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Phosphorus Removal and Phytonutrients Retention in the Refining of Solvent Extracted Palm-Pressed Mesocarp Fiber Oil

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Abstract: Phosphoric acid is used in the refining of palm oil for the removal of phosphatides. The high concentration of phosphorus in solvent extracted palm-pressed mesocarp fiber oil hinders palm oil mills to recover this phytonutrients-rich residual oil in pressed fiber which typically contains 0.1 to 0.2% of total oil yield. This study aimed to refine the palm-pressed mesocarp fiber oil and determine the optimum dosage of phosphoric acid for acid-degumming of palm-pressed mesocarp fiber oil while retaining its phytonutrients. The refining process was carried out with combination of wet degumming, acid degumming, neutralisation, bleaching and deodorization. The optimum dose of phosphoric acid was identified as 0.05 wt.% by incorporating the wet degumming process. The refined palm-pressed mesocarp fiber oil showed a reduction in phosphorus content by 97% (from 901 ppm to 20 ppm) and 97% free fatty acid content removal (from 6.36% to 0.17%), while the Deterioration of Bleachability Index increased from 1.76 to 2.48, which showed an increment of 41%. The refined oil retained the key phytonutrients such as carotenoids (1,150 ppm) and vitamin E (1,540 ppm) that can be further developed into high-value products. The oil meets the quality specification of refined, bleached, and deodorized palm oil while preserving the heat-sensitive phytonutrients, which in turn provides a new resource of nutritious oil.

Key words: carotenoids, vitamin E, degumming, vegetable oil

1 Introduction

Palm-pressed mesocarp fiber (PPMF) is the by-product of the fibrous material that exist as solid waste after separated from bunch stalk and kernel mixture during the palm oil extraction process[1)]. Generally, PPMF is found to be clean, non-carcinogenic, free from pesticides, and constructed with soft parenchyma cells. Due to its unique properties, PPMF has been used to produce various products such as mattress cushion, landscaping paper, and others[2)]. Traditionally, the leftover PPMF from palm oil extraction is used as recycled solid fuel to generate heat and steam in the palm oil industry or for power generation[1-4)]. Based on the data collected in year 2005, Malaysia, one of the world's leading oil palm producing country produced 11.9 million tonnes of PPMF from 75.5 million tonnes of fresh fruit bunch (FFB) processed which constituted about 15.7% of the FFB on dry mass basis[5)]. This amount of waste fiber production had been further increased to 14.55 million tonnes of PPMF in 2017 and this value is believed to further increase in the future[6, 7)].

PPMF has been found to have great application potentials in other fields or industries. One of it is becoming new feedstock for red palm oil as it was found to contain high concentrations of carotenes (4000 – 6000 ppm) and other phytonutrients such as vitamin E (2400 – 3500 ppm) and sterols (4500 – 8500 ppm) from the 5 – 7% of residue oil retained in the fiber after screw press extraction of crude palm oil (CPO)[5, 8-10)]. Recent research has found that the palm mesocarp fiber oil (PMFO) is a superior natural food that possess anti-oxidant properties and could be potentially used as carrier oil in emulsion[11, 12)]. Several extraction methods had been introduced, such as pressurized liquid extraction (PLE)[13)], supercritical CO_2 extraction[5)], and also solvent extraction by Soxhlet[1, 14)]. Different types of solvents have been used to extract PMFO, including ethanol[13)], propane[15)], and *n*-hexane[1)]. Among all the

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methods reported, Soxhlet extraction using *n*-hexane as solvent was the most optimized and economical method to extract the remaining palm oil from the PPMF effectively[16].

Typically, phospholipids content in CPO ranged from 20 to 80 ppm[7]. However, the phospholipids content is much higher in crude palm-pressed mesocarp fiber oil (PMFO), which was commonly noticed to be of more than 500 ppm[7]. This condition happened due to co-extraction of phospholipids by using *n*-hexane as the solvent during the extraction process of crude PMFO[7]. The phosphorus exists in the form of phospholipids, also known as gums in palm oil. These phospholipids are classified into two different groups, namely hydratable and non-hydratable phosphatides[17, 18]. These two groups of phospholipids will show their hydrophilicity and hydrophobicity properties when they are subjected to different chemical processes.

