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Establishment of an Effective Refining Process for *Moringa oleifera* Kernel Oil

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Abstract: This study systematically established the most effective refining process for *Moringa oleifera* (MO) kernel oil. Acid degumming (20.33 ± 1.37 ppm) removed significantly greater phosphorus than water degumming (31.18 ± 0.90 ppm). Neutralization was more effective than deodorization in decreasing the acid (0.06 mg KOH/g) and *p*-Anisidine (*p*-AV, 0.36 ± 0.03) values of the oil. Besides improving its color properties, acid-activated bleaching earth Type B was better than Types A and C in decreasing the oil's *p*-AV (0.43 ± 0.02), acid value (3.96 ± 0.02 mg KOH/g), and moisture content (0.01 ± 0.00% w/w). The selected refining stages successfully produced MO kernel oil with acceptable peroxide value (PV, 1.66–3.33 meq/kg), *p*-AV (1.05–1.49), total oxidation value (TOTOX, 4.38–8.15), acid value (0.03 mg KOH/g), moisture content (0.01% w/w), phosphorus content (1.28–1.94 ppm), iodine value (80.79–81.03), oleic acid (79.52–79.65%), and tocopherol content (65.26–87.00 mg/kg).

Keywords: fats and oils; oilseeds; oxidative stability; lipids; lipid chemistry/lipid analysis; processing technology; refining

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1. Introduction

Moringa oleifera (MO) is the most widely cultivated species of the Moringaceae family [1]. The plant is native to northern India, and is widely distributed throughout the tropics and subtropics, including Africa, Asia, Central, and South America due to its strong resistance to drought [2]. Almost every part of the plant (leaves, root bark, stem bark, immature pods, and mature seeds) is edible and valuable. From an economic point of view, MO is potentially one of the most valuable crops for food, industrial, agricultural, and medicinal uses, especially for addressing food security issues in countries where hunger and malnutrition are major problems [1].

One of the key parts of MO is its seed kernel, which can contain up to 35% (w/w) protein and 40% (w/w) edible oil [3]. Many studies have reported on the physicochemical and oxidative properties of MO kernel oil. These studies attributed its high stability to the presence of up to 75% oleic acid (i.e., comparable to olive oil), and significant amounts of tocopherol and sterol [4,5]. A diet rich in monounsaturated fat as a substitute for saturated fat is a strong and continuing trend due to its beneficial impact on coronary heart disease and overall heat [6]. With reference to these findings, MO kernel oil can potentially be developed and commercialized as healthy and functional food products. It can also be promoted as a new source of oil in tropical and subtropical countries since the tree grows rapidly under a wide range of climatic conditions with little or no agricultural input [2].

Nadeem and Imran [7] incorporated the oil in food products including vanaspati and shortening.

Despite these advantages and the existence of many publications on MO, the need to refine its kernel oil has not been addressed systematically in literature. Different types of oil will require different refining methods. Refining is critical to produce and preserve oil of edible quality [8]. Robust refining processes retain the most desirable components in the oil, and eliminate detrimental and off-flavor-producing compounds, which, in turn, allow the oil to remain bland and neutral in taste over the course of its shelf life lasting several months. Sánchez-Machado et al. [9] have reported on degumming and neutralization of MO kernel oil originating from South Sonora, Mexico. However, there is a significant knowledge gap. In addition to degumming and neutralization, it is also necessary to investigate deodorization, and the effect of different refining stages on the oil's acid value. Furthermore, MO kernel oil contains significant levels of tocopherol, and the effects of different refining stages on tocopherols is not known. A systematic study of refining alternatives and their effects on the final MO oil quality is, therefore, necessary in order to exploit its full potential.

The refining stages investigated in this study for MO oil are: degumming, neutralization, bleaching, and deodorization. In terms of approach, this study first establishes the most effective parameters for each individual refining stage and then investigates the effects of combining the various refining steps on the final oil quality.

2. Materials and Methods

2.1. Materials

Cold-pressed crude MO kernel oil was purchased from Greenish Export (Tamil Nadu, India) and stored at a room temperature of 25.0 ± 3.0 °C until use. Three oil batches were purchased and termed Batch X, Batch Y, and Batch Z in this study. The bleaching earths used were from Taiko Classic Bleaching Earth (Perak, Malaysia). Acetic acid, phosphoric acid, iso octane, potassium iodide, sodium hydroxide, sodium thiosulfate, starch, sodium dodecyl sulfate, isooctane, p-anisidine, potassium hydroxide, isopropyl alcohol, phenolphthalein, toluene, ethyl acetate, 1,1-diphenyl-2-picrylhydrazl (DPPH), d,1-alpha tocopherol, sodium methoxide, hydrochloric acid, heptane, sodium sulphate, methyl red, potassium iodide, cyclohexane, Wijs reagent, acetone, bromophenol blue indication solution, magnesium oxide, nitric acid, ammonium molybdate, ammonium vanadate, disodium hydrogen phosphate, and monopotassium hydrogen phosphate were purchased from Merck Chemicals (Darmstadt, Germany).

2.2. Oil Refining Methodology

Prior to refining, 15 kg of crude MO kernel oil was centrifuged (Heraeus Megafuge 8, Thermo Fisher Scientific, Third Avenue Waltham, MA, USA) at 11,309 rpm for 10 min to remove any residues present, while 400 g of crude oil MO kernel oil was used as the starting material for each refining stage.

