Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

PHYSICOCHEMICAL PROPERTIES AND BIODEGRADABILITY OF PALM OIL PRODUCTS (POP) IN MARINE ENVIRONMENT

(Sifat Fizikokimia dan Kebolehbiodegradasi Produk Minyak Sawit (POP) dalam Persekitaran Marin)

Nur Azmina Roslan*, Nur Aainaa Syahirah Ramli, Noorazah Zolkarnain, Siti Afida Ishak, and Razmah Ghazali

Malaysian Palm Oil Board (MPOB),
6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

**Corresponding author: nazmina@mpob.gov.my*

Received: 18 January 2023; Accepted: 21 June 2023; Published: 22 August 2023

Abstract

Palm oil products (POP), *i.e.*, crude palm oil (CPO), crude palm kernel oil (CPKO), refined, bleached, and deodorized (RBD) palm oil (RBDPO), and RBD palm olein (RBDPOo), are among the most highly traded POP in the world. Therefore, the quality and biodegradability of POP need to be assessed to ensure the traded POP is safe to consume and environmentally friendly. This paper investigates the physicochemical properties of POP, including moisture, slip melting point, iodine value, fatty acid content, and free fatty acid, using MPOB p2.1:2004, AOCS C c3-25, AOCS Cd 1d-92, AOCS Ce 1a-13, and MPOB p2.5:2004 test methods, respectively. Analysis showed that all the POP were within the requirements as specified by the Malaysian Standards, the Palm Oil Refiners Association of Malaysia (PORAM), the Malayan Edible Oil Manufacturers' Association (MEOMA), Codex Alimentarius, and the Malaysian Palm Oil Association (MPOA). Meanwhile, the biodegradability assessment of these POP in the marine environment was carried out according to Organization for Economic Co-operation and Development (OECD) 306, Biodegradability in Seawater test method, using the closed bottle method. The results indicated that CPO, CPKO, RBDPO, and RBDPOo can be degraded in temperate marine water at 74%, 70%, 75%, and 72%, respectively.

Keywords: biodegradation, quality, seawater, specification, standard

Abstrak

Produk minyak sawit (POP), iaitu minyak sawit mentah (CPO), minyak isirung sawit mentah (CPKO), minyak sawit ditapis, diluntur dan dinyahbau (RBDPO) dan minyak sawit olein RBD (RBDPOo), adalah antara POP yang terkenal dan diperdagangkan di dunia. Oleh itu, kualiti dan kebolehbiodegradan POP perlu dinilai untuk memastikan POP yang diperdagangkan selamat untuk dimakan dan mesra alam. Kajian ini menyiasat sifat fizikokimia POP, termasuk lembapan, takat lebur gelincir, nilai iodin, kandungan asid lemak dan asid lemak bebas menggunakan kaedah ujian MPOB p2.1:2004, AOCS C c3-25, AOCS Cd 1d-92, AOCS Ce 1a-13 and MPOB p2.5:2004. Analisis menunjukkan bahawa semua POP berada dalam perincian seperti yang ditetapkan oleh Jabatan Standard Malaysia, Persatuan Penapis Minyak Sawit Malaysia (PORAM), Persatuan Pengilang Minyak Masak Malaysia (MEOMA), Codex Alimentarius dan Persatuan Minyak Sawit Malaysia (MPOA). Sementara itu, penilaian kebolehbiodegradan POP ini dalam persekitaran marin telah dilaksanakan mengikut kaedah ujian Organisasi bagi Pembangunan

dan Kerjasama Ekonomi (OECD) 306, Biodegradasi dalam Air Laut, menggunakan kaedah botol tertutup. Keputusan menunjukkan bahawa CPO, CPKO, RBDPO and RBDPOo boleh terdegradasi dalam air marin sederhana dengan 74%, 70%, 75% and 72%, masing-masing.

Kata kunci: biodegradasi, kualiti, air laut, perincian, piawai

Introduction

Palm oil is an edible vegetable oil derived from the flesh or pulp (mesocarp) of palm fruits through the milling process and has been used for various purposes, including in food and non-food applications. Malaysia is a prominent exporter of palm oil since it represents 18.3% of the global oils and fats, thus contributing to 34.3% of the total palm oil trade in 2020 [1]. Meanwhile, in 2021, Malaysia exported 24.27 million tonnes of POP to various destinations, with more than 1.6 million tonnes exported to EU countries [2]. Further processing of crude palm oil (CPO) involves the removal of the products of hydrolysis and oxidation, color, and flavor for the production of refined products, *i.e.*, refined, bleached, and deodorized (RBD) palm oil (RBDPO) and RBD palm olein (RBDPOo), which are mainly used as cooking oil and in margarine, confectionery, non-dairy creamer, shortening, and others [3]. RBD palm kernel oil (RBDPKO) from CPKO has also been used in the same manner as CPO and in the production of oleochemical derivatives, *i.e.*, fatty acids, fatty amines, fatty alcohols, fatty methyl esters, and glycerin. These derivatives are mainly utilized as raw ingredients in the cosmetics, personal care, and household sectors for products including balms, lipsticks, soaps, detergents, and cleaning agents [3].

As product development and improvement evolve rapidly, it can be expected that there will be an increasing demand for quality improvement from consumers and their end-users. From a commercial point of view, quality is a valuable and significant attribute in edible oil commodities and their trades. Hence, it can be predicted that the trading specifications will become broader and more stringent over time. This will require reliable and accredited analysis methods to meet all statutory requirements. Failure to comply with quality specifications could affect the acceptance of the traded oils. In order to assess the quality of POP, several physicochemical analyses need to be conducted, including moisture, free fatty acid (FFA), iodine value (IV), fatty acid content (FAC), and slip melting point (SMP) [4].

Moisture is one of the most prevalent contaminants in POP. It is necessary to ensure the moisture content of POP is within the permitted limit since a high moisture content would affect hydrolytic stability [5]. In addition, some palm oil products tend to have a considerable amount of moisture and impurities due to insufficient processing, which may accelerate the microorganism's activities [6]. Besides moisture content, the amount of FFA is also a significant measure of the quality of POP since it is related to the economic value of edible oils [7]. In general, FFA is formed during oil storage through the reaction of oil and water, and a high level of FFA content implies poor POP quality [8].

The IV measures the degree of unsaturation [9] or double bonds in POP, which indicates the susceptibility of the oils to oxidation [10]. Meanwhile, the SMP is prevalently applied to analyze the melting and solidification properties of oil samples, at which the temperature of the solidified oil turns into fluid and flows into an open capillary tube [11]. The determination of SMP varies with the fatty acid chain length, trans fatty acid composition, and position of the fatty acids in the glycerol backbone, as well as unsaturation ratios [12].