Phospholipids, or commercially referred to as lecithin are vital structural and functional components due to their chemical compositions that are useful in enhancing biological functions. Lecithin is a natural bi-polar lipid used in the food industry and incorporated in food products, animal feed, and industrial products as emulsifiers. Other than food uses, phospholipidosis also played an important role in human body, mainly for metabolism processes. The metabolism processes that required phospholipids are fat absorption, cholesterol metabolism, nerve function, biosynthesis of prostaglandins, and so on[19, 20]. However, the presence of phospholipids or phosphorus is deemed to be unbeneficial in palm oil refining. In commercial palm oil extraction process, it was found that the palm oil extracted contained certain amount of phosphorus as co-extracted product. Organic phosphorus-containing compound such as phospholipids will lead to operational issues if not removed. The high concentrations of phosphorus will cause fouling in pipeline and equipment such as heat exchanger at high temperature operation, reduction in oxidative stability of palm oil, acting as trace metal ions carrier and increase in refining cost[7, 18, 21]. In this case, the degumming process is inevitable in the refinery to get rid of the phosphorus in palm oil. This degumming process aimed to remove both hydratable and non-hydratable phospholipids to increase the quality of palm oil[22].

The commonly used degumming processes are water degumming and acid degumming processes which played their roles to remove hydratable and non-hydratable phospholipids. Typically, water degumming will be carried out by the use of water on the oil sample in different dosages while acid degumming is performed with two acids, phosphorus and citric acids, at various dosages[23]. However, phosphorus acid proved to be able to reduce the phosphorus content in palm oil more effectively than citric acid. This is due to the ions that bonded with phosphatidic acid in palm oil normally referred to as the magnesium ion (Mg^{2+}) or calcium ion (Ca^{2+}), will be dislodged from the structure by the hydroxyl group of phosphoric acid. Due to the ion exchange process, the phosphatidic acid with ion loss will eventually form insoluble lipid precipitates which can be easily removed from the palm oil sample[7]. This study reported on the phosphorus removal and preservation of indigenous phytonutrients in crude PMFO through mild refining technique.

2 Materials and Methods

2.1 Materials

Fresh commercial crude PMFO was obtained from a local palm oil mill in Malaysia. Sodium hydroxide (NaOH), *n*-hexane, and *n*-heptane (HPLC grade) were purchased from Merck KGaA, Germany while 85% phosphoric acid, ammonium monovanadate and ammonium heptamolybdate tetrahydrate were bought from Friendemann Schmidt Chemical, Germany. Natural bleaching earth (NBE) was purchased from Taiko Bleaching Earth Sdn. Bhd., Malaysia, Magnesium oxide, potassium dihydrogen phosphate, and 6N of nitric acid were acquired from Chemiz (M) Sdn. Bhd., Malaysia, Potassium hydrogen phthalate was bought from Classic Chemicals Sdn. Bhd., Malaysia. Isopropanol was purchased from Qrec (Asia) Sdn. Bhd., Malaysia, Sodium methoxide solution, tetrahydrofuran solution, and pyridine solution (HPLC grade) were purchased from Sigma-Aldrich Corporation, Germany. *n*-heptane (GC grade) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) were obtained from Acros Organics, Belgium. 1-glyceryl monononadecanoin, 1,3-glyceryl dinonadecanoin and glyceryl trinonadecanoin powders were bought from Nu-Chek Prep, Inc., USA. Analytical grade α-tocopherol (α-T, 95%) used was from Sigma-Aldrich Corporation, Germany and α-tocotrienol (α-T3, 99.1%), β-tocotrienol (β-T3, 99.9%), γ-tocotrienol (γ-T3, 98.3%), and δ-tocotrienol (δ-T3, 95.5%) were from Chromadex, USA.

2.2 Experimental procedures

The refinery process of crude PMFO was carried out using two-step water-degumming, acid-degumming, bleaching, deacidification, and deodorization. About 4.0 kg crude PMFO sample was weighed, pre-heated to 90℃ and stirred with a magnetic stirrer. A two-step water-degumming process was applied to the pre-heated crude PMFO by adding 5 wt.% distilled water (85℃) and mixed for 60 min[7]. The water with gums was then removed from the oil by centrifugation at 300 rpm for 15 min. The second step of water-degumming process was carried out by repeating the same procedures. Different dosages of phosphoric acid with 85% purity were added into water-degummed PMFO and reacted at 90℃ for 30 min. The dosage of acid used were 0.05, 0.1, 0.15 and 0.2 wt.% respectively. The amount

of acid added to the oil was slightly higher as compared to calculation to achieve 100% acid concentration by using equation 1.