2.2.1. Degumming

Water degumming was carried out according to the method described by Tsaknis et al. [10]. Boiling water [20% (*w*/*w*)] was added to the crude oil and the temperature of the dispersion was raised to 75 °C and maintained for 10 min while being stirred. The dispersion was then cooled down to room temperature and centrifuged (11,309 rpm) for 15 min in 200 cm^3 tubes. The phosphatide content at this stage was determined using MPOB Test Method p2.8 Part 1(b) [11].

Acid degumming was carried out according to Chew et al. [12], with some modifications. The crude oil was mixed with 0.3% (*w*/*w*) phosphoric acid (85% concentration) for 10 min at 70 °C. The mixture was further treated with 3% (*w*/*w*) Milli-Q water with agitation at 200 rpm for 30 min. The degummed oil was recovered by centrifugation for

15 min at 11,309 rpm. The phosphatide content was then determined using MPOB Test Method p2.8 Part 1(b) [11].

2.2.2. Neutralization

Neutralization was carried out based on the method described by Chew et al. [12], with some modifications. Sodium hydroxide (NaOH) of different concentrations (10 °Bé and 15 °Bé) were tested. The crude oil was heated to 65 °C, mixed with the NaOH solution, and stirred for 30 min. The mixture was further centrifuged at 3504 rpm for 10 min, which resulted in the immediate separation of neutral oil and soap stock. The oil was washed three times with Milli-Q water (15% *w*/*w*) to remove the traces of soap in the oil. The mixture was centrifuged again at 3504 rpm for 10 min to separate the oil and the residual soap stock. The soap content in the oil was determined by using the soap-in-oil titrimetric method of AOCS Recommended Practice Cc 17-95 [13]. The soap content in the oil measured should be less than 50 ppm according to the Codex Standards [14].

2.2.3. Bleaching

Bleaching was carried out based on the method done by Chew et al. [11]. The crude oil was treated with 1.2% (*w*/*w*) bleaching earth at 95 °C at reduced pressure. The types of bleaching earth tested were Taiko Classic acid-activated of Type A and Type B, both with pH values of 7.7 and 7.3 and surface areas of 192.9 m^2/g and 260.7 m^2/g, respectively, and a natural (Type C) bleaching earth with a pH value of 9.19 and surface area of 147.0 m^2/g. The bleaching earth residual was removed by centrifuging at 11,309 rpm for 10 min.

2.2.4. Deodorization

Deodorization was carried out as described by Chew et al. [15], with some modifications. Three deodorization temperatures were tested; 200, 235, and 270 °C. The crude oil was taken in a four-necked round bottom flask equipped with a sparging tube. A reflux condenser, a conical flask, a water-jacketed trap, and a vacuum pump (RZ 2.5, Vacuubrand, Alfred-Zippe-Str, Wertheim, Germany) were connected to the deodorizer unit. The oil was heated under vacuum at 0.5–1.0 mbar to the temperatures tested for 1 h. Concurrently, a round bottom flask containing water was heated by a heating mantle (MS-DMB605, Mtops, Yangju-City, Kyunggi-do, South Korea), and the steam generated was passed into the oil through a sparging inlet tube and delivery tube.

2.3. Physicochemical and Oxidative Properties of Refined Moringa Oleifera Kernel Oil

The methods used for determining the physicochemical and oxidative properties of the refined oil samples are summarized in Table 1.

Table 1. Analytical methods used to determine the physicochemical and oxidative properties of *Moringa oleifera* kernel oil.

No.	Properties	Method Used	Reference
1	Peroxide value	Cd 8b-90	AOCS Official Methods
2	*p*-Anisidine value	Cd 18-90	AOCS Official Methods
3	Total oxidation value	Cg 3-91	AOCS Analytical Guidelines
4	Acid value	Cd 3d-63	AOCS Official Methods
5	Moisture content	Ca 2c-25	AOCS Official Methods
6	Color properties	HunterLab ColorEx colorimeter (Hunter Associates Laboratory, Sunset Hills Road Reston, VA, USA) together with EasyMatch QC Software	Chew (2016)
7	Iodine value	P3.2 (2004)	MPOB Test Method
8	Fatty acid composition	P3.5 (2004)	MPOB Test Method
9	Tocopherol composition	3.3-26 (2006)	GHRSB (O&F) Sime Darby Method

2.4. Individual and Combination of Refining Steps

2.4.1. Individual Refining Steps (Batch X)

The oil of Batch X was divided into two portions, and each portion was separately subjected to each of the refining steps indicated in Sections 2.2.1–2.2.4. The physicochemical and oxidative properties of the refined oil samples (Table 1, Analytical tests 1–6) [11,13] were determined. The analytical tests were undertaken in triplicate on each oil portion, giving a total of $n = 6$ from both portions.

2.4.2. Combination of Refining Steps (Batches Y and Z)

Based on Section 2.4.1, the individual refining steps that produce MO kernel oil with desirable physicochemical and oxidative properties were combined into a series of refining steps. These degumming-neutralization-bleaching (DNB) refining steps were further subjected to two batches of crude MO kernel oil (Batch Y and Batch Z). For each oil batch, the oil was divided into two portions, and each portion was independently treated with the combined refining steps. The physicochemical and oxidative properties of the refined oil samples were determined (Analytical tests 1–9, Table 1) [11,13,16]. These tests were carried out in triplicate on each oil portion of every batch, giving a total of $n = 6$ from both portions.

