Changes in 3-MCPD esters, glycidyl esters, bioactive compounds and oxidation indexes during kenaf seed oil refining

Sook-Chin Chew¹ · Chin-Ping Tan² · Oi-Ming Lai³ · Kar-Lin Nyam¹

Received: 18 September 2017 / Revised: 20 November 2017 / Accepted: 8 December 2017 / Published online: 16 December 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V., part of Springer Nature 2017

Abstract An optimized refining process for kenaf seed oil was conducted. The 3-monochloro-1,2-propanediol (3-MCPD) contents, triacylglycerol composition, fatty acids composition, bioactive compounds, phosphorus contents, and oxidation indexes of the kenaf seed oil during each stage of the refining process were determined. The results showed that there was no detected 3-MCPD ester in kenaf seed oil throughout the refining process. Deodorization had slightly increased the 2-MCPD ester (9.0 µg/kg) and glycidyl ester (54.8 µg/kg). Oleic (36.53%) and linoleic acids (36.52%) were presented in the largest amount in the refined kenaf seed oil, and triacylglycerols contributed to 99.96% in the oil. There was a removal of 31.6% of phytosterol content and 17.1% of tocopherol and tocotrienol contents in kenaf seed oil after refining. The refining process was totally removed the hydroperoxides, 93% of free fatty acids and 98.8% of phosphorus content in kenaf seed oil.

Keywords Kenaf seed oil · 3-Monochloro-1,2-propanediol · Triacylglycerol · Phytosterol · Tocopherol and tocotrienol

Introduction

Kenaf (*Hibiscus cannabinus* L.) is a widely cultivated species originated from Africa where it has been grown for 4000 years. Kenaf plants present a high potential due to its diverse functions in food, cosmetics, medicinal and other industrial utilization [1]. Kenaf seed, a by-product from the kenaf contains 20.8% of oil content, which consists of high amount of oleic and linoleic acids contributed to the high proportion of monounsaturated and polyunsaturated fatty acids. Thus, the research that focused on the utilization of kenaf seed oil has been increased in order to explore the kenaf seed oil as an edible oil and functional product [2, 3]. Besides that, kenaf seed oil was proved to be cytotoxic towards human breast cancer and colon cancer cell lines [4]. Owing to the above advantages, kenaf seed oil may present as an alternative and economic source of edible oil and functional oil to advantageous in the food, nutraceutical, and pharmaceutical industry. Crude oils are basically refined to produce better quality and oxidatively stable edible oil, which is preferable by the consumers [5]. However, minor components such as tocopherols and phytosterols are unavoidable to degenerate during the refining process [3]. Thus, changes of the bioactive compounds need to be monitored during the refining process of edible oil to minimize the loss of bioactive compounds. Chemical refining was proven to have a fewer effects on the desirable bioactive components [6]. Chemical refining has been widely used in the refining of vegetable oil such as wheat germ oil [7], soybean oil, canola oil [8], sunflower oil [6], and Camellia seed oil [9].

Previous studies on the optimized conditions in the refining process for kenaf seed oil has been reported [10–13]. However, there are still some minor components have not reported during the refining process of kenaf seed

✉ Kar-Lin Nyam
nyamkl@ucsiuniversity.edu.my

¹ Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia

² Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

³ Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

oil, which is greatly concerned recently. 3-Monochloro-1,2-propanediol (3-MCPD) has shown carcinogenic in a long term effect in the animal studies, including infertility and malfunction of certain organs [14]. There were different concentrations of 3-MCPD esters and glycidyl esters reported in vegetable oils and fats, including peanut oil, rapeseed oil, olive oil, and palm oil in many studies [15–18]. Palm oil was reported contained the highest amount of 3-MCPD esters among the vegetable oils [16]. The high temperature that exceeds 200 °C is pointed as the main culprit in the formation of 3-MCPD esters in the refined oil [15]. Thus, the formation of 3-MCPD esters in the oil during the refining process should be monitored and minimized.

The analysis of triacylglycerol composition of vegetable oils has received great interest to detect adulteration for quality control and origin determination. Formation of a cyclic acyloxonium ion from triacylglycerol with the present of chloride ions was proposed to one of the formation mechanism of 3-MCPD esters. Therefore, the formation of partial acylglycerol, including monoacylglycerol and diacylglycerol can further result in the formation of 3-MCPD esters and glycidyl esters [14]. There is no study reported on the 3-MCPD content and the acylglycerol composition in the kenaf seed oil. Against this background, the present study has studied the effects of the different stages of the chemical refining process using optimized parameters on the 3-MCPD esters, 2-MCPD esters and glycidyl esters, acylglycerol composition, fatty acid composition, bioactive compounds, phosphorus content, and oxidation indexes of kenaf seed oil.

Materials and methods

Materials and chemicals

Kenaf (*Hibiscus cannabinus* L.) seeds (variety: V36) were purchased from the National Kenaf and Tobacco Board (Kelantan, Malaysia). Acid-activated bleaching earth was obtained from Taiko Clay Marketing Sdn. Bhd. (Perak, Malaysia). 1,2-Bis-palmitoyl-2-chloropropanediol, 1,2-bis-palmitoyl-3-chloropropanediol, 1,2-bis-palmitoyl-3-chloropropanediol-d₅, glycidyl palmitate and glycidyl palmitate-d₅ were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Phytosterols standard (β -sitosterol, campesterol, and stigmasterol) and Supelco fatty acids methyl esters mixture were the products of Sigma-Aldrich (St. Louis, USA). Tocopherols and tocotrienols standards (α -, β -, γ -, and δ -) were the products of ChromaDex, Inc. (California, USA). Acetone, acetonitrile of HPLC grades and all other analytical grade chemicals used were purchased from Merck (Darmstadt, Germany).