$$Content\ of\ Acid = \frac{Weight\ of\ Sample \times Weight\ Percent\ of\ Acid}{0.85} \quad (1)$$

The mixture was subjected to neutralization by adding NaOH solution at 90℃ for 30 min. The weight of NaOH used was calculated using equation 2 while the weight of water used was 10 times of NaOH. The mixture was centrifuged and pumped dry to remove excess water.

$$Weight\ of\ NaOH = \left(\frac{Weight\ of\ Oil \times Initial\ FFA\ Content}{100}\right)\left(\frac{40(mw\ of\ NaOH) \times 1.2(20\%\ excess)}{282(mw\ of\ oleic\ acid)}\right) \quad (2)$$

The neutralized PMFO was then subjected to bleaching. 1.0 wt.% NBE was added to the neutralized PMFO at 100℃ for 20 min, followed by vacuum filtration of oil and NBE mixture. The bleached PMFO was then subjected to deodorization at temperature between 165 to 175℃ for 15 min. Each experiment was carried out in triplicates. Samples were collected at different stages for analysis.

2.3 Analysis of sample

All samples were analysed in triplicates and the mean results were reported.

2.3.1 Analysis of FFA, phosphorus content, total carotenes content and DOBI

The following tests were carried out according to MPOB Test Methods[24]. Free fatty acid (FFA) content was determined according to MPOB Test Method p2.5: 2004. Phosphorus content was performed according to MPOB Test Method p2.8 Part 1(b): 2004. Total carotenes content was measured according to MPOB Test Method p2.6: 2004. The UV adsorption was measured using a UV-vis spectrometer (UV-1800, Shimadzu Scientific Instrument, Kyoto, Japan) and measured at 446 nm. The Deterioration of Bleachability Index (DOBI) of oil was determined by getting the ratio of absorbance at 446 nm and 269 nm.

2.3.2 Analysis of vitamin E content

The vitamin E analyses was carried out by using a high-performance liquid chromatography (HPLC) 1100 Series with a fluorescence detector (Agilent Technologies, Santa Clara, California, United States), fitted with 5 microns normal phase silica column (150 mm × 4.6 mm i.d.) (Supelco Inc., USA). The mobile phase used was 97% of *n*-heptane and 3% of ethyl acetate at a flow rate of 1 mL/min and sample injection volume was 20 μL. A standard solution that contained α-T, α-T3, β-T3, γ-T3 and δ-T3 was used to obtain the calibration curve.

2.3.3 Analysis of fatty acid composition

The analysis of fatty acid composition (FAC) was carried out by weighing 0.3 g sample into a GC vial. 0.2 mL CH_3ONa and 1.3 mL *n*-hexane were added into the vial. The solution was homogenized using vortex mixer (VELP Scientifica, Italy) for 5 min and let settled for 10 min. The upper layer of the sample was injected into GC–FID for analysis. The equipment used was GC–FID (Perkin Elmer, Waltham, Massachusetts, United States) fitted with BPX5 GC column (30 m × 0.32 mm i.d.) (Trajan Scientific and Medical, Australia) with sample injection volume of 1 μL. Initial oven temperature was 140℃, followed by a ramping rate of 8℃/min to 220℃ and held for 2 min. The injector and detector temperature were both set at 250℃ with total run time of 12 min per sample.

2.3.4 Analysis of mono-, di- triacylglycerols

The mono-, di- and triacylglycerols content were analysed by GC–FID (Agilent Technologies, Santa Clara, California, United States) using 0.1 microns Select Biodiesel GC column (15 m × 320 μm i.d.) (CP9078, Agilent Technologies, Santa Clara, California, United States) with an injection volume of 1 μL. The flow rate of carrier gas (helium) was 25 mL/min while hydrogen and compressed air were 40 mL/min and 450 mL/min respectively. Initial GC oven temperature was set at 50℃ for 1 min, followed by a temperature ramping of 15℃/min to 180℃, 7℃/min to 230℃, 10℃/min to 370℃ and maintained for 15 min at 370℃. The standard acylglycerols stock solution was prepared by weighing 0.05 g 1-glyceryl monononadecanoin, 1,3-glyceryl dinonadecanoin, and glyceryl trinonadecanoin into a 20 mL volumetric flask and top with tetrahydrofuran to the calibration mark. 0.05 g homogenized oil sample was weighed into a 20 mL scintillation vial. 0.2 mL of each standard acylglycerols stock solution, MSTFA, and pyridine solution were added into the scintillation vial. The mixture was vortexed and left for 15 min. 8 mL *n*-heptane was added into the mixture and transferred into a GC vial.