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation ($n = 6$). All results were analyzed using Minitab Statistical Software 17 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare multiple sets of data. A comparison between two sets of data was carried out by using two-sample *t*-test [17]. All statistical tests were performed at significance level of 0.05.

3. Results and Discussion

3.1. Effect of Individual Refining Stages on the Physicochemical and Oxidative Properties of Moringa Oleifera Kernel Oil (Batch X)

3.1.1. Degumming

The main objective of degumming is to remove the phospholipid content that may be present in the crude oil. According to O'Brien [18], the phosphorus content should be reduced to less than 30 ppm by degumming so that it can be further reduced to less than 5 ppm after bleaching. With reference to Table 2, the phosphorus content in crude MO kernel oil was 45.00 ± 0.80 ppm, which indicated the need to degum the oil. Two different degumming methods were tested in this study: water degumming (WD) and acid degumming (AD). Table 2 shows that both WD and AD significantly decreased ($p < 0.05$) the phosphorus content, and this corresponds to a significantly decreased phospholipid content in the oil ($p < 0.05$). Furthermore, in comparison with WD, AD resulted in significantly lower phosphorus content ($p < 0.05$). This finding suggested that the MO kernel oil contained significant amount of non-hydratable phospholipid, which was difficult to remove by WD. Zufarov et al. [19] also reported on the unsuitability of the WD method for degumming vegetable oils such as pressed rapeseed oil and sunflower oil containing high non-hydratable phospholipids.

The crude MO kernel oil exhibited a peroxide value (PV) of up to 15.51 ± 2.51 meq/kg. However, the maximum PV of a refined oil should be 10 meq/kg PV [14]. Oil with high PV indicates the presence of primary oxidation products, most probably attributed to improper storage and/or extraction and processing conditions [20]. Based on Table 2, both WD and AD were insignificantly different ($p > 0.05$) from each other in affecting the PV (3.33 ± 0.01 meq/kg), and these two methods significantly decreased the PV of the oil samples as compared to that of crude MO kernel oil ($p < 0.05$). A significant decrease in PV upon degumming was also reported in the case of MO kernel oil from seeds of South Sonora (Mexico) origin [9], kenaf seed oil [12], and perilla seed oil [21]. Decrease in the PV consequently resulted in degummed (WD and AD) oil samples with significantly lower total oxidation (TOTOX) values, with oil sample subjected to AD showing significantly lower value as compared to that which is subjected to WD. A similar trend was also observed for the *p*-Anisidine value (*p*-AV) of the oil samples, which indicates the presence of secondary oxidation products such as aldehydes and small volatile compounds [20]. In terms of moisture content, WD resulted in MO kernel oil with a significantly higher ($p < 0.05$) moisture content as compared to both crude oil and AD-treated oil. During degumming, a significant amount of water is added to the oil, especially in the case of WD, which must be removed at the end of the process to avoid producing oil containing high moisture content [20]. In contrast to the PV, *p*-AV, TOTOX value, and moisture content, WD resulted in a significantly lower acid value than that of crude oil and AD-treated oil. This finding highlighted the potential of degumming method in decreasing the acid value of an oil. In the AD-treated oil, the significantly higher acid value may be attributed to the traces of phosphoric acid used in the digestion method itself. Based on these findings, it can be concluded that AD shows more promise in decreasing the phosphorus content of the MO kernel oil, while improving its oxidative properties.

Table 2. Effect of individual refining steps on the physicochemical and oxidative properties of *Moringa oleifera* (MO) kernel oil.

Refining Stages	Peroxide Value (meq/kg)	*p*-Anisidine Value	Total Oxidation Value	Acid Value (mg KOH/g)	Moisture Content (%)	Phosphorus Content (ppm)
Crude MO kernel oil	15.51 ± 2.51	0.56 ± 0.03	31.57 ± 5.03	4.24 ± 0.03	0.06 ± 0.00	45.00 ± 0.80
			Degumming			
Water	3.33 ± 0.01 ^{a}	0.62 ± 0.05 ^{a}	7.27 ± 0.05 ^{a}	4.16 ± 0.03 ^{a}	0.15 ± 0.01 ^{a}	31.18 ± 0.90 ^{b}
Acid	3.33 ± 0.01 ^{a}	0.55 ± 0.04 ^{b}	7.20 ± 0.04 ^{b}	4.23 ± 0.02 ^{b}	0.10 ± 0.01 ^{b}	20.33 ± 1.37 ^{a}
			Neutralization			
10 °Bé	6.37 ± 0.66 ^{a}	0.42 ± 0. 05 ^{a}	13.17 ± 1.35 ^{a}	0.06 ± 0.00 ^{b}	0.15 ± 0.01 ^{a}	Nd
15 °Bé	5.26 ± 0.68 ^{b}	0.36 ± 0.03 ^{b}	10.87 ± 1.33 ^{b}	0.06 ± 0.00 ^{b}	0.11 ± 0.01 ^{b}	Nd
			Bleaching			
Type A	4.99 ± 0.01 ^{a}	0.63 ± 0.06 ^{a}	10.60 ± 0.03 ^{a}	4.10 ± 0.02 ^{a}	0.02 ± 0.00 ^{a}	Nd
Type B	4.99 ± 0.00 ^{a}	0.43 ± 0.02 ^{b}	10.41 ± 0.02 ^{b}	3.96 ± 0.02 ^{c}	0.01 ± 0.00 ^{b}	Nd
Type C	1.65 ± 0.03 ^{b}	0.64 ± 0.11 ^{a}	3.93 ± 0.05 ^{c}	4.03 ± 0.02 ^{b}	0.01 ± 0.00 ^{b}	Nd
			Deodorization			
200 °C	0.33 ± 0.00 ^{b}	1.00 ± 0.13 ^{a}	1.67 ± 0.13 ^{b}	3.81 ± 0.15 ^{a}	0.02 ± 0.00 ^{a}	Nd
235 °C	0.33 ± 0.00 ^{b}	1.02 ± 0.09 ^{a}	1.68 ± 0.09 ^{b}	3.53 ± 0.09 ^{b}	0.01 ± 0.00 ^{b}	Nd
270 °C	0.53 ± 0.07 ^{a}	1.02 ± 0.08 ^{a}	2.07 ± 0.13 ^{a}	2.44 ± 0.10 ^{c}	0.00 ± 0.00 ^{c}	Nd