Chemical refining process

Crude kenaf seed oil was extracted from the kenaf seeds with a Soxhlet extractor according to the previously described method [3]. The refining process was conducted according to the chemical refining process using optimized parameters in each stage of the refining process specifically for kenaf seed oil that reported in the previous studies [10–13]. Crude kenaf seed oil was acid degummed by 0.09% w/w phosphoric acid (85% concentration) for 10 min in an agitated water bath at 40 °C and followed by treating the crude oil with 22.4% w/w Milli-Q water for 30 min. Then, a stoichiometric quantity of NaOH solution (16 °Be) with an excess level of 3.75% was added into the degummed oil at 40 °C for 20 min to neutralize the free fatty acid. The neutralized oil was centrifuged at $10,000 \times g$ for 10 min to remove the soapstock and the neutralized oil was washed three times with 15% w/w ultrapure water. Taiko Classic acid-activated bleaching earth (1.5% w/w) was added into the neutralized oil and heated at 70 °C for 40 min under constant stirring. Residual bleaching earth was removed by centrifugation of the bleached oil at $10,000 \times g$ for 10 min. The final deodorization step was performed with a custom-made lab-scale glass deodorizer, consisting of a 250 mL four-necked round bottom flask equipped with a sparging inlet tube. The oil was heated at 220 °C at a reduced pressure of 9–12 mbar for 1.5 h. Steam was generated and passed through a delivery tube and sparging inlet tube into the oil.

2-MCPD ester, 3-MCPD ester, and glycidyl ester determination

The 2-MCPD ester, 3-MCPD ester and glycidyl ester contents were determined in accordance with the AOCS official method Cd 29a-13 [19]. Gas chromatography (GC) (Agilent Technologies 5975C, Agilent Technologies, Santa Clara, CA, USA) equipped with a mass selective detector and a capillary column HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) (Agilent Technologies) was used. The column temperature started from 80 °C (1 min) to 170 °C at a rate of 10 °C/min, increased to 200 °C at a rate of 3 °C/min, and lastly increased to 300 °C (15 min) at a rate of 15 °C/min. A 1 μ L of the prepared sample was injected and the injector was maintained at 250 °C (splitless). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. For the quantitative analysis, the derivatized 2-MCPD and 3-MCPD compound was examined by monitoring characteristic ions (quantifier) at m/z 147 of 3-MCPD, m/z 150 of an isotopically-labelled internal standard (3-MCPD-d₅) m/z 196 of 2-MCPD. Ions at m/z 196 and 198 (3-MCPD), m/z 198 (2-MCPD), and m/z 203 (3-MCPD-d₅) were used as qualifiers. Quantifier ion

used to determine derivatized 3-MBPD compound were monitored at m/z 147 for 3-MBPD and m/z 150 for 3-MBPD- d_5 (isotopically-labelled internal standard). Qualifiers ions were at m/z 240 (3-MBPD) and m/z 245 (3-MBPD- d_5). The respective content was calculated based on a calibration curve that was constructed by plotting the peak area ratios for the calibration standards (y-axis) against the concentration of the calibration standards (x-axis).

Acylglycerol composition

The acylglycerol composition was analyzed using high performance liquid chromatography (HPLC) (Waters e2695, Milford Massachusetts, USA) equipped with evaporative light scattering detector and a pre-coated silica reversed phase Lichro CART C18 column ($5\ \mu\text{m} \times 250\ \text{mm} \times 4\ \text{mm}$) (Merck, Germany). Separation of the acylglycerol peaks was based on the gradient elution of the mobile phase, which consisted of acetone (A) and acetonitrile (B) at a flow rate of 1 mL/min with a total run time of 65 min. The gradient elution was started to increase to 15% A, 85% B (0–8 min), changed to 65% A, 35% B (8–35 min), maintained at 65% A, 35% B (35–55 min), changed to 15% A, 85% B (55–60 min), and maintained at 15% A, 85% B (60–65 min). The temperature for column, drift tube and nebulizer of the detector was set at 35, 60 and 36 °C, respectively. Nitrogen was used as nebulizer at 35 psi. Prior to the HPLC analysis, 100 μL of the oil sample was dissolved in 1 mL of acetone and filtered through a 0.45 μm PTFE membrane filter. Aliquots of 20 μL were injected for acylglycerol analysis. Tridodecanoate, tripalmitin and trioleate standard were used as reference standard for peak identification from the chromatogram. ECN (Equivalent Carbon Number) value was used to identify the peaks and calculated by using the equation of $\text{ECN} = \text{CN (carbon number)} - 2\text{DB (number of double bond)}$. The relative amount of each acylglycerol was quantified by integrating the area under the peak and dividing the area by the total areas for all acylglycerols [20].

Fatty acid composition

Fatty acid composition was determined according to the previously established method [3] by using GC (Agilent Technologies 7890A, Agilent Technologies) connected with a flame ionization detector (FID) and a polar capillary column BPX70 ($30\ \text{m} \times 0.32\ \text{mm i.d.}, 0.25\ \mu\text{m}$ film thickness) (SGE International Pty. Ltd., Victoria, Australia).

Chromatographic determination of phytosterols and tocopherols

The phytosterols content in kenaf seed oil were analyzed according to the previously established method [3, 21] using a GC (Agilent Technologies 7890A, Agilent Technologies), coupled with a HP-5 capillary column column ($30\ \text{m} \times 0.32\ \text{mm i.d.}, 0.25\ \mu\text{m}$ film thickness) (Agilent Technologies) and a FID. The tocopherols and tocotrienols contents were analyzed according to the previously described method [13] using a HPLC (Agilent Technologies 1200 Series, Agilent Technologies), equipped with a UV-vis detector and a reversed phase Cosmosil 5PFP packed column ($5\ \mu\text{m} \times 250\ \text{mm} \times 4.6\ \text{mm}$) (Nacalai Tesque, Inc., Japan), which uses pentafluorophenyl-bonded as the stationary phase.