2.4 Statistical analysis

All the data were presented in mean and statistical analyses were performed by using GraphPad Prism 7. Data were analysed by two-way ANOVA, followed by Tukey's Multiple Comparison Test. The p value less than 0.05 was considered statistically significant.

3 Results and Discussion

3.1 Mass balance

In 2020, there are 15 crude PMFO extraction plants (using hexane) in Malaysia from a total of 457 palm oil mills. Crude PMFO was reported to contain high phospholipids and other phytonutrients content which need to be refined[7]. Table 1 shows the mass balance of the refining process of crude PMFO with different dosages of phosphoric acid, ranging from 0.5 to 2.0 wt.%. The average weight loss of water degumming was 15.1%, in which the first step of water degumming contributed to 14.2% of the

Table 1 Mass balance of refining process of crude palm-pressed mesocarp fiber oil (PMFO).

	Weight (g)	Percentage Loss (%)
Crude PMFO	4001.7	–
First Step Water-Degummed PMFO	3432.3 ± 10.3	14.2
Second Step Water-Degummed PMFO	3399.3 ± 5.7	15.1

	Batch 1 - 0.05 wt%		Batch 2 - 0.10 wt%		Batch 3 - 0.15 wt%		Batch 4 - 0.20 wt%	
	Weight (g)	Percentage Loss (%)	Weight (g)	Percentage Loss (%)	Weight (g)	Percentage Loss (%)	Weight (g)	Percentage Loss (%)
Water-Degummed PMFO	500.3	–	515.8	–	513.7	–	514.7	–
Acid-Degummed and Neutralized PMFO	407.7 ± 3.6	18.5	421.4 ± 2.9	18.3	417.1 ± 2.7	18.8	416.9 ± 4.1	19.0
Bleached and Deodorized PMFO	396.2 ± 6.1	20.8	408.0 ± 4.0	20.9	407.9 ± 5.4	20.6	402.9 ± 4.1	21.7

Note: The data represent the mean ± SD of three independent experiments.

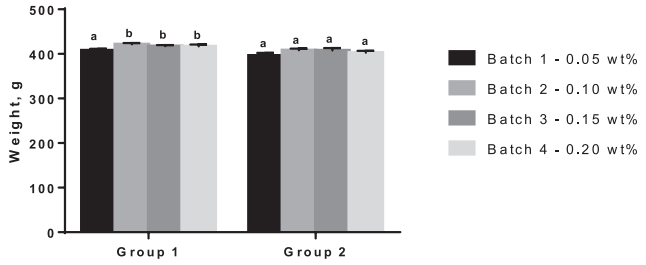

Fig. 1 Yield of palm-pressed mesocarp fiber oil (PMFO) at different stages of refining using different dosages of phosphoric acid. Different alphabet letters indicate $p<0.05$ within the group; Group 1 represents Acid-Degummed and Neutralized PMFO; Group 2 represents Bleached and Deodorized PMFO.

oil loss. The yield of acid-degummed and neutralized PMFO at different phosphoric acid dosages were of no significant differences ($p<0.05$) except when 0.05% dosage was used (Fig. 1). Statistically, the yield of PMFO after bleaching and deodorization were not affected by phosphoric acid dosage ($p<0.05$) for all the range studied. The findings indicated that 94% of the hydratable phospholipids were effectively removed in the first water degumming step with another 6% removed in the second step. Subsequent acid degumming dan neutralization incurred weight loss between 18.3 to 19.0% and additional 1.8 to 2.7% weight loss were observed during bleaching and deodorization steps. The oil loss could be reduced during commercial operation with the use of high centrifugal force separator for effective separation of heavy and light phases. FFA was removed in the form of sodium soap via neutralization step. The removal of soapstock also removed some neutral oil and losses could be minimized by the addition of 1% demulsifiers to break the emulsion[25].