- Nd, not determined.
- Means ± standard deviation followed by the same alphabets in each column for each refining stage are not significantly different ($p > 0.05$) as determined by the analysis of variance (ANOVA) and Tukey's Multiple Comparison Test.
- Neutralization was carried out by using NaOH solutions of different densities (10 °Bé and 15 °Bé).
- Bleaching was carried out by using three types of bleaching earth: Type A, Type B (acid-activated), and Type C (neutral).

3.1.2. Neutralization

The major purpose of neutralization is to remove free fatty acids [22]. In addition, phosphatides and gums may also be removed during this stage [18]. According to Codex Standards [14], the free fatty acid is described as acid value, and the maximum permissible acid value for a refined oil is 0.6 mg KOH/g oil. In this study, the acid value of the crude MO kernel oil was 4.24 ± 0.03 mg KOH/g (Table 2) and this indicates the need to refine the oil. Neutralization by using NaOH of both 10 °Bé and 15 °Bé resulted in significantly lower acid values of 0.06 mg KOH/g; a 98.67% reduction from that of crude oil. During neutralization, the free fatty acids in the crude oil are saponified by the alkali and removed together with the soap stock, thereby decreasing the acid value and improve oil quality [23]. Treatments with 10 °Bé and 15 °Bé NaOH resulted in similar amounts of soap recovered; 37.88 ± 0.07 ppm and 37.90 ± 0.08 ppm, respectively. These values are less than the maximum allowable soap content of 50 ppm [14]. Without adequate washing, a relatively large amount of soap may still be present in the oil and may be adsorbed by the bleaching earth during bleaching. This, in turn, will lower the adsorption capacity of the bleaching earth for other constituents in the oil and, therefore, decrease its performance [24]. An earlier study by Yoon [25] also reported a decrease in acid value upon neutralization with a 12 °Bé solution of NaOH in the case of chufa or tiger nut oil.

The PV, *p*-AV, and TOTOX value of the MO kernel oil also decreased significantly during neutralization, with the use of 15 °Bé NaOH resulting in significantly lower values than 10 °Bé NaOH. These findings can be correlated with the significant decrease in the free fatty acid content as explained earlier. In contrast, Sánchez-Machado et al. [9] reported that neutralization did not significantly affect the PV of MO kernel oil, yet increased the oil's *p*-AV. Neutralization also significantly decreased the PV of perilla [21] and kenaf [12] seed oils, and increased the *p*-AV of these oils. On the other hand, both Yoon [25] and Lee et al. [21] reported a significant increase ($p < 0.05$) in the PV of neutralized chufa oil and camellia seed oil, respectively. These varying results may be due to the different concentration of caustic agents and neutralization process employed, in addition to the differences in the quality of the crude oil samples used. According to O'Brien [18], factors affecting the effectiveness of a neutralization process include the concentration of Baume caustic used, mixing energy, and effective contact of the caustic with the oil for a controlled time and at a fixed temperature.

3.1.3. Bleaching

The primary function of bleaching is to remove color, besides other oxidation products, phospholipids, pigments, and traces of soap from earlier refining stages [18,26]. During bleaching, primary oxidation products (peroxides, hydroperoxides) become unstable and, therefore, decompose into secondary oxidation products (aldehydes, ketones, and small molecules). This occurs as a result of combined catalytic properties of bleaching earth and the high temperature of 80–120 °C used during the process [27,28]. In this study, the process was carried out at 95 °C and the effect of different types of bleaching earth on the quality of MO kernel oil are reported in Table 2, according to which all bleaching earths resulted in a significant decrease in the PV, TOTOX values, acid values, and moisture content. Only bleaching earth Type B significantly decreased the *p*-AV, and resulted in the lowest acid value in the treated oil. On the other hand, only bleaching earth Type C was able to produce oil with a TOTOX value below 10, which is an indicator of good quality oil [29]. In contrast to bleaching earth Types B and C, the use of bleaching earth Type A resulted in MO kernel oil with the highest acid value and moisture content. The presence of moisture in crude oil induces hydrolysis of triglycerides, which further leads to the formation of free fatty acids, and therefore higher acid value [30,31]. Sánchez-Machado et al. [9] reported a slightly different result in which the bleached MO kernel oil exhibited significantly lower PV yet higher *p*-AV as compared to its crude counterpart.