Chemical analysis

AOCS Official Methods were used for the determination of peroxide value (PV) (Method Cd 8-53), *p*-Anisidine value (*p*-AV) (Method Cd 18-90), and free fatty acid (FFA) (Method 26.042) [22] of the oil samples. Equation of total oxidation value (TOTOX) = $2\text{PV} + p\text{-AV}$ was used to calculate the TOTOX value [23]. Iodine value (IV) was determined by titration using previously established method [24]. The phosphorus content of oil samples was measured according to the Malaysian Palm Oil Board Test Method p 2.8 [25].

Statistical analysis

All experiments were conducted in duplicate and measurements were replicated two times. The results were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using MINITAB 16 software (Minitab Inc, Pennsylvania, USA) to determine the significant difference at the level of $p < 0.05$.

Results and discussion

2-MCPD esters, 3-MCPD esters, and glycidyl esters

Since 3-MCPD esters can be generated through the 2-MCPD and glycidyl esters, and 3-MCPD esters can be metabolized to liberate free 3-MCPD in the human gastrointestinal tract [17], thus the contents of 2-MCPD, 3-MCPD and glycidyl esters in the food product are highly concerned. Table 1 shows that there was no detected 3-MCPD ester in the kenaf seed oil during the whole refining process. A tolerable daily intake (TDI) of 2 $\mu\text{g/kg}$ body weight for free 3-MCPD defined by the European

Table 1 2-, 3-MCPD esters and glycidyl ester contents of kenaf seed oil during the refining process

Oil	2-MCPD ester ($\mu\text{g/kg}$)	3-MCPD ester ($\mu\text{g/kg}$)	Glycidyl ester ($\mu\text{g/kg}$)
Crude	3.5	ND	ND
Degummed	ND	ND	ND
Neutralized	1.0	ND	ND
Bleached	ND	ND	ND
Deodorized	9.0	ND	54.8

ND not detected

Scientific Committee on Food in 2001 [17]. However, the Panel on Contaminants in the Food Chain established a TDI of 0.8 $\mu\text{g/kg}$ body weight for free and esterified 3-MCPD after reviewed 7175 data for free and esterified 2- and 3-MCPD and glycidyl esters in food [26]. Previous studies reported different contents of 3-MCPD in different types of oil, including rapeseed oil (1000 $\mu\text{g/kg}$), palm oil (4400 $\mu\text{g/kg}$), and peanut oil (440 to 620 $\mu\text{g/kg}$) [14, 15, 17]. This study showed that the crude and refined kenaf seed oil were not contaminated by 3-MCPD esters and safe for consumption.

A slight amount of 2-MCPD esters was found in the crude, neutralized and deodorized kenaf seed oil. On the other hand, there were no detected glycidyl esters in the kenaf seed oil in the previous stages of the refining process. Deodorization has increased the glycidyl esters slightly in kenaf seed oil. Different types of oils may contain different precursors to contribute these types of food contaminants [15]. MAG, DAG, and chlorine donors were found to be the precursors in the formation of 3-MCPD esters. Glycidyl esters are produced mainly by intramolecular elimination of a fatty acid from DAG [17]. However, MAG, DAG, and chlorides are polar compounds, which can be removed partially through the water and acid degumming. The absence of 3-MCPD esters and glycidyl esters in crude kenaf seed oil might attribute to the lower percentage of MAG and DAG in this study [16]. The formation of glycidyl esters in the deodorization stage might be due to the heating of DAG and MAG at high temperature that exceeds 200 °C. The mean chronic exposure to glycidol was reported in a range of 0.1–0.5 $\mu\text{g/kg}$ body weight per day across the dietary surveys for adolescents and adult population groups [26]. Refined kenaf seed oil is safe from 3-MCPD esters and contains less 2-MCPD esters and glycidyl esters compared to others refined oils. Refined kenaf seed oil contains lesser amount of glycidyl esters than other types of oil that reported in the previous studies, including palm oil (3000–5000 $\mu\text{g/kg}$), sunflower oil (269 $\mu\text{g/kg}$), soybean oil (171 $\mu\text{g/kg}$), rapeseed oil (166 $\mu\text{g/kg}$), and peanut oil (148 $\mu\text{g/kg}$) [14, 26]. Palm oil was reported to consist of the highest content of 3-MCPD esters and glycidyl esters among the edible oil [14]. The previous study reported that intrinsic composition of the

crude palm oil was the main factor to contribute the formation of 3-MCPD rather than the parameters used in the refining process [16]. This showed that the intrinsic part of the kenaf seed oil and the deodorization parameters did not contribute to the formation of 3-MCPD esters. There was only the deodorization increase the 2-MCPD esters and glycidyl esters in refined kenaf seed oil to 9.0 and 54.8 $\mu\text{g/kg}$, respectively.

Acylglycerol composition and fatty acid composition

A total of 20 peaks were separated and identified in 65 min for crude and refined kenaf seed oil as shown in Fig. 1(A) and (B), respectively. Table 2 shows the monoacylglycerol (MAG), diacylglycerol (DAG) and triacylglycerol (TAG) composition of kenaf seed oil during each stage of the refining process. The highest amount of TAG was occupied by POL and OOL (ECN = 46) with 22.97–24.46% in the percent composition. LLS (ECN = 46), LLP (ECN = 44), OLL (ECN = 44), and POO/SOL (ECN = 48) occupied more than 10% respectively, which totally contributed to approximately 53% in the acylglycerol composition of kenaf seed oil. LLH (ECN = 43), PLP (ECN = 46), OOO/ALL (ECN = 46), PPO (ECN = 48), and LLN/LLB (ECN = 50) were totally contributed to about 22% in the acylglycerol composition in kenaf seed oil. Other TAGs, MAG, and DAG were contributed to less than 1% respectively in the kenaf seed oil. MAG and DAG contributed to only 0.2% in the crude kenaf seed oil. MAG had been totally removed during the degumming process. DAG had significantly reduced ($p < 0.05$) in the neutralization stage. After the whole refining process, there was only 0.04% of DAG in the refined kenaf seed oil. TAG contributed to the main components (99–99.96%) in kenaf seed oil. The main TAGs mostly contained 44, 46 and 48 acyl carbon atoms, which is most likely due to the kenaf seed oil contains a high level of linoleic acid, oleic acid, and palmitic acid. Most of the fatty acids in kenaf seed oil identified using GC was found to contribute to the TAGs.