Fatty acids can be classified according to their degree of saturation, *i.e.*, saturated or unsaturated fatty acids. Unsaturated fatty acids are either monounsaturated or polyunsaturated. Fatty acids play an important role in the oil and fat industry due to their health implications in the human diet and properties in industrial processes. Previous literature demonstrated the importance of the fatty acid content (FAC) of the oil in determining the biodegradation of vegetable oils [13-14]. The valuable data on the FAC of POP is also crucial since the type of fatty acids will determine the oil's nutritional status and storage stability. A previous study showed that POP was

stable up to 10 months of storage time at a low temperature of 6 ± 2 °C, indicating that these POP are suitable to be used as control samples for validation of test results [15].

Apart from the physicochemical properties, biodegradability is also considered a critical criterion for evaluating the behavior of substances or products in the environment. Assessment of the biodegradability of POP is deemed worthy since it is well known that vegetable oils, especially palm oil, are one of the most traded commodities transported *via* sea, and there is the possibility of oil spillage even though it is comparatively rare in the marine environment in comparison to mineral oil. Several incidents of accidental oil spillage caused by vessel accidents have been reported, with the most recent incidents occurring in Chinese waters, where about 9,000 tonnes of palm oil stearin were spilled. [16]. Vegetable oils are bulk liquid substances regulated as chemicals under the International Convention for the Prevention of Pollution from Ships (MARPOL) Annex II [17].

According to the GESAMP Composite List 2019 [18], most vegetable oils, including canola, sunflower, olive, and palm oils, are readily biodegradable. However, the conditions, as well as the rate of biodegradation, are yet to be revealed. Research conducted on the biodegradation of natural oils in seawater, *i.e.*, mustard, canola, and olive oil [19], revealed that canola oil could be easily degraded, followed by mustard and olive oils. The different trends in bacterial numbers were observed for the different types of oil. Most of the oil samples showed high bacterial numbers within 7 to 15 days of the biodegradation test, indicating that the oil samples are nontoxic and that the biodegradation process occurred rapidly in seawater. There was also research conducted on the microbes that possess the unique ability to bioremediate fat-containing waste. In this study, the marine strain, *Rhodococcus erythropolis* PR4, showed the potential to convert animal fats and vegetable oils into fatty acid methyl ester (FAME) derivatives in a relatively short time [20]. There was also a study on an isolated soil microbe, *Acinetobacter sp.* strain SOD-1, capable of degrading salad oil, which consists of soybean and rapeseed oil [21]. *Pseudomonas otitidis* strain was able to hydrolyze sunflower cooking oil to produce lipase [22]. Previous research on the degradation of CPO and CPKO in seawater using the modified shake flask method revealed an increase in bacterial population that peaked on days 7 and 21 before declining, indicating that palm oil is being used as a substrate for bacterial growth amidst degradation, which is aided by the lipase enzyme produced by selected bacteria [23].

The biodegradation test is crucial to assessing the biodegradation of substances in various environmental compartments. According to the OECD, Biodegradability in Seawater (OECD 306) and Marine BODIS test guidelines are frequently used to determine the biodegradability of substances in seawater, where the degradation of a substance is assessed by the measurement of dissolved oxygen over 28 days of the test period. This study aims to assess the physicochemical properties of POP under investigation, *i.e.*, moisture, FFA, IV, FAC, and SMP, using the American Oil Chemists Society (AOCS) and the Malaysian Palm Oil Board (MPOB) test methods, as well as the biodegradability of POP in the marine environment according to standard method OECD 306, Biodegradation in Seawater *via* the closed bottle method. This study can be used as a database in the event of an accidental oil spill.

Materials and Methods

The standard grade of palm oil products (POP), CPO, CPKO, RBDPO, and RBDPOo were obtained from Sime Darby Jomalina Sdn. Bhd., Selangor, Malaysia. All chemicals and solvents were utilized as received without further purification. Sodium acetate was used as a reference substance to check the validity of the test procedure and the suitability of the seawater used. A non-ionic surfactant, octylphenoxypolyethoxyethanol (IGEPAL CA-630), was used to emulsify the oil samples since they are insoluble in water.

Natural seawater was collected directly from Lowestoft, United Kingdom, and transferred into the laboratories through a pipe that extends below the sand some distance into the bay. The seawater was decanted and then filtered using Whatman 54 filters. Prior to the start

of the test, the seawater was equilibrated to the test temperature, which is 15-20 °C. Meanwhile, for the preparation of mineral stock solution A, potassium dihydrogen orthophosphate, KH_2PO_4 (8.50 g), dipotassium hydrogen orthophosphate, K_2HPO_4 (21.75 g), disodium hydrogen orthophosphate heptahydrate, $Na_2HPO_4.7H_2O$ (50.30 g), and ammonium chloride, NH_4Cl (0.50 g) were dissolved in water and made up to 1 L. Solution B was prepared by dissolving magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ (22.50 g), in water and made up to 1 L. Solution C was prepared by dissolving calcium chloride dihydrate, $CaCl_2.2H_2O$ (36.40 g), in water and made up to 1 L. As for solution D, the solution needs to be freshly prepared by dissolving iron (III) chloride hexahydrate, $FeCl_3$ (0.15 g), in water and made up to 1 L. Mineral stock solutions used in the OECD 306 biodegradation test were prepared using analytical-grade reagents and deionized Ecotox Maxima-grade water. The mineral stock solutions A, B, and C were made in advance and stored at 4 ºC ± 2 ºC, while solution D was freshly made and not stored. All solutions were added at 1 mL/L to a total volume of 40 L of seawater. The prepared seawater mineral medium was aerated for 20 minutes and allowed to stand for 25 hours at 15-20 °C prior to use.

The equipment used in this study includes 300 mL Biological Oxygen Demand (BOD) bottles with glass stoppers, 4 L bottles with a liter mark, a dissolved oxygen meter (Multi 3410 WTW), an oven (ULM 500, Memmert), an analytical balance (HR 250A, A&D), a hot plate (SP 131320-33, Thermo Scientific Cimarec), gas chromatography with capillary column HP-88 (Agilent HP 6890 Series), a capillary tube for SMP with a length of 75 mm ± 0.02 mm, id 1.1 to 1.2 mm, od 1.3 to 1.4 mm, wall 0.2 mm ± 0.02 mm (Fisherbrand Microhematocrit), and a water bath (F34, Julabo).

