table 2. The data illustrate excellent range of correlation coefficient (R^2) values of more than 0.999. This shows good linearity of the analytical method which reflects feasibility of the method. Values of the RSD are less than 10% for all concentrations (0.5, 1.0, 5.0, 10.0, 20.0 $\mu\text{g mL}^{-1}$) which are within the acceptable range according to the Association of Official Analytical Chemists (AOCS, 2016).

The LOD and LOQ for the method are shown in Table 3. The LOD and LOQ were found to be 0.03 and 0.10 $\mu\text{g mL}^{-1}$, respectively. These results demonstrate that the validated method is very sensible for detecting and quantifying chloride.

TABLE 2. CALIBRATION CURVE DATA OF CHLORIDE STANDARD

Concentration of chloride standard ($\mu\text{g mL}^{-1}$)	Injection volume (μL)	Mean value ($\mu\text{g mL}^{-1}$)	SD	RSD (%)
0.5	40	0.49	0.02	3.10
1.0	40	1.01	0.07	6.57
5.0	40	4.93	0.15	3.10
10.0	40	9.90	0.20	2.02
20.0	40	19.93	0.15	0.77

Note: SD - standard deviation; RSD - relative standard deviation.

TABLE 3. LINEAR EQUATION, LOD AND LOQ OBTAINED FOR QUANTIFICATION OF TC

Parameter	Data
LOD ($\mu\text{g mL}^{-1}$)	0.03
LOQ ($\mu\text{g mL}^{-1}$)	0.10
Calibration curve	$y = 0.9035x + 0.006$
R^2	0.999

Note: LOD - limit of detection; LOQ - limit of quantification; R^2 - correlation coefficient.

The accuracy and precision of the results, estimated in percent average of standard addition recoveries, was higher than 80% at all spiked levels (Table 4). Recoveries were found to be 84% to 95%, 82% to 86%, 81% to 92% and 87% to 91% for CPO, RBDPO, sunflower oil and canola oil, respectively. Average recoveries for all samples were in the range of 81% and 95%, which demonstrated the conformity to the guidelines for the recovery analysis from 70% and 110%. This coincides with the requirement of the Association of Official Analytical Chemists (AOCS, 2016). It is not possible to compare the experimental mean recoveries with reference values since there is no Reference Material (RM) for chloride in edible oil for these matrices.

TABLE 4. RECOVERIES OF CHLORIDE IN SPIKED SAMPLES

Sample	Concentration of spiked chloride ($\mu\text{g mL}^{-1}$)	Mean recovery \pm SD	Recovery (%)
CPO	0.5	0.42 \pm 0.01	84
	1.0	0.86 \pm 0.02	86
	5.0	4.77 \pm 0.24	95
RBDPO	0.5	0.41 \pm 0.01	82
	1.0	0.85 \pm 0.01	85
	5.0	4.32 \pm 0.31	86
Sunflower oil	0.5	0.44 \pm 0.01	88
	1.0	0.81 \pm 0.01	81
	5.0	4.63 \pm 0.09	92
Canola oil	0.5	0.45 \pm 0.02	90
	1.0	0.87 \pm 0.01	87
	5.0	4.55 \pm 0.17	91

Note: CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil; SD - standard deviation.

The precision of the methods was assessed by repeatability and reproducibility, as described in Materials and Methods. RSD were calculated under RSD repeatability (RSD_r) and RSD reproducibility (RSD_R) conditions, to evaluate the precision of the method. The RSD for repeatability of chloride was found to be 0.8% to 2.4% in unspiked CPO, 0.4% to 3.6% in CPO, 1.8% to 2.7% in unspiked RBDPO, 0.9% to 2.6% in RBDPO, 2.3% to 3.2% in unspiked sunflower oil, 1.5% to 3.5% in sunflower oil, 0.9% to 2.1% in unspiked canola oil and 1.5% to 3.8% in canola oil. The RSD_R was found to be between 1.2% to 5.1% (Table 5). However, the developed method was within the acceptable range of RSDs of less than 20%, with the performance criteria requirement of the Association of Official Analytical Chemists (AOCS, 2016) and less than 15%, in agreement with the Food and Drug Administration guidelines (FDA, 2012). Hence, this indicates that the results are satisfactory and the method can be successfully applied for accurate and routine analysis of chloride in edible oils.