Table 3 shows the fatty acids composition of kenaf seed oil in each stage of the refining process. The crude and refined kenaf seed oil exhibited a fatty acid profile with

Fig. 1 Chromatogram of acylglycerol composition of (A) crude and (B) deodorized kenaf seed oil. The peaks were followed the order that stated in Table 2

oleic acid (C18:1n9) and linoleic acid (C18:2n6) as the most abundant fatty acids and followed by palmitic acid (C16:0). There was a slight decrease in the saturated and monounsaturated fatty acids and a slight increase in the

polyunsaturated fatty acids. The fatty acid profile of hazelnut oil and groundnut oil did not change significantly by the refining process [27, 28]. The previous study reported deodorization caused a significant decrease in the

Table 2 Acylglycerol composition of kenaf seed oil during the refining process

Acylglycerol (%)		Crude	Degummed	Neutralized	Bleached	Deodorized
MAG		0.09 ± 0.01	ND	ND	ND	ND
DAG		0.10 ± 0.01 ^a	0.09 ± 0.01 ^b	0.04 ± 0.00 ^c	0.05 ± 0.00 ^c	0.04 ± 0.00 ^c
ECN TAG						
40	LLLn	0.23 ± 0.02 ^a	0.24 ± 0.02 ^a	0.23 ± 0.03 ^a	ND	ND
	LnLnO	0.03 ± 0.00 ^b	0.04 ± 0.00 ^{ab}	0.04 ± 0.00 ^a	ND	ND
42	LLL	0.37 ± 0.03 ^a	0.39 ± 0.02 ^a	0.36 ± 0.01 ^a	0.07 ± 0.00 ^b	0.04 ± 0.01 ^b
	OLLn	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	ND	ND
43	LLH	4.74 ± 0.17 ^a	4.61 ± 0.13 ^{ab}	4.44 ± 0.17 ^{abc}	4.12 ± 0.29 ^c	4.23 ± 0.14 ^{bc}
44	OOLn	0.06 ± 0.00 ^{bc}	0.05 ± 0.01 ^c	0.07 ± 0.01 ^b	0.10 ± 0.01 ^a	0.06 ± 0.01 ^{bc}
	OLL	13.42 ± 0.41 ^a	12.86 ± 0.39 ^{ab}	12.42 ± 0.32 ^b	12.05 ± 0.30 ^b	12.46 ± 0.65 ^b
	LLP	12.75 ± 0.33 ^a	12.21 ± 0.33 ^a	12.06 ± 0.30 ^a	11.85 ± 0.20 ^a	12.43 ± 0.76 ^a
46	LLS	14.55 ± 0.25 ^a	14.42 ± 0.35 ^a	14.13 ± 0.41 ^a	14.28 ± 0.25 ^a	15.09 ± 1.33 ^a
	POL/OOL	22.97 ± 0.61 ^{ab}	22.43 ± 0.38 ^{ab}	22.24 ± 0.39 ^b	22.46 ± 0.55 ^{ab}	24.45 ± 1.92 ^a
	PLP	5.26 ± 0.16 ^b	5.39 ± 0.12 ^{ab}	5.58 ± 0.21 ^{ab}	5.64 ± 0.09 ^{ab}	5.87 ± 0.46 ^a
48	OOO/ALL	7.85 ± 0.46 ^b	8.31 ± 0.23 ^{ab}	8.70 ± 0.51 ^a	8.85 ± 0.39 ^a	6.68 ± 0.29 ^{ab}
	POO/SOL	12.18 ± 0.78 ^a	12.65 ± 0.43 ^a	12.74 ± 0.48 ^a	12.98 ± 0.27 ^a	12.59 ± 0.60 ^a
	PPO	3.94 ± 0.42 ^c	4.38 ± 0.46 ^{bc}	4.79 ± 0.36 ^{ab}	5.22 ± 0.12 ^a	4.00 ± 0.35 ^{ab}
50	LLN/LLB	0.91 ± 0.12 ^b	1.27 ± 0.08 ^{ab}	1.32 ± 0.11 ^a	1.48 ± 0.11 ^a	1.31 ± 0.14 ^a
	OSS/OLA	0.44 ± 0.04 ^c	0.55 ± 0.06 ^{bc}	0.60 ± 0.01 ^b	0.75 ± 0.00 ^a	0.63 ± 0.08 ^a
52	LLLg/OLN/OLB	ND	ND	0.07 ± 0.00 ^b	0.05 ± 0.00 ^c	0.07 ± 0.02 ^a
54	OLLg/OON/OOB	ND	ND	0.07 ± 0.00 ^b	0.06 ± 0.00 ^c	0.06 ± 0.00 ^a

ND not detected

A arachidic acid, B behenic acid, H heptadecenoic acid, L linoleic acid, Lg lignoceric acid, Ln linolenic acid, N nervonic, O oleic acid, P palmitic acid, S stearic acid

^{abc}Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p < 0.05$) according to Tukey's test

oleic acid from bleached kenaf seed oil [3]. However, deodorization did not affect so much in the unsaturated fatty acids in this study. There were no major changes in the fatty acids composition of kenaf seed oil in each stage of the refining process. Unsaturated fatty acids accountable for 75.07 and 75.15% of the total fatty acids in the crude and refined kenaf seed oil, respectively. The high amount of unsaturated fatty acids makes the kenaf seed oil suitable to use as cooking oil for diet enrichment.