The color properties of the bleached MO kernel oil samples are shown in Table 3, while photo images of the oil samples are as shown in Figure 1. In general, the *L** and *b** values were least affected by both bleaching earth Type A and Type C, while bleaching earth Type B resulted in oil with significantly lower *L**, *a**, and *b** values. This may be due to the higher surface area of bleaching earth Type B (260.7 m^2/g) as compared to Type A (192.9 m^2/g) and Type C (147.0 m^2/g), which allows for higher sorption performance in removing the coloring pigments [32]. As such, activated bleaching earth, Types A and B, are preferable to Type C since they display a higher adsorption capacity.

Table 3. Changes in the color properties of various *Moringa oleifera* (MO) kernel oil samples after bleaching with bleaching earth. Batch X oil samples represent oil that was individual refined; Batches Y and Z represent oils that have undergone combined refining stages.

	Color Properties		
	***L** Value**	***A** Value**	***b** Value**
		Batch X	
Crude MO kernel oil	74.29 ± 1.74 ^{a}	13.95 ± 0.65 ^{b}	117.30 ± 3.00 ^{a}
Type A	71.69 ± 1.13 ^{b}	16.47 ± 0.69 ^{a}	115.06 ± 1.40 ^{a}
Type B	67.32 ± 1.27 ^{c}	11.91 ± 0.36 ^{c}	106.83 ± 2.06 ^{b}
Type C	73.34 ± 0.75 ^{ab}	16.94 ± 0.63 ^{a}	117.12 ± 1.38 ^{a}
		Batch Y	
Crude MO kernel oil	68.97 ± 0.36 ^{a}	16.13 ± 0.04 ^{a}	112.15 ± 0.99 ^{a}
Refined MO kernel oil	79.62 ± 0.52 ^{b}	4.09 ± 0.71 ^{b}	109.70 ± 3.26 ^{a}
		Batch Z	
Crude MO kernel oil	54.34 ± 0.14 ^{a}	18.84 ± 0.06 ^{a}	90.60 ± 0.41 ^{a}
Refined MO kernel oil	72.85 ± 0.41 ^{b}	0.35 ± 0.07 ^{b}	84.80 ± 1.55 ^{b}

- Means ± standard deviation followed by the same alphabet in each column of each MO kernel oil batch are not significantly different ($p > 0.05$) as determined by the analysis of variance (ANOVA) with Tukey's Multiple Comparison Test, and by the two-sample *t*-test.
- Types of bleaching earth (Batch X): Type A and Type B (acid-activated); Type C (neutral).
- The degumming step was carried out by using acid degumming.
- The neutralization step was carried out by using sodium hydroxide solution of 15 °Bé.
- The bleaching stage (for Batch Y and Batch Z) was carried out by using bleaching earth Type B.

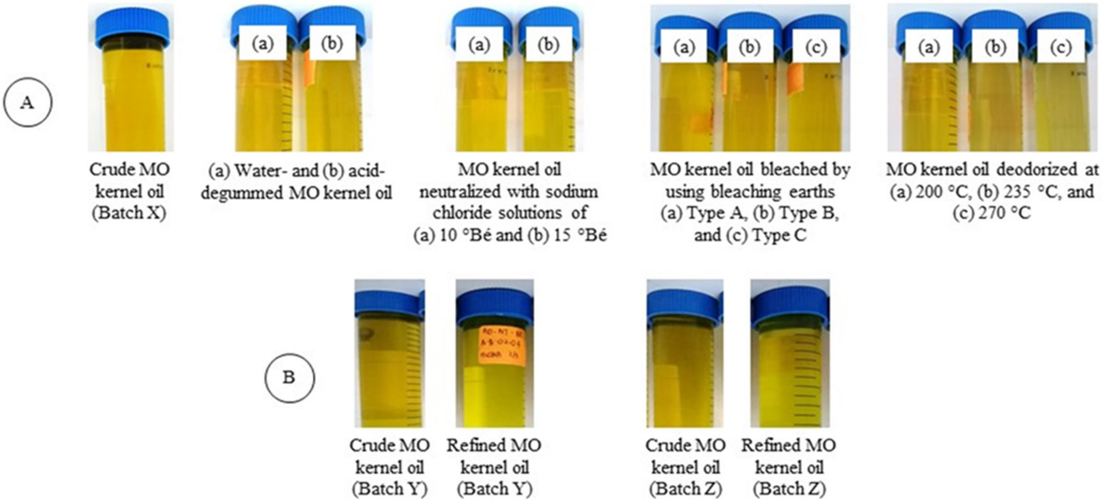

Figure 1. Images of *Moringa oleifera* (MO) kernel oil samples. (**A**) The oil after different refining stages. Neutralization was carried out by using sodium hydroxide solutions of different density (10 °Bé and 15 °Bé). Bleaching was carried out by using three types of bleaching earth: Type A, Type B (acid-activated), and Type C (neutral). (**B**) The oil of different batches (Batch Y and Batch Z) after acid degumming, neutralization with 15 °Bé, sodium hydroxide solution, and bleaching with bleaching earth Type B.