Determination of moisture content

The moisture content of the POP was determined using the MPOB test method, MPOB p2.1:2004 [24]. This method involves heating the oil sample in an oven at 103 ± 2 °C, until all moisture and volatile substances are removed. The oil sample in a petri dish was dried and cooled in desiccators before it was weighed (approximately 10 g to the nearest 0.001 g). Then, the oil sample was heated at 103 °C for 2.5 h, left to cool to room temperature (23 to 25 °C) in a desiccator for 30 min, and further weighed. All of these stages were repeated until the weight remained constant. All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation. The moisture content was calculated using equation (1),

$$\text{Moisture (\%)} = \frac{\text{Mb}-\text{Md}}{\text{Mb}-\text{M}} \times 100 \quad (1)$$

Where: M = weight of the petri dish, Mb = weight of the petri dish and oil and Md = weight of the petri dish and oil after drying.

Determination of free fatty acid (FFA)

The MPOB p2.5:2004 test method [24] was used to measure the FFA in POP as a palmitic acid percentage. The oil was melted and homogenized before being weighed into a conical flask. Subsequently, neutralized isopropanol (50 mL) was poured into the flask containing the oil sample. Afterwards, a few drops of phenolphthalein were added to the mixture. The conical flask was then placed on a hot plate at 40 °C and titrated with a sodium hydroxide (NaOH) standard solution with continuous swirling before the first permanent pink color appeared. The FFA content was determined according to equation (2):

$$\text{FFA as palmitic acid (\%)} = \frac{25.6 \times \text{M} \times \text{V}}{\text{m}} \quad (2)$$

Where: M = molarity of NaOH solution, V = volume (mL) of NaOH solution, and m = weight (g) of the oil sample.

All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation.

Determination of iodine value (IV)

The AOCS Cd 1d-92 method was used to measure IV of the POP [25]. The oil sample was melted, homogenized, and filtered, and then the temperature was left to reduce to 68–71 °C before being weighed into a conical flask. The flask was then filled with cyclohexane: acetic acid (1:1) solution (15 mL) and Wijs solution (25 mL). The substances were thoroughly stirred before being stored for an hour in dark conditions at room temperature. Thereupon, a 10% potassium iodide (KI) solution (20 mL) was added, followed by distilled water (100 mL). The mixture was then titrated with a 0.1M sodium thiosulfate ($Na_2S_2O_3$) standard solution until the yellow color almost disappeared. The mixture was then added with a starch indicator solution (1–2 mL), followed by titration with $Na_2S_2O_3$ until the blue color diminished. These steps were repeated for the blank sample. All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation. The IV was expressed using equation (3):

$$\text{IV} \left(\frac{\text{g}}{\text{100g}}\text{oil}\right) = \frac{12.69 \times C \times (V1\text{-}V2)}{M} \quad (3)$$

Where: C = molarity (M) of the $Na_2S_2O_3$ solution, V1 = blank volume (mL) of $Na_2S_2O_3$ solution, V2 = test sample volume (mL) of $Na_2S_2O_3$ solution, and M = weight (g) of the oil sample.

Determination of fatty acid composition (FAC)

The AOCS Ce 2-66 and AOCS Ce 1a-13 methods were used in the preparation of fatty acid methyl esters (FAME) and quantitative analysis of FAC, respectively [25]. The preparation of FAME is important in order to enhance the volatility of the methyl ester derivatives. The FAC of the POP was determined using Agilent 6890 gas chromatography with a flame ionization detector (FID) and a capillary column HP-88 (60 m × 250 µm × 0.2 µm). The flow rate of the column was set at 0.8 mL/min. Meanwhile, the injector temperature and FID temperature were set at 250 °C. Initially, the oven temperature was programmed at 150 °C, then heated to 210 °C for 10 min with a heating rate of 3 °C/min. The injection volume was 1 µL, with a total run time of 30 min. FAME standards were used to analyze the GC chromatogram peaks. All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation.

Determination of slip melting point (SMP)

The SMP of POP was determined using the AOCS C c3-25 method, which applied the open capillary tube technique [25]. The palm oil samples need to be melted and homogenized first before they are filtered at 60°C in the oven and left in the oven for 10 min. Capillary tubes in triplicate were immersed in the melted oil portion until the oil ascended to a height of about 10 mm in the tube. Subsequently, the tubes with the oil sample were rapidly chilled by holding the ends of the tubes against ice until the sample hardened. These tubes were inserted into a beaker and stored in a refrigerator at 10 ± 1°C for 16 h. The tubes were taken out of the refrigerator after 16 h and affixed to a thermometer, whereby the lower ends of the tubes were even with the bottom of the thermometer's mercury bulb. The thermometer was immersed in a 400-mL beaker of distilled water, with its bottom reaching the level of the immersion mark. The water bath's initial temperature was fixed at 8–10 °C below the slip point of the oil sample. Then, the water bath was stirred with a magnetic stirrer, and the temperature was adjusted at a rate of 1 °C/min, then slowed to 0.5 °C/min as the slip point was nearly reached. The heating was carried on until the oil ascended in each tube, and the temperature was recorded. The average temperature of triplicate capillary tubes was calculated as the SMP. All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation.

Determination of chemical oxygen demand (COD) value

The COD test was carried out following Standard Methods for the Analysis of Water and Wastewater [26]. COD is an indicative measure of the oxygen required to oxidize soluble and particulate organic matter in water.

The sample was oxidized by refluxing in the presence of sulphuric acid/silver sulphate, and potassium dichromate. Unreduced potassium dichromate was determined by titration with a standardized iron (II) ammonium sulphate solution. The amount of dichromate reduced is proportional to the amount of oxygen required to oxidize the test substance and is expressed as mgO_2/mg. The test material was added to duplicate round bottom flasks (0.0121 g and 0.0125 g) containing deionized water, sulphuric acid/silver sulphate, mercuric sulphate, and potassium dichromate. In order to check the integrity of the test procedure, readily oxidizable reference material was tested. Blank vessels containing only reagents and deionized water were also tested. The vessels were refluxed for two hours, cooled, rinsed, and titrated with a standardized iron(II) ammonium sulphate solution (FAS) using a redox indicator.