Phytosterols, tocopherols and tocotrienols contents

The effect of refining process on the total phytosterol content is illustrated in Table 4. The removal of phytosterols is due to the acid-catalyzed dehydration on the surface of the bleaching earth leads to the formation of steradienes during bleaching process. Besides, high temperature during the deodorization process also promotes the dehydration of phytosterols [27, 29]. However, the most significant removal (13.1%) in the total phytosterols content occurred in the neutralization stage. Parts of the

total phytosterols are transferred by liquid–liquid partitioning to the soapstock during neutralization stage [27]. Bleaching stage in this study caused a lower impact on the total phytosterols content in kenaf seed oil than others stages in the refining process. Ortega-García et al. [29] reported a decrease of 14.2% of the total sterols content in the bleaching stage of safflower oil, but the bleaching stage in this study resulted in a decrease of 4.1% in kenaf seed oil. The whole refining process was totally removed 31.6% of phytosterols content from the crude kenaf seed oil. Previous study showed the conventional refining process had reduced 22.5% of the total phytosterol content in kenaf seed oil, which is lesser than this study [3]. This might be due to the parameters used in the neutralization stage of the conventional refining process were not efficient to form the soapstock to remove the FFA content. Thus, lesser phytosterol content was removed through the soapstock, as the FFA value still remained high in the refined kenaf seed oil after the conventional refining process [3].

Table 4 summarizes the tocopherols and tocotrienols contents of kenaf seed oil during different stages of the

Table 3 Fatty acid composition of kenaf seed oil during the refining process

Fatty acid (%)	Crude	Degummed	Neutralized	Bleached	Deodorized
C14:0	0.14 ± 0.00 ^b	0.14 ± 0.00 ^b	0.14 ± 0.00 ^b	0.14 ± 0.00 ^b	0.15 ± 0.00 ^a
C16:0	19.33 ± 0.12 ^c	19.48 ± 0.10 ^c	19.51 ± 0.04 ^{bc}	19.72 ± 0.08 ^{ab}	19.76 ± 0.13 ^a
C16:1	0.53 ± 0.03 ^a	0.50 ± 0.00 ^a	0.51 ± 0.00 ^a	0.51 ± 0.00 ^a	0.51 ± 0.00 ^a
C17:1	0.22 ± 0.00 ^a	0.21 ± 0.00 ^a	0.22 ± 0.01 ^a	0.21 ± 0.00 ^a	0.22 ± 0.00 ^a
C18:0	4.32 ± 0.05 ^{ab}	4.35 ± 0.03 ^a	4.37 ± 0.01 ^a	4.28 ± 0.01 ^b	3.84 ± 0.04 ^c
C18:1n9	36.14 ± 0.12 ^{bc}	36.13 ± 0.08 ^{bc}	36.01 ± 0.02 ^c	36.26 ± 0.04 ^b	36.53 ± 0.10 ^a
C18:2n6	36.23 ± 0.05 ^b	36.23 ± 0.05 ^b	36.27 ± 0.01 ^b	36.44 ± 0.03 ^a	36.52 ± 0.06 ^a
C18:3n6	0.96 ± 0.07 ^a	0.85 ± 0.01 ^b	0.86 ± 0.01 ^b	0.85 ± 0.00 ^b	0.88 ± 0.01 ^b
C18:3n3	0.35 ± 0.02 ^a	0.32 ± 0.00 ^b	0.32 ± 0.01 ^b	0.33 ± 0.00 ^{ab}	0.33 ± 0.01 ^{ab}
C20:0	0.65 ± 0.02 ^a	0.63 ± 0.01 ^{ab}	0.62 ± 0.01 ^b	0.63 ± 0.00 ^{ab}	0.64 ± 0.02 ^{ab}
C22:0	0.31 ± 0.01 ^a	0.32 ± 0.01 ^a	0.31 ± 0.00 ^a	0.31 ± 0.00 ^a	0.31 ± 0.01 ^a
C24:0	0.17 ± 0.01 ^{ab}	0.18 ± 0.00 ^a	0.17 ± 0.00 ^{ab}	0.16 ± 0.00 ^{bc}	0.15 ± 0.01 ^c
C24:1	0.65 ± 0.01 ^b	0.66 ± 0.01 ^b	0.68 ± 0.00 ^a	0.15 ± 0.01 ^c	0.16 ± 0.00 ^c
SFA	24.93 ± 0.07 ^c	25.10 ± 0.07 ^b	25.13 ± 0.03 ^{ab}	25.25 ± 0.06 ^a	24.85 ± 0.06 ^c
MUFA	37.53 ± 0.10 ^a	37.50 ± 0.07 ^a	37.42 ± 0.02 ^a	37.13 ± 0.04 ^b	37.42 ± 0.10 ^a
PUFA	37.54 ± 0.11 ^{bc}	37.40 ± 0.05 ^d	37.45 ± 0.02 ^{cd}	37.62 ± 0.03 ^{ab}	37.73 ± 0.04 ^a

SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

^{abcd}Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p < 0.05$) according to Tukey's test

Table 4 Phytosterol, tocopherol and tocotrienol contents of kenaf seed oil during the refining process