3.1.4. Deodorization

The main objective of deodorization is to remove free fatty acids and oxidative products such as aldehydes and ketones, and undesirable flavor components [8]. As the deodorization temperature increased from 200 °C to 235 °C and 270 °C, the acid value decreased significantly ($p < 0.05$). This is in line with the significant decrease in the moisture content of the oil, which indicates that, at higher temperatures, greater amounts of free fatty acids, moisture and other volatile impurities are eliminated. However, at 270 °C, the acid value of the oil was 2.44 ± 0.10 mg KOH/g oil, which is approximately four times greater than the maximum permissible value of 0.6 mg KOH/g.

Despite this unfavorable finding in terms of acid value, Table 2 shows that deodorization at all temperatures led to a nearly complete decomposition of primary oxidation products, as indicated by significant decreases in PV from 15.51 ± 2.51 meq/kg for crude oil to 0.33–0.53 meq/kg for the refined oil. The decrease in PV subsequently resulted in the TOTOX value decreasing to less than 10, which is considered acceptable [29]. However, the *p*-AV increased significantly ($p < 0.05$). It is speculated that the primary oxidation products decompose into secondary oxidation products as deodorization continues, thus decreasing PV while increasing *p*-AV. As deodorization temperature increased, the PV and TOTOX values significantly increased ($p < 0.05$), while the *p*-AV values remained similar. To conclude, deodorization at 200 °C and 235 °C for 60 min seem adequate based on the PV, TOTOX, and moisture content of the deodorized oil samples.

3.2. Effect of DNB Refining on the Physicochemical and Oxidative Properties

Based on the individual refining stages conducted on the MO kernel oil (Batch X), it can be concluded that degumming is necessary for MO kernel oil in order to remove phospholipids, and that AD is better suited than WD for MO kernel oil. On the other hand, deodorization decreased the PV, TOTOX value, and moisture content of MO kernel oil, but not its acid value, which is also an important property of an edible oil [14]. Therefore, in comparison to deodorization, neutralization is more effective for decreasing the acid value of MO kernel oil, while PV, *p*-AV, and TOTOX values remain at acceptable levels. Specifically, the use of 15 °Bé NaOH is better than 10 °Bé NaOH. Additionally, selecting neutralization over deodorization assures that only refining stages of lower temperature, which is more cost-saving, are applied to the MO kernel oil. In the case of bleaching, bleaching earth Type B was better at decreasing the *p*-AV, acid value, and moisture content of the MO kernel oil. Furthermore, this bleaching earth improved the color properties of the oil. Based on these findings for individual refining stages, the crude oil was treated by combining AD, neutralization (using 15 °Bé NaOH), and bleaching (using bleaching earth Type B). The effect of this selection of DNB refining was further tested on two different batches of MO kernel oil, i.e., Batch Y and Batch Z (Table 4).

Table 4. Changes in the physicochemical and oxidative properties of refined *Moringa oleifera* (MO) kernel oil (Batches Y and Z refined by degumming, neutralization, and bleaching).

Refining Stages	Peroxide Value (meq/kg)	*p*-Anisidine Value	Total Oxidation Value	Acid Value (mg KOH/g)	Moisture Content (%)	Phosphorus Content (ppm)	Iodine Value
Batch Y							
Crude MO kernel oil	4.98 ± 0.01 ^{a}	0.15 ± 0.02 ^{a}	10.11 ± 0.02 ^{a}	6.99 ± 0.02 ^{a}	0.07 ± 0.00 ^{a}	71.10 ± 0.21 ^{a}	80.98 ± 0.04 ^{a}
Refined MO kernel oil	1.66 ± 0.00 ^{b}	1.05 ± 0.06 ^{b}	4.38 ± 0.06 ^{b}	0.03 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	1.28 ± 0.10 ^{b}	81.03 ± 0.11 ^{a}
Changes (%)	−66.67	+ 85.71	−56.68	−99.57	−85.71	−98.20	+ 0.06
Batch Z							
Crude MO kernel oil	6.66 ± 0.00 ^{a}	0.46 ± 0.00 ^{a}	13.78 ± 0.02 ^{a}	6.98 ± 0.03 ^{a}	0.07 ± 0.00 ^{a}	68.84 ± 0.67 ^{a}	80.94 ± 0.05 ^{a}
Refined MO kernel oil	3.33 ± 0.00 ^{b}	1.49 ± 0.02 ^{b}	8.15 ± 0.02 ^{b}	0.03 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	1.94 ± 0.32 ^{b}	80.79 ± 0.18 ^{a}
Changes (%)	−50.00	+69.13	−40.86	−99.57	−85.71	−97.18	−0.19

- Means ± standard deviation followed by the same letter between values of crude and refined MO kernel oils for each oil batch are not significantly different ($p > 0.05$) as determined by a two-sample *t*-test.
- The degumming step was carried out by using acid degumming.
- The neutralization step was carried out by using sodium hydroxide solution of 15 °Bé.
- The bleaching step was carried out by using acid-activated bleaching earth Type B.