Biodegradation tests using the OECD 306 test method

The OECD 306, Biodegradability in Seawater test method [27] is a modification of the established OECD 301D Closed Bottle and the OECD 301E Modified Screening Test [28]. The OECD 306 test evaluates the biodegradation of a substance over a set period by analyzing indirect parameters in a closed system containing seawater, the test substance, and nutrients. No additional microorganisms are added to the system. The biodegradation test was performed at Chemex Environmental International Limited, United Kingdom (UK). The natural seawater was collected from the Centre for Environment, Fisheries, and Aquaculture Science laboratories in the UK. The biodegradation test was conducted according to the OECD 306 test method at 15-20°C. The study on the effect of POP in a temperate environment is deemed necessary due to the high export of these products to EU countries.

Sodium acetate was used as a reference substance to ensure the test procedure's validity and the suitability of the inoculum used. Since the test materials are insoluble in water, a surfactant was introduced to emulsify the samples. About 0.50g of each POP was mixed with 0.25g of a non-ionic surfactant (IGEPAL CA-630). The resulting emulsion stock solution was gradually diluted by adding deionized water and then made up to volume (500 mL) to give a concentration of 1.0 g/L. The sample stock solution was then prepared by adding 1.0 g/L of the emulsion stock solution to 3.5 L of seawater mineral medium at 2.0 mL/L to give a final test concentration of 2.0 mg/L. Several replicates of BOD bottles were filled to absolute volume (300 mL) with the sample stock solution.

In order to account for any degradation of the surfactant in the test vessels, an additional 100 mL of 0.10 g of IGEPAL CA-630 stock solution was prepared and used in a series of surfactant control bottles. The surfactant control bottles were prepared by adding the surfactant stock solution to seawater mineral medium (1 ml/L) to give a final concentration of 1.0 mg/L, representing the final concentration of the surfactant used in the test sample bottles. These were used as the blank values for the calculation of the BOD for the test material. Initial dissolved oxygen (DO) concentrations were measured using a dissolved oxygen meter. The bottles were incubated in the dark at 18.7–19.2 ºC for the test period. Further measurements of DO concentration were made on bottles removed after 7, 14, 21, and 28 days. All analyses were conducted in duplicate, and the results were presented in the form of mean ± standard deviation. The BOD and percentage of degradation were calculated using equations (4) and (5), respectively.

$$\text{BOD } (mgO_2)\text{calculation } = \frac{(DOt0 - DOtt) - (DOb0 - DObt)}{\text{Concentration of test material } (\frac{mg}{L})} \times 100 \quad (4)$$

Where: DOt0 = DO of the test sample on day 0, DOtt = DO of the test sample on day t, DOb0 = DO of the blank on day 0, DObt = DO of the blank on day t*

* If materials are added using a surfactant, the DO of the surfactant control at days 0 and t are used in the place of the blank.

$$\text{Percentage of degradation (\%)} = \frac{\text{BOD} \times 100}{\text{COD}} \quad (5)$$

Results and Discussion

The physicochemical properties of the selected POP were compared with known standards for crude and refined oils such as the Malaysian Standards (MS 814:2007, Palm Oil – Specification; MS 816:2007, Palm Olein – Specification; MS 80:2011, Palm Kernel Oil – Specification), the Palm Oil Refiners Association of Malaysia (PORAM), the Malayan Edible Oil Manufacturers' Association (MEOMA), Codex Alimentarius, and the Malaysian Palm Oil Association (MPOA). The primary data were obtained from analyses conducted in the laboratory, and they were also compared with extracted data from the certificate of analysis (COA) issued by Sime Darby Jomalina Sdn. Bhd. The properties of POP from the laboratory were expressed in the form of mean value and standard deviation from three replicates, as in Tables 1–4.

Table 1. Comparison of quality characteristics of CPO

Characteristics	Specification	Primary data	COA
Moisture, %max	0.25[b]	0.17 ± 0.01	0.19
FFA, as palmitic acid, %max	5.0[a]	5.3 ± 0.00	4.1
Iodine value (Wijs)	50.1 – 54.9[a]	57.5 ± 0.17	52.6
Slip melting point	33.8 – 39.2[a]	35.4 ± 0.00	36.4

a - MS814:2007; b - PORAM/MPOA

Table 2. Comparison of quality characteristics of CPKO

Characteristics	Specification	Primary data	COA
Moisture, %max	0.5[a, b]	0.2 ± 0.02	0.1
FFA, as lauric acid, %max	5.0[a, b]	3.5 ± 0.00	2.1
Iodine value (Wijs)	19.0[a]; 16.5 – 18.75[b]	18.2 ± 0.15	18.76
Slip melting point	27.3 – 28.0[b]	27.8 ± 0.00	27.8

a - MEOMA; b - MS 80:2011

Table 3. Comparison of quality characteristics of RBDPO

Characteristics	Specification	Primary data	COA
Moisture, %max	0.1[a, b]	0.05 ± 0.01	0.02
FFA, as palmitic acid, %max	0.1[a, b]	0.20 ± 0.00	0.07
Iodine value (Wijs)	50 – 55[a]	51.7 ± 0.20	52.3
Slip melting point	33 – 39[a]	36.8 ± 0.00	36.6

a – PORAM; b – MPOA

Table 4. Comparison of quality characteristics of RBDPOo

Characteristics	Specification	Primary data	COA
Moisture, %max	0.1[b]	0.04 ± 0.01	0.040
FFA, as palmitic acid, %max	0.1[a, b]	0.2 ± 0.00	0.062
Iodine value (Wijs), min - max	56.0 – 59.1[b]	57.4 ± 0.00	58.82
Slip melting point, max	24[a]	20.4 ± 0.00	17.4
	19.2 – 23.6[b]		