Bioactive compound (mg/100 g oil)	Crude	Degummed	Neutralized	Bleached	Deodorized
Campesterol	52.94 ± 2.14 ^a	46.55 ± 1.88 ^b	44.87 ± 1.71 ^{bc}	44.95 ± 3.25 ^{bc}	41.19 ± 1.48 ^c
Stigmasterol	28.45 ± 0.53 ^a	24.70 ± 1.34 ^b	16.01 ± 1.56 ^c	14.43 ± 0.94 ^c	14.17 ± 1.53 ^c
β-Sitosterol	467.95 ± 5.70 ^a	421.02 ± 15.22 ^b	366.93 ± 11.04 ^c	350.80 ± 13.56 ^c	320.52 ± 16.30 ^d
Total phytosterol	549.34 ± 7.54 ^a	492.27 ± 15.63 ^b	427.81 ± 11.51 ^c	410.18 ± 16.90 ^c	375.87 ± 15.96 ^d
Tocotrienol					
α	2.17 ± 0.15 ^a	1.74 ± 0.06 ^b	1.44 ± 0.07 ^c	1.14 ± 0.10 ^d	1.22 ± 0.10 ^{cd}
γ	2.04 ± 0.08 ^a	1.98 ± 0.08 ^a	1.82 ± 0.07 ^b	1.48 ± 0.06 ^c	1.42 ± 0.06 ^c
δ	3.00 ± 0.07 ^a	2.90 ± 0.03 ^b	2.90 ± 0.02 ^b	2.88 ± 0.05 ^b	2.86 ± 0.02 ^b
Tocopherol					
α	21.93 ± 0.94 ^a	22.65 ± 0.38 ^a	19.63 ± 0.38 ^b	17.71 ± 1.55 ^c	16.98 ± 0.83 ^c
β	6.44 ± 0.07 ^a	6.24 ± 0.02 ^b	6.21 ± 0.08 ^b	6.00 ± 0.02 ^c	5.94 ± 0.03 ^c
γ	27.22 ± 1.56 ^a	26.29 ± 0.33 ^{ab}	24.15 ± 0.21 ^{bc}	23.39 ± 2.24 ^c	23.54 ± 0.74 ^{bc}
δ	1.31 ± 0.06 ^a	1.32 ± 0.06 ^a	1.14 ± 0.03 ^c	1.29 ± 0.03 ^{ab}	1.20 ± 0.03 ^{bc}
Total tocotrienol and tocopherol	64.11 ± 2.23 ^a	63.13 ± 0.36 ^a	57.29 ± 0.38 ^b	53.89 ± 1.18 ^c	53.15 ± 1.28 ^c

^{abcd}Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p < 0.05$) according to Tukey's test

refining process. γ-Tocopherol represents as the most abundant tocopherol in kenaf seed oil, followed by α-tocopherol and β-tocopherol. δ-Tocopherol and α-, γ- and δ-tocotrienols contributed to 13.3 and 12.6% of the total Vitamin E in crude and refined kenaf seed oil, respectively. The results showed that degumming and deodorization stage had no significant effect on the total tocopherols and

tocotrienols contents of kenaf seed oil due to the optimized parameters used in the refining process. Neutralization and bleaching stages had significantly affected the total tocopherols and tocotrienols contents of kenaf seed oil, especially neutralization stage. The reduction of total tocopherols and tocotrienols contents during neutralization might be due to the tocopherol compounds are unstable in

the presence of alkali treatment [6] and absorption on soaps formed [27]. On the other hand, adsorption of tocopherol on the bleaching earth might occur in the bleaching stage [6]. Previous studies reported the deodorization conditions were greatly affected on the tocopherol contents in vegetable oil [7, 29]. However, this study showed the deodorization did not significantly affect the total tocopherols and tocotrienols contents of kenaf seed oil. Optimal processing parameters are important to avoid the degradation of the bioactive compound. The whole refining process removed 17.1% of the total tocopherols and tocotrienols contents in kenaf seed oil. Compared to other studies, the refining process removed 28.5% of the total tocopherol content in safflower oil [29] and 29.2% in sunflower oil [6].

Phosphorus content

Phospholipids are required to be removed from the crude oil as its ability to carry trace metal ions, which results in oil darkening during storage [30]. The phosphorus content of kenaf seed oil was significantly reduced ($p < 0.05$) by degumming and neutralization process as shown in this study (Table 5). Bleaching and deodorization did not contribute to the removal of phosphorus content of kenaf seed oil. The previous study reported that hydratable phospholipid such as phosphatidyl choline (21.9%) was identified as the major phospholipid in kenaf seed oil. Non-hydratable phospholipids such as phosphatidyl ethanolamine and phosphatidyl acid were also presented in kenaf seed oil [31]. Acid degumming is required to convert unhydratable phospholipids to hydratable by chelating metal cations while water degumming is required to precipitate hydratable phospholipids [7]. Degumming process removed 89.3% of the phosphorus content from the crude kenaf seed oil. Neutralization reduced the phosphorus content in degummed oil significantly ($p < 0.05$) also. This is because absorption of the residual phospholipids and coagulation of phosphatides by alkali occurred in the

neutralization stage [30]. The phosphorus content is required to be lower than 10 ppm in the oil before continuing to the deodorization process to prevent oil darkening [32]. However, previous study reported that the phosphorus content in the degummed kenaf seed oil was 35.01 ± 3.88 mg/kg [10] using the previously described method in Chew et al. [3]. The optimized refining process in this study was able to remove 98.8% of the phosphorus content of the crude oil.

Oxidation indexes

The results of PV, p -AV, TOTOX values, FFA and IV of kenaf seed oil in each stage of the refining process are summarized in Table 5. There was a significant difference ($p < 0.05$) in each stage of the refining process for the PV of kenaf seed oil. The whole refining process was totally removed the hydroperoxides content in the kenaf seed oil. Each stage of the refining process was helped to reduce the hydroperoxide, especially the bleaching stage. Acid-activated bleaching earth decomposed the hydroperoxides into the secondary oxidation products, including aldehydes, ketones, and conjugated polyenes via catalytic decomposition [33]. This is the reason for the significant increase of p -AV of kenaf seed oil in the bleaching stage. Previous study reported that the soapstock might absorb the hydroperoxides during the neutralization stage [11]. However, an increase of PV in the neutralization stage was reported in the neutralization stage in the previous study [8], which might due to the high temperature used in the neutralization (85–90 °C). The previous study reported that increase of temperature from 40 to 85 °C might increase the PV of kenaf seed oil [11]. PV was completely reached zero in the deodorization stage. There was a significant decrease ($p < 0.05$) in the p -AV of kenaf seed oil in the deodorization stage. Most of the secondary oxidation products are volatile and can be removed by the sparge steam process under a vacuum-steam distillation at deodorization stage with high temperature applied [29].