Table 4 shows that the DNB refining significantly decreased the PV of batch Y and batch Z to acceptable levels of 1.66 ± 0.00 meq/kg and 3.33 ± 0.00 meq/kg, respectively. However, the *p*-AV increased to 1.05 ± 0.06 (Batch Y) and 1.49 ± 0.02 (Batch Z). This may be attributed to the high temperature (95 °C) used during bleaching, which, according to Strieder et al. [33], can cause an increase in an oil's *p*-AV. Moreover, the catalytic properties of acid-activated bleaching earth may have decomposed the hydroperoxides and resulted in the formation of secondary oxidation products (such as aldehydes and ketones), and therefore, increased the *p*-AV [34]. This trend was also reported by Sánchez-Machado et al. [9], whereby the *p*-AV of MO kernel oil significantly increased upon refining. According to Rossell [35], an oil can be considered to be of good quality when its *p*-AV is below 10. These studies confirmed that the *p*-AVs of both DNB refining of MO kernel oil samples studied were acceptable, despite being higher than those of crude oil samples. A significant decrease was also observed in TOTOX values in the cases of Batch Y (4.38 ± 0.06 representing a 56.68% reduction) and Z (8.15 ± 0.02 representing a 40.86% reduction). As discussed earlier, these values are less than 10, and therefore considered to be acceptable. In comparison with other properties, the greatest decrease (99.57%) was observed in the acid values of both Batches Y and Z after treatment. The maximum acid value allowed for refined oil based on Codex Standards [14] is 0.6 mg KOH/g, while the acid value of DNB refined MO kernel oil obtained in this study was 0.03 ± 0.00 mg KOH/g, which was equivalent to 0.02% free fatty acid. This finding indicated a removal of 99.43% free fatty acid content from the oil. This free fatty acid content is also significantly lower than the BIS Standard Value stated to be 0.25%. The presence of excess free fatty acid and other fatty materials can cause an offensive odor and taste in an oil during longer term storage [36]. Therefore, lower free fatty acid content can enhance consumers' acceptability of the oil. A similar level of reduction (85.71%) in Batches Y and Z was also observed in the moisture contents, which, at 0.01 ± 0.00%, is lower than the BIS Standard Value (0.10%), and contributed to decreased acid value of the oil. Additionally, the removal of moisture and volatile matter from an oil is helpful in increasing its stability [27]. Significant reductions were also observed in the phosphorus content of both Batch Y (by 98.20%) and Batch Z (by 97.18%) to acceptable levels of 1.28 ± 0.10 ppm and 1.94 ± 0.32 ppm, respectively. This finding highlighted the need to combine different refining stages with degumming for greater removal of phospholipids. Lee et al. [22] and Yoon [25] also reported that most phospholipids were removed from camellia seed oil and chufa oil following degumming, neutralization, and bleaching.

The color properties of refined MO kernel oil (Batches Y and Z) are shown in Table 3, while Figure 1 displays the images of the oil samples. A significant increase ($p < 0.05$) in the L^* value and a decrease ($p < 0.05$) in the a^* value in both oil batches indicated improvement in their color properties from dark orange (crude) to light yellowish orange (DNB refined). This is most likely due to the use of phosphoric acid during degumming, caustic soda during neutralization, and bleaching earth during the bleaching stage, which were able to remove some of the coloring pigments and other impurities from the MO kernel oil [37]. To date, the types of pigment present in this oil have not been reported. Meanwhile, there was no significant change in the b^* value upon refining of Batch Y, while the value significantly decreased in the case of Batch Z.

3.3. Effect of DNB Refining on the Iodine Value and Fatty Acid Composition

The content of unsaturated fatty acids in an oil is indicated by iodine value (IV). In this study, it was found that the IV of Batches Y and Z did not change significantly after refining (Table 4) and ranged from 80.94–81.03. This shows that the DNB refining process employed did not change the degree of unsaturation of the oil. In an earlier study conducted by Mat Yusoff et al. [4], lower IVs of 54–65 g I_2/100 g of oil were reported. These differences may be due to the different oil extraction methods employed. In this study, the oil was cold-pressed from the kernels, while Mat Yusoff et al. [4] extracted the oil by using solvent and aqueous enzymatic methods. The oil oxidation rate may be affected by the solvents, enzymes, and extraction temperatures used, and this can further lead to differences in IVs [38].

Although there was no significant change in the IV, slight differences were observed in the fatty acid composition (FAC) of the two batches (Table 5). Batch Y showed no significant difference between all types of fatty acid, except for linoleic acid, which significantly increased upon refining. This change did not significantly influence the total saturated and unsaturated fatty acids in the oil. However, a significant decrease was observed in myristic, palmitoleic, and linoleic acids for Batch Z, while significant increases were observed in its palmitic and oleic acid contents. These changes resulted in a significant decrease in the total saturated fatty acids and a significant increase in the total unsaturated fatty acid. Table 5 also highlights oleic acid as the dominant fatty acid in batches Y and Z. However, the values obtained were higher (79.22–79.65%) as compared to those reported by Mat Yusoff et al. [4] in the case of crude MO kernel oil (~75%) and by Sánchez-Machado et al. [9] in the case of refined MO kernel (71–72%) produced using different refining stages of neutralization, degumming, and bleaching. Similarly, the total content of oleic, palmitic, stearic, and linolenic acids comprised approximately 98% of the total fatty acids in both Batches Y and Z, while only ~87% of these fatty acids were present in refined MO kernel oil as reported by Sánchez-Machado et al. [9]. According to Gulla and Waghray [39], different storage periods and initial conditions of the oil batches prior to analysis may contribute to these differences.

Table 5. Fatty acid and tocopherol compositions of *Moringa oleifera* (MO) kernel oil (Batches Y and Z).