a – PORAM; b - MS 816:2007

Table 5. Comparison of FAC of POP

POP	Characteristics	Specification	Primary data
CPO	C12:0	0.1 – 0.5[a]	0.2 ± 0.00
	C14:0	0.9 – 1.5[a]	1.1 ± 0.01
	C16:0	39.2 – 45.8[a]	44.8 ± 0.04
	C18:0	3.7 – 5.1[a]	4.6 ± 0.05
	C18:1	37.4 – 44.1[a]	39.0 ± 0.08
	C18:2	8.7 – 12.5[a]	9.2 ± 0.05
	C18:3	0.0 – 0.6[a]	0.2 ± 0.01
CPKO	C12:0	48[b]	47 ± 0.16
		45 – 55[c]	
	C14:0	16.7[b]	16.7 ± 0.10
		14.0 – 18.0[c]	
	C16:0	8.5[b]	8.9 ± 0.10
		6.5 – 10[c]	
	C18:0	2.1[b]	2.4 ± 0.03
		1.0 – 3.0[c]	
	C18:1	14.9[b]	16.6 ± 0.09
		12.0 – 19.0[c]	
	C18:2	2.5[b]	2.7 ± 0.02
		1.0 – 3.5[c]	
RBDPO	C12:0	0.1 – 0.5[a]	0.2 ± 0.00
	C14:0	0.9 – 1.5[a]	1.1 ± 0.00
	C16:0	39.2 – 45.8[a]	44.0 ± 0.06
	C18:0	3.7 – 5.1[a]	4.4 ± 0.00
	C18:1	37.4 – 44.1[a]	39.3 ± 0.02
	C18:2	8.7 – 12.5[a]	9.8 ± 0.03
	C18:3	0.0 – 0.6[a]	0.2 ± 0.00
RBDPOo	C12:0	0.2 – 0.4[d]	0.2 ± 0.00
	C14:0	0.9 – 1.2[d]	1.0 ± 0.02
	C16:0	38.2 – 42.9[d]	38.5 ± 0.04
	C18:0	3.7 – 4.8[d]	4.2 ± 0.07
	C18:1	39.8 – 43.9[d]	43.7 ± 0.08
	C18:2	10.4 – 12.7[d]	11.2 ± 0.12
	C18:3	0.1 – 0.6[d]	0.2 ± 0.00

a - MS814:2007; b - MS 80:2011; c - Codex (2019); d - MS 816:2007

Moisture content

Lipids are primarily hydrophobic; however, small amounts of moisture can easily be absorbed and preserved. The presence of moisture is often unfavorable since it facilitates microbial growth and stimulates the hydrolysis of lipids, thus increasing the FFA content. In addition, it can adversely affect the oil's taste, scent, and appearance, thus reducing customer acceptance and marketability.

Tables 1–4 depict the moisture content of the POP obtained from primary and secondary sources. The maximum permissible amount of moisture is 0.25%, 0.5%, 0.1%, and 0.1% for CPO, CPKO, RBDPO, and RBDPOo, respectively [5]. It was observed that the moisture content of POP from both sources was below the permissible limit set in the specifications. It is important to ensure the moisture content is within the

limit specified by the standards since an abundance of ample moisture could facilitate microbial growth. Previous studies have shown that microorganisms species such as *Penicillium sp*. and *Aspergillus niger* can be detected in the CPO when the moisture content value is higher than 0.69% [29].

Free fatty acid (FFA)

FFA is formed by the hydrolysis of triglycerides in the oil. FFA is an undesirable component since it gives the oil an unpleasant metallic taste. High FFA content will ultimately result in rancidity, which consequently changes the sensory and nutritional quality of POP [30].

It was observed that the FFA values of CPO (Table 1), CPKO (Table 2), RBDPO (Table 3), and RBDPOo (Table 4) are still below the maximum limit stated in the specifications. However, the FFA values observed through data from the laboratory analysis were slightly higher, and this may be due to the long storage period of oils that causes hydrolysis due to the presence of moisture. In addition, CPO releases FFA naturally through the activity of enzymes in the palm fruit and by microbial lipases, which further increases the FFA value [8]. It was specified that the maximum FFA content for CPO is 5%, as stated by MPOA. Meanwhile, PORAM stipulates that the maximum FFA content for RBDPO and RBDPOo is 0.1% [31]. As for CPKO, MEOMA and MS 80:2011 standardized the FFA value not exceeding 5% [5].

Iodine value (IV)

The IV is an indicator of the relative degree of unsaturation in oil components, as calculated by the uptake of halogen. A higher IV value indicates greater unsaturation, and the oil is more prone to oxidation [32]. According to MS 814:2007, the admissible range of IV for CPO is 50.1–54.9 [33]. As for CPKO, the permissible range is 19.0 according to MEOMA [5] and 16.5–18.75 according to MS 80:2011 [34]. Meanwhile, PORAM standards stipulate that the permissible IV range for RBDPO is 50-55, and the minimum IV for RBDPOo is 56 (PORAM, 2012). According to the results obtained in Tables 1 to 4, it can be assumed that most POP possess good quality in terms of IV since the value is within the specified maximum limit. As for CPO, the IV obtained from the laboratory analysis slightly exceeds the allowable range stated in MS 814:2007. However, the IV of CPO, when tested at Sime Darby Jomalina Sdn Bhd, is still within the limit, suggesting that the CPO might have undergone some changes in its properties during storage before the procurement of oils. Significant changes in the IV of CPO after 3 months of storage at room temperature have been reported in a previous study [35]. The difference in storage duration and condition, a higher storage temperature, and the presence of sunlight can lead to a higher IV [36]. Another study suggested that high temperatures increase the oxidation rate of unsaturated fatty acids [15].

Slip melting point (SMP)

Based on the data in Tables 1 to 4, the SMP of the tested POP met the requirement specified by MS 814:2007 with a value of 33.8°C–39.2°C for CPO [33], MS 80:2011 with a value of 27.3°C–28.0°C for CPKO [34], and PORAM with a value of 33°C–39°C for RBDPO and a maximum of 24 °C for RBDPOo [31].

Fatty acid composition (FAC)

Table 5 confirmed the presence of all main components of fatty acids in oils. It was observed that the FAC of CPO, RBDPO, and RBDPOo were within the range stated by MS 814:2007 and MS 816:2007 for palm oil and palm olein, respectively [33; 37]. The specifications of the processed POP were also included in the standards mentioned. As for CPKO, the FAC value slightly exceeded the allowable range stated in MS 80:2011 but is still within the range based on the Codex standard [38].

Chemical oxygen demand (COD)

The COD value of POP obtained from the accredited laboratory that conducted the biodegradation test is shown in Table 6. It was observed that all of POP showed quite similar COD values. The COD value is essential to determining the amount of oxidizable organic material in the sample, which will reduce the dissolved oxygen (DO) levels. Low DO concentrations in water can lead to anaerobic conditions, which are unfavorable to aquatic life [39].