Table 5 Physical analysis and chemical analysis of kenaf seed oil during the refining process

Analysis	Crude	Degummed	Neutralized	Bleached	Deodorized
PV (meq/kg)	1.80 ± 0.00^a	1.57 ± 0.05^b	1.15 ± 0.06^c	0.23 ± 0.03^d	0.00 ± 0.00^e
p -AV	2.15 ± 0.14^c	2.35 ± 0.15^c	3.52 ± 0.05^b	4.63 ± 0.11^a	3.61 ± 0.09^b
TOTOX	5.75 ± 0.14^{ab}	5.50 ± 0.23^b	5.83 ± 0.13^a	5.08 ± 0.06^c	3.61 ± 0.09^d
FFA (%)	0.71 ± 0.02^a	0.67 ± 0.01^b	0.09 ± 0.00^c	0.09 ± 0.00^c	0.05 ± 0.00^d
IV (g I ₂ /100 g oil)	89.56 ± 1.53^b	89.50 ± 3.29^b	94.10 ± 1.50^{ab}	95.07 ± 3.11^{ab}	97.22 ± 0.56^a
Phosphorus content (mg/kg)	62.87 ± 2.89^a	6.70 ± 0.44^b	0.76 ± 0.01^c	0.73 ± 0.02^c	0.73 ± 0.04^c

^{abcde}Means $\pm$ standard deviations followed by different superscript letters within the same row are significantly different ($p < 0.05$) according to Tukey's test

Taking into account the PV and *p*-AV for the overall oxidation status, deodorization reduced the most TOTOX value of kenaf seed oil as deodorization helped to decompose the hydroperoxides and distil the volatile secondary oxidation products.

In this study, the whole refining process was removed 93% of FFA from the crude oil. The highest removal of FFA was occurred in the neutralization stage by adding a required amount of NaOH to neutralize it. However, FFA can be stripped from the oil in the deodorization stage due to FFA more volatile than the neutral triglycerides in the oil [30]. The FFA value of refined kenaf seed oil in this study had met the CODEX standard and food safety legislation in Malaysia for refined oil, in which the refined oil shall not contain more than 0.2% of FFA for the edible purpose [34]. Previous study reported the FFA of refined kenaf seed oil was 0.61% using the refining process adapted from previous studies, which was not desirable to meet the standards of edible oil [3]. Different parameters are required to optimize to suit different types of oils due to the different components present. IV indicates the degree of unsaturation in the oils. The results showed that there was an increase in the IV of kenaf seed oil during the refining process, except a slight decrease in the degummed oil. The results were compatible with the trend of the polyunsaturated fatty acid composition of kenaf seed oil, especially linoleic acid during the refining process (Table 3). This is because polyunsaturated fatty acids contain higher number of double bonds.

A chemical refining process with optimized parameters has been developed and conducted on kenaf seed oil. The results showed that there was no 3-MCPD ester detected in the kenaf seed oil during the refining process. TAG contributed to 99.96% in acylglycerol composition of refined kenaf seed oil with POL and OOL were the main TAGs identified. Fatty acid profile revealed that linoleic acid (36.52%) and oleic acid (36.53%) contributed to the most abundant fatty acids in refined kenaf seed oil, followed by palmitic acid (19.76%). The refining process did not make a major change in the fatty acid profiles of kenaf seed oil. Minor component contents were gradually decreased during the refining process, including reduction of 31.6% of phytosterols content and 17.1% of total tocopherols and tocotrienols contents. This refining process was completely removed the hydroperoxides and reduced 37.2% of TOTOX value, 93% of FFA and 98.8% of phosphorus content from the crude kenaf seed oil. This work showed that the chemical refining process offers a good quality in the refined kenaf seed oil that is high in phytosterols and tocopherols contents, and free from 3-MCPD esters.