3.2 Quality parameter of crude PMFO

Table 2 shows the properties of crude PMFO and treated PMFO at different stages of refining process. Water degumming process was carried out to remove hydratable and non-hydratable gums or phospholipids in crude PMFO to ensure a good quality oil can be produced. The crude PMFO contains 1,318 ppm carotenoids and 1,711 ppm vitamin E which were two-fold higher compared to CPO[5,8]. The total vitamin E content showed that PMFO is a good source of tocotrienols which contained 50% tocotrienols[21]. Tocotrienols have been shown to possess beneficial health properties, act as superior anti-oxidant for potential treatment of inflammatory diseases, immunity, etc[26–28]. The FFA of the crude PMFO was 6.36 wt.% which exceeded the maximum permissible level in CPO. The high FFA content was attributed to the excessive hydrolysis of residual oil in the de-oiled pressed mesocarp fiber which was exposed to steam during digestion of fruits. The cleavage of triacylglycerols molecule produced FFA, monoacylglycerols (MG) and diacylglycerols (DG) as evidenced in high MG and DG contents of 0.27 and 6.24 wt.% respectively in crude PMFO. Although MG and DG are natural emulsifiers used in food formulation, they need to be adequately removed as they affect the crystallisation during dry fractionation process[29].

3.3 Water degumming, acid degumming and neutralization

The two-step water degumming process reduced the initial phosphorus content in the crude PMFO from 901

Table 2 Properties of crude and treated palm-pressed mesocarp fiber oil (PMFO) at different stages of refining process.

Properties	Crude PMFO	Water-degummed PMFO	Acid-degummed and neutralized PMFO				Bleached and deodorized PMFO			
			Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4
Phosphorus (ppm)	901 ± 12	33 ± 2	23 ± 0^{a}	24 ± 1^{a}	23 ± 2^{a}	23 ± 0^{a}	9 ± 1^{a}	8 ± 0^{a}	8 ± 0^{a}	9 ± 1^{a}
Carotenes (ppm)	1,318 ± 0	1,362 ± 3	1,473 ± 9^{b}	1,452 ± 23^{b}	1,364 ± 2^{a}	1,357 ± 3^{a}	1,168 ± 3^{c}	1,203 ± 8^{d}	1,098 ± 10^{a}	1,131 ± 10^{b}
Vitamin E (ppm)	1,711 ± 1	1,737 ± 8	1,510 ± 30^{a}	1,567 ± 3^{b}	1,564 ± 47^{b}	1,614 ± 12^{c}	1,552 ± 16^{b}	1,579 ± 64^{c}	1,565 ± 3^{bc}	1,465 ± 5^{a}
FFA (%)	6.36 ± 0.32	5.50 ± 0.01	0.25 ± 0.04^{a}	0.25 ± 0.04^{a}	0.22 ± 0.00^{a}	0.20 ± 0.04^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}
Monoglycerides (%)	0.3 ± 0.0	–	–	–	–	–	ND	0.1 ± 0.01	ND	ND
Diglycerides (%)	6.4 ± 0.2	–	–	–	–	–	5.2 ± 0.0^{a}	5.9 ± 0.1^{a}	5.4 ± 0.3^{a}	5.4 ± 0.2^{a}
Triglycerides (%)	87.0 ± 0.1	–	–	–	–	–	94.6 ± 0.0^{a}	93.9 ± 0.1^{a}	94.4 ± 0.3^{a}	94.5 ± 0.2^{a}
DOBI	1.76 ± 0.01	2.85 ± 0.01	3.39 ± 0.00^{a}	3.25 ± 0.14^{a}	3.20 ± 0.00^{a}	3.14 ± 0.07^{a}	2.64 ± 0.01^{a}	2.65 ± 0.01^{a}	2.38 ± 0.01^{a}	2.27 ± 0.03^{a}
FAC (%)										
C8:0	0.7 ± 0.4	0.2 ± 0.0	0.5 ± 0.3^{ab}	0.8 ± 0.1^{b}	0.5 ± 0.1^{ab}	0.3 ± 0.2^{a}	0.5 ± 0.2^{a}	0.3 ± 0.2^{a}	0.5 ± 0.4^{a}	0.7 ± 0.4^{a}
C10:0	0.5 ± 0.3	0.2 ± 0.0	0.3 ± 0.2^{a}	0.6 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.2^{a}	0.5 ± 0.1^{a}	0.4 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}
C12:0	2.2 ± 0.0	2.1 ± 0.1	2.0 ± 0.1^{a}	1.8 ± 0.1^{a}	1.9 ± 0.0^{a}	1.9 ± 0.2^{a}	1.9 ± 0.2^{a}	1.8 ± 0.1^{a}	1.8 ± 0.2^{a}	1.9 ± 0.1^{a}
C14:0	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.1^{a}	1.5 ± 0.0^{a}	1.5 ± 0.0^{a}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}	1.5 ± 0.0^{a}	1.5 ± 0.1^{a}	1.6 ± 0.0^{a}
C16:0	41.0 ± 0.1	41.3 ± 0.4	41.0 ± 0.0^{a}	40.9 ± 0.2^{a}	41.3 ± 0.0^{a}	41.3 ± 0.9^{a}	41.2 ± 0.0^{a}	41.2 ± 0.2^{a}	40.8 ± 0.0^{a}	41.1 ± 0.1^{a}
C16:1	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.4 ± 0.3^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.5 ± 0.3^{a}
C18:0	3.9 ± 0.0	4.1 ± 0.1	4.1 ± 0.2^{a}	4.3 ± 0.1^{a}	4.2 ± 0.1^{a}	4.2 ± 0.1^{a}	4.2 ± 0.1^{a}	4.3 ± 0.2^{a}	4.4 ± 0.1^{a}	4.2 ± 0.2^{a}
C18:1	39.8 ± 0.4	40.6 ± 0.2	40.7 ± 0.2^{a}	40.6 ± 0.4^{a}	40.3 ± 0.4^{a}	40.7 ± 0.7^{a}	40.5 ± 0.1^{a}	40.9 ± 0.0^{a}	41.1 ± 0.6^{a}	40.5 ± 0.5^{a}
C18:2	9.1 ± 0.1	8.8 ± 0.1	8.7 ± 0.1^{a}	8.5 ± 0.3^{a}	8.7 ± 0.0^{a}	8.7 ± 0.1^{a}	8.5 ± 0.0^{a}	8.5 ± 0.1^{a}	8.4 ± 0.1^{a}	8.4 ± 0.1^{a}
C18:3(1)	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
C18:3(2)	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}
C18:3(3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± .0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
C20:0	0.1 ± 0.0	0.1 ± 0.0	ND	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}