The study also demonstrated that the TC recovery increased with the function of combustion temperature and oxygen flow rate. A preliminary study revealed that the TC percentage recovery from CPO increased in concomitant with the increase of the combustion temperature and oxygen flow rate (Table 6). The combustion temperature of 950°C and 200 mL min^{-1} of oxygen flow rate is the optimal condition for the chloride analysis and this observation is in line with the findings by Cavallo *et al.* (2010). This combustion temperature of more than 950°C with the oxygen flow rate at 200 mL min^{-1} ensures enough energy to break the chemical

TABLE 5. REPEATABILITY (RSD_r) AND REPRODUCIBILITY (RSD_R) FOR CHLORIDE DETERMINATION IN OPTIMAL INSTRUMENT CONDITIONS

Sample	Spike ($\mu\text{g mL}^{-1}$)	Repeatability (RSD_r) %			Reproducibility (RSD_R) %
		Day 1	Day 2	Day 3	
Unspiked CPO	-	1.5	2.4	0.8	2.6
CPO	0.5	2.8	1.3	3.6	2.3
	1.0	0.4	2.8	2.8	2.1
	5.0	3.2	1.8	1.7	2.6
	-	2.1	2.7	1.8	3.2
RBDPO	0.5	1.2	2.6	1.5	2.1
	1.0	0.9	1.5	1.4	1.2
	5.0	1.6	1.3	2.1	1.5
	-	3.2	2.5	2.3	2.7
Sunflower oil	0.5	2.0	3.3	3.1	2.7
	1.0	1.7	2.7	3.5	4.2
	5.0	1.5	3.1	2.3	5.1
	-	0.9	2.1	1.5	3.1
Canola oil	0.5	3.8	1.8	2.6	2.8
	1.0	1.8	3.2	2.6	1.7
	5.0	3.6	1.5	1.8	5.1
	-	0.9	2.1	1.5	3.1

Note: CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil.

bond between organic and inorganic chloride in order to reach the maximum chloride recovery. From this study, the developed method can accurately determine the TC content which comprises of organochloride and inorganic chloride in edible oil samples. However, a comprehensive study needs to be conducted to support this theory and to gain more valuable data.

TABLE 6. CHLORIDE RECOVERY OF DIFFERENT COMBUSTION TEMPERATURES AND OXYGEN FLOW RATES

Combustion temperature (°C)	Oxygen flow rate (mL min ⁻¹)	% Recovery		
		0.5 µg mL ⁻¹	1.0 µg mL ⁻¹	5.0 µg mL ⁻¹
750	150	66	58	70
850		71	73	69
950		81	75	82
750	200	73	79	69
850		77	74	82
950		89	86	92

Harmonisation of Sample Preparation of Crude Palm Oil

Research by the Analytical Methods Committee (AMC) showed that sample preparation is the most common cause of errors in trace analysis and that contamination is always a major concern during sample preparation (Paul, 2013). Appropriate sample preparation techniques are always required in analytical analysis to enrich target analytes, or transform the sample into a suitable form for subsequent instrumental analysis (Hu and He, 2012).

Before the analysis of TC, the preparation of the CPO needs to be optimised to obtain homogeneous and representative test portions to avoid incorrect data interpretation. Data on TC obtained in different samples of pre-treated CPO is shown in *Table 7*.

TABLE 7. CONCENTRATION OF TC IN CPO AT DIFFERENT PRETREATED PARAMETERS

No.	Pre-treated parameter					
	50°C/10 min	50°C/20 min	60°C/10 min	60°C/20 min	70°C/10 min	70°C/20 min
1	2.6	3.5	2.4	3.8	3.3	3.6
2	3.4	1.9	2.8	3.2	3.1	3.2
3	2.7	2.5	1.6	2.4	2.8	3.4
4	2.8	2.8	3.1	2.6	2.6	3.0
5	1.9	2.4	3.5	2.9	3.4	2.9
6	3.5	1.7	2.6	3.3	3.9	3.7
7	3.9	3.7	2.7	1.8	2.4	3.2
Mean	3.0	2.6	2.7	2.9	3.1	3.3
SD	0.7	0.8	0.6	0.7	0.5	0.3
RSD (%)	22.6	28.5	22.2	23.0	16.8	9.0

Note: TC - total chloride; CPO - crude palm oil; SD - standard deviation; RSD - relative standard deviation.