MO Oil Composition	**Batch Y**		**Batch Z**	
	Crude	**Refined**	**Crude**	**Refined**
	Fatty acid composition (%)			
Lauric acid (C12:0)	0.13 ± 0.00 ^{a}	0.14 ± 0.01 ^{a}	0.13 ± 0.00 ^{a}	0.13 ± 0.01 ^{a}
Myristic acid (C14:0)	0.34 ± 0.09 ^{a}	0.33 ± 0.11 ^{a}	0.57 ± 0.09 ^{a}	0.29 ± 0.06 ^{b}
Palmitic acid (C16:0)	7.08 ± 0.02 ^{a}	7.14 ± 0.10 ^{a}	7.07 ± 0.01 ^{a}	7.14 ± 0.03 ^{b}
Palmitoleic acid (C16:1)	1.32 ± 0.08 ^{a}	1.25 ± 0.05 ^{a}	1.31 ± 0.01 ^{a}	1.26 ± 0.01 ^{b}
Stearic acid (C18:0)	6.91 ± 0.01 ^{a}	6.86 ± 0.05 ^{a}	6.91 ± 0.01 ^{a}	6.95 ± 0.06 ^{a}
Oleic acid (C18:1)	79.54 ± 0.00 ^{a}	79.52 ± 0.13 ^{a}	79.22 ± 0.10 ^{a}	79.65 ± 0.09 ^{b}
Linoleic acid (C18:2)	0.61 ± 0.01 ^{a}	0.70 ± 0.01 ^{b}	0.69 ± 0.00 ^{a}	0.61 ± 0.01 ^{b}
Arachidic acid (C20:0)	0.15 ± 0.01 ^{a}	0.17 ± 0.01 ^{a}	0.16 ± 0.01 ^{a}	0.16 ± 0.01 ^{a}
Linolenic acid (C18:3)	3.93 ± 0.01 ^{a}	3.92 ± 0.05 ^{a}	3.97 ± 0.01 ^{a}	3.86 ± 0.11 ^{a}
Total saturated fatty acids	14.61 ± 0.07 ^{a}	14.63 ± 0.08 ^{a}	14.83 ± 0.09 ^{a}	14.67 ± 0.04 ^{b}
Total unsaturated fatty acids	85.40 ± 0.08 ^{a}	85.38 ± 0.09 ^{a}	85.18 ± 0.09 ^{a}	85.34 ± 0.05 ^{b}
	Tocopherol composition (%)			
Alpha-tocopherol	34.27 ± 1.35 ^{a}	62.22 ± 0.55 ^{b}	30.80 ± 0.25 ^{a}	42.04 ± 0.84 ^{b}
Beta-tocopherol	2.29 ± 0.09 ^{a}	2.70 ± 0.35 ^{b}	2.21 ± 0.01 ^{a}	2.47 ± 0.34 ^{b}
Gamma-tocopherol	18.24 ± 0.66 ^{a}	20.07 ± 0.23 ^{b}	17.68 ± 0.03 ^{a}	18.79 ± 0.26 ^{b}
Delta-tocopherol	2.32 ± 0.05 ^{a}	1.96 ± 0.24 ^{b}	2.26 ± 0.01 ^{a}	1.95 ± 0.27 ^{b}
Total tocopherol content (mg/kg)	57.13 ± 2.14 ^{a}	87.00 ± 0.83 ^{b}	52.95 ± 0.27 ^{a}	65.26 ± 1.14 ^{b}

- Means ± standard deviation followed by the same letter between values of crude and refined MO kernel oils for each oil batch are not significantly different ($p > 0.05$) as determined by a two-sample *t*-test.
- The degumming step was carried out by using acid degumming.
- The neutralization step was carried out by using sodium hydroxide solution of 15 °Bé.
- The bleaching step was carried out by using acid-activated bleaching earth Type B.

3.4. Effect of DNB Refining on the Tocopherol Composition

Tocopherol composition is one of the important criteria to determine the quality of an oil. Changes in the concentration of each type of tocopherol, alpha, beta, gamma, and delta-tocopherol, upon refining, are shown in Table 5. Similar trends were observed in both oil batches, where all types of tocopherol significantly increased upon refining, except for delta-tocopherol, which significantly decreased. Therefore, in both oil batches, the total tocopherol content significantly increased after refining. Pestana et al. [40] reported significant increase in the alpha-tocopherol concentration in rice bran oil after the bleaching stage. According to Chew et al. [12], the concentration of gamma-tocopherol and the total tocopherol in kenaf seed oil significantly increased after degumming and bleaching. Rossi et al. [41] stated that the use of acid-activated clay during bleaching of palm oil could lead to the release or regeneration of tocopherol between its linked and free forms, depending on the clay concentration. This can explain the higher tocopherol content in the refined MO kernel oil as compared to the crude oil as observed in the current study.

4. Conclusions

The combination of acid degumming, neutralization with 15 °Bé NaOH, and bleaching with Type B (acid-activated) bleaching earth had effectively produced edible MO kernel oil with acceptable physicochemical and oxidative properties. Neutralization had a greater effect in reducing both the acid value and p-anisidine value of the oil than that of deodorization. This, in addition to its high oleic acid (~80%) and tocopherol content (65–87 mg/kg), contributed to high oxidative stability of the oil. Therefore, the incorporation of the refined MO kernel oil into food products may positively contribute to the product's quality, shelf life, and nutritional properties.

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