Acknowledgements Financial support of this work by internal funding from CERVIE UCSI University (Proj-In-FAS-024) is gratefully acknowledged.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ashori A, Harun J, Raverty W, Yusoff M. Chemical and morphological characteristics of Malaysian cultivated kenaf (*Hibiscus cannabinus*) fibre. *Polym.-Plast. Technol. Eng.* 45(1): 131–134 (2006)
2. Chew SC, Tan CP, Long K, Nyam KL. *In-vitro* evaluation of kenaf seed oil in chitosan coated-high methoxyl pectin-alginate microcapsules. *Ind. Crops Prod.* 76: 230–236 (2015)
3. Chew SC, Tan CP, Long K, Nyam KL. Effect of chemical refining on the quality of kenaf (*Hibiscus cannabinus*) seed oil. *Ind. Crops Prod.* 89: 59–65 (2016)
4. Wong YH, Lau HW, Tan CP, Long K, Nyam KL. Binary solvent extraction system and extraction time effects on phenolic antioxidants from kenaf seeds (*Hibiscus cannabinus* L.) extracted by a pulsed ultrasonic-assisted extraction. *Scientific World J.* 1–8 (2014)
5. Yoon SH. Optimization of the refining process and oxidative stability of chufa (*Cyperus esculentus* L.) oil for edible purposes. *Food Sci. Biotechnol.* 25: 85–90 (2016)
6. Suliman TEMA, Jiang J, Liu YF. Chemical refining of sunflower oil: effect on oil stability, total tocopherol, free fatty acids and colour. *Int. J. Eng. Sci. Technol.* 5: 449–454 (2013)
7. Wang T, Johnson LA. Refining high-free fatty acid wheat germ oil. *J. Am. Oil Chem. Soc.* 78: 71–76 (2001)
8. Farhoosh R, Einafshar S, Sharaye P. The effect of commercial refining steps on the rancidity measures of soybean and canola oils. *Food Chem.* 115: 933–938 (2009)
9. Lee SY, Jung MY, Yoon SH. Optimization of the refining process of camellia seed oil for edible purposes. *Food Sci. Biotechnol.* 23: 65–73 (2014)
10. Chew SC, Tan CP, Nyam KL. Optimization of degumming parameters in chemical refining process to reduce phosphorus contents in kenaf seed oil. *Sep. Purif. Technol.* 188: 379–385 (2017)
11. Chew SC, Tan CP, Nyam KL. Optimization of neutralization parameters in chemical refining of kenaf seed oil by response surface methodology. *Ind. Crops Prod.* 95: 742–750 (2017)
12. Chew SC, Tan CP, Nyam KL. Optimization of bleaching parameters in refining process of kenaf seed oil with a central composite design model. *J. Food Sci.* 82: 1622–1630 (2017)
13. Chew SC, Tan CP, Nyam KL. Application of response surface methodology for optimizing the deodorization parameters in chemical refining of kenaf seed oil. *Sep. Purif. Technol.* 184: 144–151 (2017)
14. Wong YH, Muhamad H, Abas F, Lai OM, Nyam KL, Tan CP. Effects of temperature and NaCl on the formation of 3-MCPD esters and glycidyl esters in refined, bleached and deodorized palm olein during deep-fat frying of potato chips. *Food Chem.* 219: 126–130 (2017)
15. Franke K, Strijowski U, Fleck G, Pudiel F. Influence of chemical refining process and oil type on bound 3-chloro-1, 2-propanediol contents in palm oil and rapeseed oil. *LWT- Food Sci. Technol.* 42: 1751–1754 (2009)

16. Zulkurnain M, Lai OM, Latip RA, Nehdi IA, Ling TC, Tan CP. The effects of physical refining on the formation of 3-monochloropropane-1,2-diol esters in relation to palm oil minor components. *Food Chem.* 135: 799–805 (2012)
17. Li C, Li L, Jia H, Wang Y, Shen M, Nie S, Xie M. Formation and reduction of 3-monochloropropane-1,2-diol esters in peanut oil during physical refining. *Food Chem.* 199: 605–611 (2016)
18. Özdikicierler O, Yemişçioğlu F, Gümüşkesen AS. Effects of process parameters on 3-MCPD and glycidyl ester formation during steam distillation of olive oil and olive pomace oil. *Eur. Food Res. Technol.* 242: 805–813 (2016)
19. AOCS. 2- and 3-MCPD fatty acid esters and glycidyl fatty acid esters in edible oils and fats by acid transesterification. In: Official Methods and Recommended Practices of The American Oil Chemists' Society. Firestone D (ed). AOCS Press, Champaign III, USA (2013)
20. Lee YY, Tang TK, Phuah ET, Ab Karim NA, Mohd Aiwi SM, Lai OM. Palm-based medium-and-long-chain triacylglycerol (P-MLCT): production via enzymatic interesterification and optimization using response surface methodology (RSM). *J. Food Sci. Technol.* 52: 685–696 (2015)
21. Chew SC, Tan CP, Nyam KL. Comparative storage of crude and refined kenaf (*Hibiscus cannabinus* L.) seed oil during accelerated storage. *Food Sci. Biotechnol.* 26: 63–69 (2017)
22. AOCS. Official Methods and Recommended Practices of The American Oil Chemists' Society. AOCS Press, Champaign (2000)
23. O'Connor CJ, Lal SND, Eyres L. Handbook of Australasian edible oils. Oils and Fats Specialist Group of NZIC, Auckland (2007)
24. International Union of Pure and Applied Chemistry. Standard Methods for The Analysis of Oils, Fat and Derivatives. Blackwell Scientific Publications, Oxford (1987)
25. MPOB. In: PORIM Test Methods. Vol. 1. PORIM (ed). Palm Oil Research Institute of Malaysia, Malaysia (2005)
26. EFSA. Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA J.* 14(5): 4426 (2016)
27. Karabulut I, Topcu A, Yorulmaz A, Tekin A, Ozay DS. Effect of the industrial refining process on some properties of hazelnut oil. *Eur. J. Lipid Sci. Technol.* 107: 476–480 (2005)
28. Aluyor EO, Aluyor P, Ozigagu CE. Effect of refining on the quality and composition of groundnut oil. *Afr. J. Food Sci.* 8: 201–205 (2009)
29. Ortega-García J, Gámez-Meza N, Noriega-Rodriguez JA, Dennis-Quiñonez O, García-Galindo HS, Angulo-Guerrero JO, Medina-Juárez LA. Refining of high oleic safflower oil: Effect on the sterols and tocopherols content. *Eur. Food Res. Technol.* 223: 775–779 (2006)
30. Ghazani SM, Marangoni AG. Minor components in canola oil and effects of refining on their constituents: A review. *J. Am. Oil Chem. Soc.* 90: 923–932 (2013)
31. Mohamed A, Bhardwaj H, Hamama A, Webber C. Chemical composition of kenaf (*Hibiscus cannabinus* L.) seed oil. *Ind. Crops Prod.* 4: 157–165 (1995)
32. Tyagi K, Ansari MA, Tyagi S, Tyagi A. A novel process for physically refining rice bran oil through degumming. *Adv. Appl. Sci. Res.* 3: 1435–1439 (2012)
33. Zschau W, Grp GSFSS. Bleaching of edible fats and oils – cooperative work of the German Society for Fat Science (DGF). *Eur. J. Lipid Sci. Technol.* 103(8): 505–550 (2001)
34. Legal Research Board. Food Act 1983 (Act 281) & Regulations. International Law Book Services, Malaysia (2013)