Note: Each data represents the mean ± SD of three independent experiments where means with different letters in the same row indicate significant differences ($p < 0.05$) among the same samples; " – " means not tested for particular parameter; ND means not detected; DOBI is Deterioration of Bleachability Index; FFA is free fatty acid and FAC is fatty acid composition.

ppm to 33 ppm or 96% of phosphorus removal. The results showed that the hot water degumming process is an effective method to remove hydratable phosphatides as it formed gums that is insoluble in oil. The gums formed during the process eventually settled and could be easily removed by centrifugation[23]. Temperature and water dosage were found to influence the efficiency of hydratable gum removal in water degumming process[30]. In this study, the optimum condition of water degumming process using 5% of 90℃ water was based on previous published study and consistent results were obtained[7].

The acid degumming and neutralization techniques were applied to further improve the quality of the oil. After water-degumming, the DOBI value of PMFO increased from 1.76 to 2.85, indicating improvement in oil quality. Increase in DOBI indicates reduction of bleaching earth usage during bleaching process[18]. Subsequently, different dosages of phosphoric acid at 0.05, 0.10, 0.15, and 0.20 wt.% were used to study its effect on reducing non-hydratable gum retained in the oil. The phosphorus content of all acid degumming and neutralized PMFO were found to be between 23 to 24 ppm from the initial value of 33 ppm. The total percentage of phosphorus reduction was 97.45% with a further reduction of 1.45% after acid degumming and neutralization. Meanwhile, the carotene content in the oil had increased due to oil loss after neutralization. In another study, carotenoids content reduction of 2% was observed during neutralization of crude palm oil (CPO)[31]. This could be due to lower FFA content in CPO as compared to PMFO. On average, vitamin E content was found to reduce by 8% before and after acid degumming and neutralization. Slight reduction in vitamin E (1.6%) was also reported in neutralization of CPO[31]. Higher loss in vitamin E could be attributed by higher dose of NaOH used to neutralized FFA in PMFO compared to CPO. The neutralization process had successfully reduced the FFA in the oil to less than 0.25 wt.%. Additionally, the DOBI value of the acid-degummed PMFO was above 3.1 across different dosages of phosphoric acid. The high DOBI value indicates the excellent quality of the degummed PMFO and minimum bleaching earth will be needed for further processing.