From the study, it was discovered that the suitable temperature and duration melting time for sample pre-treatment was at 70°C and 20 min. Data obtained from this parameter showed that the TC level was consistent with low SD value and RSD percentage. On the contrary, the inconsistency of data obtained from other parameters may be due to samples being not completely melted and thus, affecting the homogeneity of chloride in the samples. This inconsistency has caused a wide range of SD and RSD percentages.

Based on statistical terms, the smaller the SD and RSD percentages, the closer the numbers are to the mean (In *et al.*, 2015). In addition, the percentage RSD also provides general information about the performance of a method, where 10% or less generally indicates a good method performance. It is proposed that the melting temperature of 70°C and duration for melting procedure at 20 min as suitable to ensure proper homogeneity and consistency in obtaining chloride in a sample.

Analysis of TC Content in Commercial Cooking Oils

The developed method was applied to determine the TC present in palm oil. The average concentrations of TC in each of the palm oil samples used in the study are shown in *Table 8*. The results show a wide variation in TC concentrations among the samples analysed and the chloride content of CPO is higher than RBDPO.

The high chloride level in the CPO may be due to uptake of chloride to the palm fruit from soil, water and fertiliser (Ibrahim *et al.*, 2015). During the processing of the palm fruit, the steam used for sterilisation may affect the chloride level in CPO (Craft *et al.*, 2012). Among the many potential sources of inorganic chloride in the oil palm supply chain, potassium chloride (KCl) and ammonium chloride

(NH₄Cl) are regularly used as fertilisers to support the growth of oil palms and increase bunch yield (Freudenstein *et al.*, 2013). Other inorganic chlorides used as fertiliser such as calcium chloride (CaCl₂), magnesium chloride (MgCl₂), ferrous chloride (FeCl₂) and ferric chloride (FeCl₃), are also commonly used in oil palm plantation (Abdollah, 2010). These inorganic sources of chloride are suggested to accumulate in the palm fruits during oil palm growth. The muriate of potash fertiliser used in oil palm plantations as well as the coagulant used for water treatment in palm oil mills were also believed to be the source of chloride in CPO (Andrew, 2018). Ferric chloride (FeCl₃) is a well-known coagulant used for water treatment in Malaysia (Chua *et al.*, 2020) and therefore acts as a potential source of chloride-containing compounds in the water within the oil palm environment.

TABLE 8. CONCENTRATIONS OF TC IN 20 PALM OIL SAMPLES ANALYSED USING TCA

No.	Matrix	TC ($\mu\text{g mL}^{-1}$) (mean \pm SD)
1	CPO	6.86 \pm 0.49
2	CPO	4.51 \pm 0.51
3	CPO	5.67 \pm 1.08
4	CPO	3.91 \pm 0.32
5	CPO	2.26 \pm 0.29
6	CPO	4.91 \pm 1.01
7	CPO	2.88 \pm 0.58
8	CPO	3.21 \pm 0.52
9	CPO	4.73 \pm 1.51
10	CPO	8.16 \pm 0.93
11	CPO	3.97 \pm 1.18
12	CPO	3.53 \pm 0.62
13	CPO	1.92 \pm 0.33
14	CPO	4.85 \pm 0.81
15	CPO	2.31 \pm 0.48
16	CPO	2.91 \pm 0.32
17	RBDPO	0.41 \pm 0.12
18	RBDPO	1.01 \pm 0.15
19	RBDPO	0.75 \pm 0.12
20	RBDPO	0.66 \pm 0.09

Note: TC - total chloride; TCA - total chloride analyser; CPO - crude palm oil; RBDPO - refined, bleached and deodourised palm oil; SD - standard deviation.

The study also showed that 93.75% of the CPO samples contained TC concentrations varying from 1.92 to 8.16 $\mu\text{g mL}^{-1}$. Empty fruit bunches (EFB) and palm oil mill sludge (POMS) contain a small amount of residual oil that is worth recovered. Hence, some

mills recover and blend these residual oils into CPO to increase oil extraction rate (OER). This practice has affected the quality parameters of CPO such as free fatty acids (FFA), peroxide value (PV), phosphorus level and also increases the level of chloride in CPO (Nizam and Mahmud, 2021).