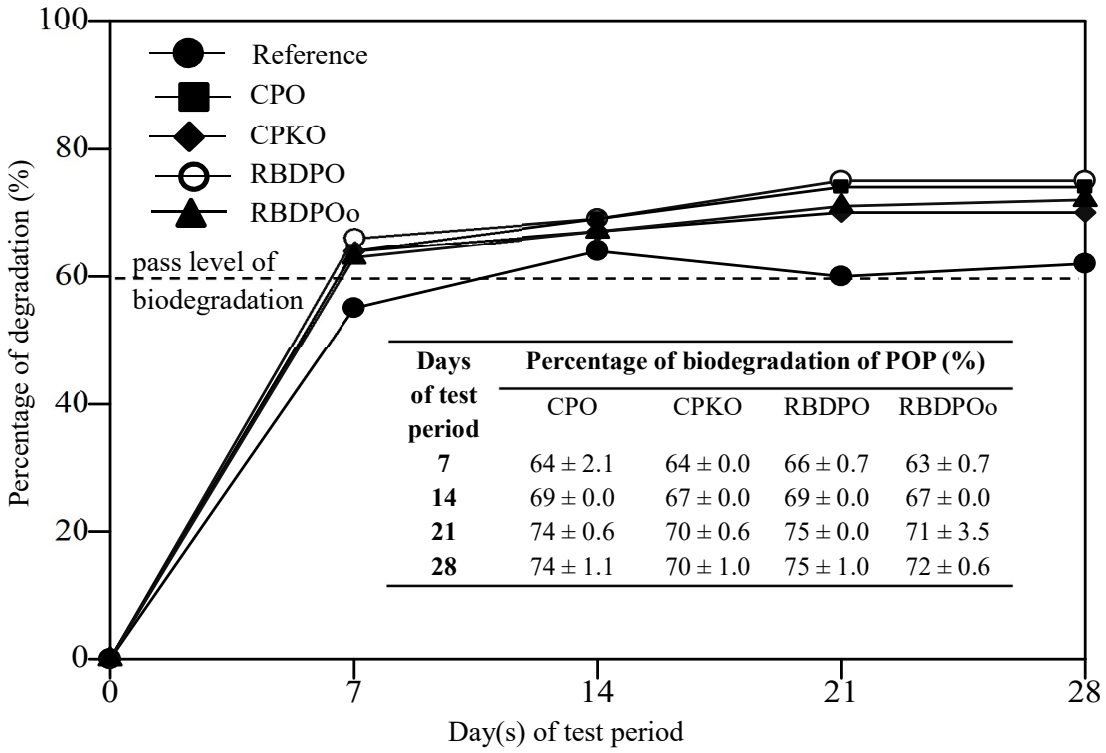
Table 6. COD value of POP

POP	ThOD/COD Value (mgO_2/g)
CPO	2456
CPKO	2489
RBDPO	2382
RBDPOo	2662

The oxygen demand value for the reference substance, sodium acetate, was calculated based on the test method (OECD, 1992), giving its theoretical oxygen demand (ThOD) value of 780 mgO_2/g.

Biodegradability assessment

All tested POP are insoluble in water; therefore, a surfactant (IGEPAL CA 630) was used to emulsify the sample. The effects of surfactant degradation were considered when calculating the degradation of the POP. It was observed that CPO, CPKO, RBDPO, and RBDPOo showed a positive result with more than 60% degradation relative to the COD value, with 60% being reached by day 7 and a maximum of 74%, 70%, 75%, and 72%, respectively, recorded on day 28 (Figure 1). A positive result of more than 60% ThOD removal within 28 days indicates that the material has the potential to biodegrade in the marine environment (OECD, 1992). This is supported by previous research on bacterial degradation of palm olein that revealed RBD palm olein was biodegradable in seawater, as indicated by the decreased DO value with increasing microbial counts over the 5 days of study. In addition, the fatty acid concentration was also found to decrease after the incubation period in seawater [40].



Days of test period	Percentage of biodegradation of POP (%)			
	CPO	CPKO	RBDPO	RBDPOo
7	64 ± 2.1	64 ± 0.0	66 ± 0.7	63 ± 0.7
14	69 ± 0.0	67 ± 0.0	69 ± 0.0	67 ± 0.0
21	74 ± 0.6	70 ± 0.6	75 ± 0.0	71 ± 3.5
28	74 ± 1.1	70 ± 1.0	75 ± 1.0	72 ± 0.6

Figure 1. Biodegradation of POP during 28-day test period

A previous study demonstrated the importance of the FAC of the oil in determining the biodegradation rate [13]. It was observed that the biodegradation process resulted in the formation of biodegradation intermediates and autoxidation products [13]. Autoxidation is a natural process of spontaneous oxidation in the presence of oxygen and can be expedited with free fatty acids, mono- and diacylglycerols, metals, e.g., iron, and thermally oxidized compounds [41]. Autoxidation of vegetable oils depends on their degree of unsaturation [42], and oils with a high unsaturated fatty acid content are more susceptible to autoxidation. The relative oxidation rates of stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were reported to be 1, 100, 1200, and 2500 at 25 °C, respectively. According to Romero and Morton [43], the reaction rates of the autoxidation of oleic-linoleic acid mixtures decreased with decreasing linoleic acid molar fractions. In addition, several studies suggested the transformation of fatty acids during the degradation process [44-45]. Therefore, it can be deduced that there is a correlation between the fatty acid composition of oil and its degradation rate.

The structure of fatty acid molecules, *i.e*., characterized by the length of the carbon chain, the number of double bonds, and the exact position of these double bonds, can define and determine the biological reactivity of the fatty acid molecules [19]. Therefore, the chemical structure affects the biodegradability of a substance [46]. Table 7 shows the percentage of saturated, monounsaturated, and polyunsaturated fatty acids in POP. Based on the literature, the saturated fatty acids composition in palm oil is around 50%, with 44% palmitic acid (C16:0), and approximately 5% of stearic acid (C18:0) and remaining are the trace amounts of myristic acid (C14:0). The unsaturated fatty acids are approximately 40% of oleic acid (C18:1) and 10% consists of polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) [47-48]. Meanwhile, for palm kernel oil, the saturated fatty acids and unsaturated fatty acids are roughly 79.1% and 20.9%, respectively [49].

Table 7. Percentage of saturated, monounsaturated and polyunsaturated fatty acids in POP

Fatty Acid Composition (%)	**POP**			
	CPO	**CPKO**	**RBDPO**	**RBDPOo**
Total saturated	50.49	74.75	49.75	43.85
Total monounsaturated	38.98	16.59	39.28	43.67
Total polyunsaturated	9.43	2.71	9.95	11.36

CPKO has the highest amount of saturated fatty acids and the lowest amount of monounsaturated and polyunsaturated fatty acids. This explained why CPKO showed a slightly low percentage of biodegradability since it is the most stable oil with the highest saturated fatty acid content compared to other POP. However, CPKO still showed the potential to be degraded in the marine environment. Meanwhile, RBDPO showed a high biodegradation percentage, mainly due to its high unsaturated fatty acid content. Unsaturated fatty acids are less stable than saturated fatty acids, making them more susceptible to oxidation [41].