The amount of phosphoric acid required in acid degumming process depends on the initial phosphorus content in the starting oil. Phosphoric acid dosage at 0.05 wt.% was found to be sufficient for the reduction of phosphorus

Table 3 Vitamin E profile of crude and treated palm-pressed mesocarp fiber oil (PMFO) at different stages of refining process.

Vitamin E	Crude PMFO	Water-Degummed PMFO	Acid-Degummed and Neutralized PMFO				Bleached and Deodorized PMFO			
			Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4
α-Tocopherol (ppm)	762 ± 4	799 ± 0	760 ± 11^{a}	804 ± 4^{b}	814 ± 20^{b}	825 ± 0^{b}	776 ± 4^{bc}	826 ± 65^{d}	801 ± 10^{cd}	722 ± 8^{a}
α-Tocotrienol (ppm)	374 ± 5	391 ± 0	377 ± 6^{a}	396 ± 0^{ab}	406 ± 9^{b}	400 ± 10^{b}	376 ± 2^{a}	368 ± 1^{a}	374 ± 1^{a}	356 ± 1^{a}
β-Tocotrienol (ppm)	66 ± 0	45 ± 4	ND	ND	ND	ND	ND	ND	ND	ND
γ-Tocotrienol (ppm)	361 ± 12	367 ± 12	320 ± 13^{ab}	319 ± 7^{a}	307 ± 15^{a}	341 ± 12^{b}	319 ± 8^{a}	309 ± 6^{a}	327 ± 2^{a}	314 ± 11^{a}
δ-Tocotrienol (ppm)	147 ± 4	135 ± 1	53 ± 1^{a}	47 ± 6^{a}	37 ± 2^{a}	49 ± 10^{a}	82 ± 3^{a}	75 ± 4^{a}	62 ± 4^{a}	73 ± 2^{a}
Total (ppm)	1711 ± 1	1737 ± 8	1510 ± 30^{a}	1567 ± 3^{bc}	1564 ± 47^{b}	1614 ± 12^{c}	1552 ± 16^{b}	1579 ± 64^{b}	1565 ± 3^{b}	1465 ± 5^{a}

Note: Each data represents the mean ± SD of three independent experiments where means with different letters in the same row indicate significant differences ($p < 0.05$) among the same samples; ND means not detected.

content in water degummed PMFO from 24 ppm to less than 10 ppm. Further increased in phosphoric acid does not significantly reduce its content further ($p < 0.05$). It was reported that high dosage of phosphoric acid with the presence of chloride precursor resulted in the formation of high 3-monochloropropane-1,2-diol (3-MCPD) ester[32]. In this study, the formation of 3-MCPD ester is not much of concern because water degumming had been proven able to remove chloride in oil effectively prior to the next step of acid degumming process[33, 34]. The 2- and 3-MCPD esters content in PMFO after water degumming treatment was less than 0.3 ppm[12]. Minimal dosage of phosphoric acid used in turn reduces the possibility of 3-MCPD formation[35].

3.4 Bleaching and deodorization

The bleaching and deodorization processes were carried out to remove impurities such as trace metals and volatile materials in the PMFO. Bleaching reduces the heat-sensitive micronutrients in PMFO such as vitamin E and carotenoids. On average, the vitamin E and carotenoids contents in PMFO before bleaching and deodorization were 1,563 ppm and 1,411 ppm respectively, compared to 1,540 ppm and 1,150 ppm respectively after bleaching and deodorization. The reduction in vitamin E content was 1.5% while reduction in carotenoids content was 18.5%.

Generally, the use of bleaching earth will cause decolorization of palm oil as part of the color pigment will be removed during bleaching by adsorption process[36]. Carotenoids were adsorbed onto the bleaching earth during bleaching while thermally degraded during deodorization at high temperature[37]. The reduction in carotenoids content during bleaching varied between 20 to 50% depending on the type of clay used, whereby natural clay retained highest carotenoids compared to acid activated clay[38]. Some refinery processes only remove a maximum of 20% carotenoids content from the palm oil, but more than 98% of carotenoids will be destroyed or removed during deodorization if the process was left for 20 min at 240℃[39]. Our study reported a higher retention of carotenoids of 81.5% after bleaching and deodorization at 165℃ for 20 min compared to 63.5% retention of carotenoids at deodorization temperature of 130℃ for 1 hour[40]. This was due to the retention time differences in deodorization which affects greatly on the degradation rate of carotenoids[40].