CPO normally undergoes the refining process through degumming, bleaching and deodourisation. During bleaching process some of the chlorinated compounds are adsorbed onto the bleaching earth and hence reduced the chloride content in RBDPO (Ramli *et al.*, 2020).

The analysis of chloride in other commercial edible oils also showed a wide variation in TC level. Table 9 shows that in almost all edible oils of soft oil cluster, the chloride level was below 1 $\mu\text{g mL}^{-1}$. However, for the nut oil cluster, only walnut oil and peanut oil showed chloride levels of more than 1 $\mu\text{g mL}^{-1}$. The differences in the levels of chloride in each of the edible oil may be due to the oil processing during extraction and refining processes. For fruit oils, some vegetative parts of the fruits may contribute to the chloride content, as seen in CPO and if the vegetative parts are removed during processing, the chloride content is expected to be low, as in the extra virgin olive oil. However, further pressing of the olive pressed mass showed an increase in the chloride content as in the olive oil and pomace oil.

Most of the seed oils are extracted using solvent and followed by chemical refining. The alkaline and bleaching earth in the refining process have the capacity to remove the chloride in the oils. Hence, soft oils contain low chloride as shown in Table 9. Nut oils as in peanuts contain high levels of chloride because of the exposure and direct contact with the soil. Analysis of blended oils shown in Table 9 indicates that blending of soft oils with palm olein had slightly increased the chloride content, while blending between soft oils does not affect the chloride content.

The TC content in various refined oils as illustrated in Table 9, reflects the 3-MCPDE content in the oils. Mixture of palm olein with soft oils had exhibited higher TC content as compared to other blended soft oils. It was reported by Abd. Razak *et al.* (2012) that blended palm olein, sesame and peanut showed the highest 3-MCPDE content compared to other soft oils including canola, corn, sunflower, rice bran and soybean oils. The TC content in these soft oils is among the lowest. It was also found that palm olein and olive pomace oil contained the second and third highest 3-MCPDE content, respectively (Abd. Razak *et al.*, 2012), as reflected on the higher TC content in these types of oils. Hence, TC content in edible oils as tabulated in Table 9 is very useful in predicting the amount of 3-MCPDE content in the oils.

TABLE 9. CONCENTRATIONS OF TC IN 20 COMMERCIAL REFINED OIL SAMPLES ANALYSED USING TCA

No.	Sample	TC ($\mu\text{g mL}^{-1}$) (mean \pm SD)
Soft oils		
1	Canola oil	0.670 \pm 0.062
2	Sunflower oil	0.577 \pm 0.024
3	Rice bran oil	0.523 \pm 0.006
4	Roasted sesame oil	0.715 \pm 0.006
5	High oleic sunflower oil	0.317 \pm 0.021
6	Grapeseed oil	0.269 \pm 0.030
7	Black seed oil	0.249 \pm 0.042
Fruit oils		
8	Extra virgin olive oil	ND
9	Olive oil	0.563 \pm 0.075
10	Olive pomace oil	0.990 \pm 0.002
11	Palm olein	1.400 \pm 0.073
Lauric oils		
12	Coconut oil	0.287 \pm 0.042
13	Cold pressed virgin coconut oil	0.448 \pm 0.045
14	Palm kernel oil	ND
Nut oils		
15	Walnut oil	2.053 \pm 0.144
16	Peanut oil	1.104 \pm 0.134
Blended oils		
17	Mixture palm olein and sunflower oil	0.853 \pm 0.037
18	Mixture canola oil and corn oil	0.203 \pm 0.018
19	Mixture canola oil and sunflower oil	0.442 \pm 0.063
20	Mixture canola oil and soybean oil	0.130 \pm 0.015

Note: TC - total chloride; TCA - total chloride analyser; ND - not detected; SD - standard deviation.

CONCLUSION

The validated method for chloride determination in edible oils by the TCA presented satisfactory values of linearity, LOD and LOQ. In the same way, the acceptance criteria for validating the methodology was achieved, with a percentage RSD lower than 10% in the precision assays and a recovery of above 80%. This method can potentially be applied for the determination of chloride in edible oils. The validated methods were successfully applied for the determination of TC in commercial cooking oils purchased in Malaysia. The monitoring and elimination of chloride in crude edible oil should be implemented by the industry in order to ensure that edible oils produced are safe for human consumption and free from process developed contaminants, in this case 3-MCPDE.

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