In terms of microbial degradation, the initial stage for the degradation of POP would be the dissociation of ester bonds from fatty acids through the action of enzymes, including esterases and lipases. These two enzymes are synthesized by various microorganisms [50-51]. Meanwhile, the cleavage of carboxyl ester bonds is catalyzed by lipases, which are hydrophobic proteins. The carboxyl ester bonds are tri-, di-, and monoacylglycerols, the major components of animal, plant, and microbial fats and oils [52]. Both saturated and unsaturated fatty acids are biodegraded *via* a process known as β-oxidation after the initial stage of degradation.

Conclusion

The quality and biodegradability of POP are the key points in terms of their industrial use. The quality of four POP, *i.e*., CPO, CPKO, RBDPO, and RBDPOo, was determined through moisture, FFA, IV, FAC, and SMP

analyses. The properties of the POP were within the specified values as stipulated in the Malaysian Standards and also PORAM, MEOMA, Codex, and MPOA standards. Meanwhile, some of the values slightly deviated from the specified value, possibly due to the storage condition and time prior to the analysis of the sample. As for the biodegradability assessment of these POP, all samples achieved more than 60% biodegradability and, therefore, can be considered biodegradable in the marine environment. Hence, POP are not expected to cause any harm to the environment.

Acknowledgement

The authors would like to express their gratitude to the Director General of MPOB for granting permission to publish this research. The authors would also like to thank all analysts at the Analytical Testing Services Laboratory of the Advanced Oleochemical Technology Division (AOTD) for their technical assistance and services in completing this study.

References

1. Parveez, G. K. A., Tarmizi, A. H. A., Sundram, S., Loh, S. K., Ong-Abdullah, M., Palam, K. D. P., Salleh, K. M., Ishak, S. M. and Idris, Z. (2021). Oil palm economic performance in Malaysia and R&D progress in 2020. *Journal of Oil Palm Research,* 33: 181-214.
2. MPOB (2022). Monthly export of oil palm products - 2021. MPOB. Access from https://bepi.mpob.gov.my/index.php/en/export/export-2021/monthly-export-of-oil-palm-products-2021, accessed on 10 April 2022.
3. Maluin, F. N., Hussein, M. Z. and Idris, A. S. (2020). An overview of the oil palm industry: Challenges and some emerging opportunities for nanotechnology development. *Agronomy,* 10**:** 356.
4. Gupta, M. K. (2017). Chapter 1 - Requirement for successful production and delivery of the refined vegetable oils. *In:* GUPTA, M. K. (ed.) *Practical Guide to Vegetable Oil Processing (Second Edition)*. AOCS Press, p.1-5.
5. Chong, C. L. (2012). 15 - Measurement and maintenance of palm oil quality. *In:* LAI, O.-M., TAN, C.-P. & AKOH, C. C. (eds.) *Palm Oil*. AOCS Press, p.431-470.
6. Dongho, D., Gouado, I., Sameza, L. M., Mouokeu, R. S., Demasse, A. M., Schweigert, F. J. and Ngono, A. R. (2017). Some factors affecting quality of crude palm oil sold in Douala, Cameroon. *Journal of Food Research,* 6: 50-58.
7. O'brien, R. D. (2009). Fats and oils: Formulating and processing for applications, Third Edition, CRC Press.
8. Che Man, Y. B., Moh, M. H. and Van De Voort, F. R. (1999). Determination of free fatty acids in crude palm oil and refined-bleached-deodorized palm olein using fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society,* 76: 485-490.
9. Peamaroon, N., Jakmunee, J. and Moonrungsee, N. (2021). A Simple colorimetric procedure for the determination of iodine value of vegetable oils using a smartphone camera. *Journal of Analysis and Testing,* 5**:** 379-386.
10. Tarmizi, A. H., Lin, S. W. and Kuntom, A. (2008). Palm-based standard reference materials for iodine value and slip melting point. *Analytical Chemistry Insights,* 3**:** 127-133.
11. Beare-Rogers, J. L., Dieffenbacher, A. and Holm, J. V. (2001). Lexicon of lipid nutrition (IUPAC Technical Report). *Pure and Applied Chemistry,* 73: 685-744.
12. Karabulut, I., Turan, S. and Ergin, G. (2004). Effects of chemical interesterification on solid fat content and slip melting point of fat/oil blends. *European Food Research and Technology,* 218**:** 224-229.
13. Salam, D. A., Suidan, M. T. and Venosa, A. D. (2016). Biodegradation and toxicity of vegetable oils in contaminated aquatic environments: Effect of antioxidants and oil composition. *Science of the Total Environment,* 547: 95-103.
14. Módenes, A. N., Sanderson, K., Trigueros, D. E. G., Schuelter, A. R., Espinoza-Quiñones, F. R., Neves, C. V., Zanão Junior, L. A. and Kroumov, A. D. (2018). Insights on the criteria of selection of vegetable and mineral dielectric fluids used in power transformers on the basis of their biodegradability and toxicity assessments. *Chemosphere,* 199: 312-319.