Reduction of vitamin E was insignificant during bleaching as vitamin E removal was mainly influenced by deodorization temperature and amount of steam injection[41]. High loss in vitamin E was reported in physical refining of vegetable oils with reduction between 25 to 37%[38, 42]. The oxidative stability of oil increased significantly after bleaching due to volatile components removal and retention of natural anti-oxidants[43]. Researchers had reported possibility of 99% vitamin E retention after bleaching with increased in of α-tocopherol and α-tocotrienol content while a reduction in γ-tocotrienol content was observed[38]. In this study, β- and δ-tocotrienols were found to reduce slowly over the refining process as shown in Table 3. However, further reduction of vitamin E content in deodorized PMFO was prevented using mild deodorization temperature (165 to 175℃), which was 100℃ lower compared to 265℃ used in normal physical deodorization process.

Under normal physical refining process at deodorization temperature above 230℃, the formation of glycidol ester (GE) was found to be significant[44]. Mitigation of GE has been shown by researchers through neutralization process to replace FFA stripping at high temperature[44, 45]. Double deodorization using lower temperature also shown 87% reduction in GE formation[46]. Previous study reported on GE content in the refined PMFO subjected to mild deodorization temperature was 0.325 ppm[12].

The neutralization step has removed 96% of FFA in the crude PMFO. The bleaching and deodorization of PMFO reduced further the FFA content from 0.25 to 0.17 wt.% for all samples tested. The reduction of FFA was not observed at mild deodorization temperature below 150℃ but FFA was reduced at temperature above 160℃[40, 47]. Controlling dosage of NaOH during neutralization and removing the remaining FFA through deodorization preserved

maximum content of phytonutrients in the PMFO.

The compositional change of acylglycerols and partial acylglycerols was noticed in the final refined PMFO. Monoacylglycerols and diacylglycerols are natural emulsifiers that are used in food formulations. However, high diacylglycerols content affects the nucleation of fat molecules during dry fractionation process and the presence of different levels of monoacylglycerols changes the crystallization profile of oil[29, 48]. The deodorization process has removed 85% of monoacylglycerols present in the crude PMFO from its initial content of 0.27 wt.% to less than 0.05 wt.%. Diacylglycerols content was reduced from 6.42 to 5.9 wt.% and below. The removal of FFA and partial acylglycerols has indirectly improved the triacylglycerols content to more than 90 wt.%. The vaporization of these volatile components happened due to the high temperature and vacuum during the deodorization process[49]. The FAC of final oil was within the range found in the original fresh palm fruit. No significant differences ($p<0.05$) in FAC were found within same batches of treatment groups as indicated in Table 2.

3.5 Economic viability

The capital expenditure for a 5 tonnes per day PMFO refining plant with GMP encapsulation and bottling facility is estimated at USD 15 million. The operation expenditure such as overhead cost (10%), plant maintenance (2%) and finance charges (8%) is estimated at USD 3 million per year. Assuming 50% plant capacity utilization and the PMFO is sold as supplement at USD 10 for 180 capsules × 1 g per box, the estimated ROI would be 0.43 year. Under the same assumptions, if the PMFO is sold as nutritious oil at USD 10 per 750 mL, the estimated ROI would be 1.8 year. The ROI is subjected to the external factors such as consumer awareness and market accessibility of the product.

4 Conclusion

A combination of water and acid degumming process is necessary for the treatment of crude PMFO to reduce its phosphorus content to below 10 ppm. The water used was 5 wt.% based on crude PMFO and optimum dosage of phosphoric acid was 0.05 wt.%. The indigenous carotenoids and vitamin E were preserved by adopting neutralization process with improved oil quality. As a result, the phosphorus content had been reduced by 98% and more than 80% of carotenoids and vitamin E had been retained in the final PMFO. The FFA content could be removed by up to 97% in this study. Refined PMFO is a new source of red palm oil that can be further processed into respective fractions for various food applications.

Authors' Contributions

Lau, H.L.N. designed, conceptualized and supervised the overall research works. Tee, Y.S. conducted the experiments, analyzed data and prepared the manuscript. Chan, M.K. improved and edited the manuscript. Teh, S.S. edited the manuscript and performed statistical analysis of the data.

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Conflict of Interest

None of the authors had conflict of interest.

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