15. Ramli, N. A. S., Mohd Noor, M. A., Musa, H. and Ghazali, R. (2018). Stability evaluation of quality parameters for palm oil products at low temperature storage. *Journal of the Science of Food and Agriculture,* 98: 3351-3362.
16. Times, T. S. (2017). Beaches shut in Hong Kong after palm oil spill. Access from https://www.straitstimes.com/asia/east-asia/beaches-shut-in-hong-kong-after-palm-oil-spill. [Accessed online 17 May 2022].
17. Group of Experts on the Scientific Aspects of Marine Environmental Protection (2019). Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships. International Maritime Organization.
18. Group of Experts on the Scientific Aspects of Marine Environmental Protection (2019). GESAMP Composite List 2019. Access from https://wwwcdn.imo.org/localresources/en/OurWork/Environment/Documents/GESAMP%20Composite%20List%20of%20hazard%20profiles-2019.pdf. [Accessed online 17 May 2022].
19. Al-Darbi, M. M., Saeed, N. O., Islam, M. R. and Lee, K. (2005). Biodegradation of natural oils in seawater. *Energy Sources,* 27: 19-34.
20. Kis, Á., Laczi, K., Zsíros, S., Rákhely, G. and Perei, K. (2015). Biodegradation of animal fats and vegetable oils by *Rhodococcus erythropolis* PR4. *International Biodeterioration & Biodegradation,* 105: 114-119.
21. Sugimori, D., Nakamura, M. and Mihara, Y. (2002). Microbial degradation of lipid by Acinetobacter sp. strain SOD-1. *Bioscience, Biotechnology, and Biochemistry,* 66: 1579-1582.
22. Ramani, K., Saranya, P., Jain, S. C. and Sekaran, G. (2013). Lipase from marine strain using cooked sunflower oil waste: production optimization and application for hydrolysis and thermodynamic studies. *Bioprocess and Biosystems Engineering,* 36: 301-315.
23. Arulasu, M. T., Ganesen, S. S. K., Lee, H. S., Tuan, A. S., Ghazali, R., Bhubalan, K. (2022). Microbial degradation of palm oil in natural seawater and identification of oil degrading bacterial consortium. *Malaysian Journal of Analytical Sciences,* 26: 976-988.
24. Kuntom, A. (2005). MPOB test methods: A compendium of test[s] on palm oil products, palm kernel products, fatty acids, food related products and others, Malaysia, Malaysian Palm Oil Board, Ministry of Plantation Industries and Commodities Malaysia.
25. Mehlenbacher, V. C. and Sallee, E. M. (2009). Official methods and recommended practices of the AOCS, American Oil Chemists Society.
26. APHA (2018). 5220 Chemical Oxygen Demand (COD); standard methods for the examination of water and wastewater. United States of America: American Public Health Association.
27. OECD (1992). Test No. 306: biodegradability in seawater, OECD Publishing.
28. Ott, A., Martin, T. J., Whale, G. F., Snape, J. R., Rowles, B., Galay-Burgos, M. and Davenport, R. J. (2019). Improving the biodegradability in seawater test (OECD 306). *Science of the Total Environment,* 666**:** 399-404.
29. Okechalu, J. N., Dashen, M. M., Lar, P. M., Okechalu, B. and Gushop, T. (2011). Microbiological quality and chemical characteristics of palm oil sold within Jos Metropolis, Plateau State, Nigeria. *Journal of Microbiology and Biotechnology Research,* 1(2): 107-112.
30. Wu, P.-F. and Nawar, W. W. (1986). A technique for monitoring the quality of used frying oils. *Journal of the American Oil Chemists' Society,* 63: 1363-1367.
31. Palm Oil Refiners Association of Malaysia (2012). *PORAM Handbook,* Malaysia, Palm Oil Refiners Association of Malaysia.
32. Sanders, T. H. (2003). Ground nut oil. *In:* Caballero, B. (ed.) Encyclopedia of food sciences and nutrition (second edition). Oxford: Academic Press, p.2967-2974.
33. Department of Standard Malaysia (2007). MS 814:2007 palm oil – specification (second revision). Malaysia: Department of Standards.
34. Department of Standard Malaysia (2011). MS 80:2011 palm oil – specification. Malaysia: Department of Standards.
35. Akusu, M. O., Achinewhu, S. C. and Mitchell, J.

(2000). Quality attributes and storage stability of locally and mechanically extracted crude palm oils in selected communities in Rivers and Bayelsa States, Nigeria. *Plant Foods for Human Nutrition,* 55**:** 119-126.

36. Akinola, S. A., Omafuvbe, B. O., Adeyemo, R. O., Ntulume, I. and Ailero, A. A. (2020). Effect of storage on the quality of processed palm oil collected from local milling points within Ile-Ife, Osun State, Nigeria. *Journal of Food Science and Technology,* 57: 858-865.
37. Department of Standard Malaysian (2007). MS 816:2007 palm oil – specification (second revision). Malaysia: Department of Standards.
38. Codex (2019). Codex standard for named vegetable oils (Codex-Stan 210-1999), Amendment 2019. Codex Alimentarius
39. Verberk, W. C. E. P., Buchwalter, D. B. and Kefford, B. J. (2020). Energetics as a lens to understanding aquatic insect's responses to changing temperature, dissolved oxygen and salinity regimes. *Current Opinion in Insect Science,* 41: 46-53.
40. Bhubalan, K., Hui-Wan, R. A. C., Renganathan, P., Tamothran, A. M., Ganesen, S. S. K., Ghazali, R. (2019). Bacterial degradation of palm olein in seawaterand identification of some cultivable strains. *Malaysian Applied Biology,* 48: 207-213.
41. Choe, E. and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety,* 5**:** 169-186.
42. Belitz, H., Grosch, W. and Schieberle, P. (1999). Lipids. Chapter 3. *Food Chemistry.* p.152-236.
43. Romero, A. J. R. and Morton, I. D. (1975). A kinetic study of the competitive oxidation of oleic acid-linoleic acid mixtures. *Journal of the Science of Food and Agriculture,* 26**:** 1353-1356.
44. Pereira, M. G., Mudge, S. M. and Latchford, J. (2003). Polymerisation versus degradation of sunflower oil spilled in the marine environments. *Marine Pollutution Bulletin,* 46: 1078-1081.
45. Pereira, M. G., Mudge, S. M. and Latchford, J. (2003). Vegetable oil spills on salt marsh sediments; comparison between sunflower and linseed oils. *Marine Environmental Research,* 56: 367-385.
46. Aluyor, E. O., Obahiagbon, K. O. and Ori-Jesu, M. (2009). Biodegradation of vegetable oils: A review. *Scientific Research and Essays,* 4: 543-548.
47. Prada, F., Ayala-Diaz, I. M., Delgado, W., Ruiz-Romero, R. and Romero, H. M. (2011). Effect of fruit ripening on content and chemical composition of oil from three oil palm cultivars (*Elaeis guineensis* Jacq.) grown in Colombia. *Journal of Agricultural and Food Chemistry,* 59: 10136-10142.
48. Noh, A., Rajanaidu, N., Kushairi, A., Mohd Rafii, Y., Mohd Din, A., Mohd Isa, Z. and Saleh, G. (2002). Variability in fatty acids composition, iodine value and carotene content in the MPOB oil palm germplasm collection from Angola. *Journal of Oil Palm Research,* 14: 18-23.
49. Bahadi, M., Mohamad Yusoff, M. F., Salimon, J., Ahmed Jumaah, M. and Derawi, D. (2019). Physicochemical characteristics of malaysian crude palm kernel oil. *Malaysian Journal of Chemistry,* 21: 17-27.
50. van Broekhuizen, P., Theodori, D., Le Blansch, K., and Ullmer, S. (2003). Lubrication in inland and coastal water activities. CRC Press.
51. Fingas, M. (2014). Vegetable oil spills. Handbook of oil spill science and technology. John Wiley & Sons, Inc. p.79-91.
52. Saifuddin, N. and Chua, K. (2006). Biodegradation of lipid-rich wastewater by combination of microwave irradiation and lipase immobilized on chitosan. *Biotechnology,* 5**:** 315-323.