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Thermal Stability Studies of Moringa Olifera Seed Oil

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Moringa olifera seed oil (MOSO) is a pleasant smelling, golden yellow liquid with a yield of 40.00 ± 0.20 % and a specific gravity of 0.89 ± 0.01. The oil had an acid value of 17.54 ± 0.15 mg KOH/g, a free fatty acid value of 8.77 ± 0.30 %, an iodine value of 48.22 ± 0.11 g I_2/100 g oil, a peroxide value of 6.00 ± 0.01 mEq/kg, a saponification value of 257.36 ± 0.13 mg KOH/g, an ester value of 239.82 ± 0.37 mg KOH/g and a heat of combustion value of 8976.94 ± 1.19 gcal/g. The peroxide value, colour and smell of MOSO makes it desirable as an edible oil, while the acid and free fatty acid values do not. The thermal stability of this oil was investigated by studying its acid value, peroxide value and iodine value at 50 °C, 70 °C and 90 °C on an hourly basis for up to 8 hours. The acid value increased marginally, the peroxide value increased rapidly, while the iodine value decreased steadily as heating time and temperature increased. The results showed that heating time and temperature did not considerably affect the acid and iodine values. However, the oil was very prone to auto-oxidation at the temperatures under consideration. The study also showed that with appropriate treatment, this oil can be used for edible as well as industrial applications, including as feedstock for producing fuel.

Keywords: *Moringa olifera* seed oil (MOSO); properties; oil, thermal stability

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Fats and oils constitute an essential part of the human diet. Vegetable oils or vegetable fats are oils extracted from seeds, or from other parts of fruits [1]. Soybean oil, palm oil, rapeseed oil, and cocoa butter are examples of oils from seeds. Olive oil and rice bran oil are examples of oils from other parts of fruits. In common usage, "vegetable oil" and "fat" may refer exclusively to compounds called lipids. Fats are of animal origin and solid at room temperature, while oils are of plant origin and are liquid at room temperature [2].

Vegetable oils are basically edible lipids used in cooking, frying, baking, etc. From a nutritional point of view, fats and oils play an important role in the body as they are concentrated sources of energy that contain fat-soluble vitamins and essential fatty acids, as well as being carriers of flavour and many bioactive compounds necessary for physiological functions [3]. In fact, they are the third most important nutrient for the body, after carbohydrates and proteins [4]. In this, regard fat and oils are an integral component of healthy diets. However, with a rapidly increasing population, the issue of food security worsens on a daily basis and the estimated annual global requirement of 40 million tons of fats and oils becomes grossly inadequate. In Asia and Africa alone, an increase in population of about 2 billion is expected in the next decade [5], while the world population is expected to grow by 25 % in the next 30 years [6]. Despite all this, fats and oils must be consumed in sufficient concentrations to meet dietary requirements [7]. More worrisome is the fact that fats and oils are increasingly used in other industrial applications, leading to a further strain on their nutritional availability.

There are about 500,000 edible oil producing plants, with only a few being utilized for commercial edible and non-edible processing. In view of the existing global food insecurity, the increase in demand for fats and oils, and the food-fuel conflict of the commodity, new oil and fat sources must be explored. *Moringa olifera* is a fast-growing soft wood tree extensively found in the tropical and sub-tropical regions of Asia and Africa. The plant can also survive in drastic climatic conditions, such as prolonged droughts [8]. Studies show that while oil yields from soy beans and cotton seeds (the world's leading oil seeds) are only about 18-20 %, the *Moringa olifera* seed has an oil content of about 40 % [9]. The *Moringa olifera* seed is therefore a very sustainable source of vegetable oil. This plant can produce 3,000 kg of seed from 1 ha, from which about 1,200 kg of edible oil can be extracted, compared to soy bean plants which produce 350-400 kg of oil from 1 ha [10]. Furthermore, the *Moringa olifera* plant bears fruit within one year of cultivation and the cost of production of its seed oil is low compared to other oil sources.

Several studies on the extraction and characterization of *Moringa olifera* seed oil (MOSO) have been conducted [11, 12, 13], but studies focusing on the thermal stability properties of this oil are lacking. The thermal stability must be determined in order to regulate the edible and industrial usage of MOSO.

MATERIALS AND METHODS

1. Seed Collection and Oil Extraction

Moringa olifera seed samples were collected from Okeogun, Ondo state. The samples were collected between 19 and 21 December 2021 and preserved at 4 °C in a deep freezer prior to analysis. The seeds were removed from the pods, sorted, sun-dried and ground into a fine powder using a blender. Thereafter, oil was extracted from a 50 g sample by Soxhlet extraction for 8 hours at 50 °C with hexane as solvent [14]. The extract was dried using a rotary evaporator.

2. Physicochemical Characteristics

The colour and smell of the oil were determined by visual and olfactory observation. Percentage yield was obtained as the ratio of the weight of the oil extracted to the weight of the sample multiplied by 100. The specific gravity was determined according to Pearson (1980) [15], using 25 ml density bottles. The saponification value (SV), acid value (AV) and iodine value (IV) were determined using standard methods [16]. Peroxide value was determined by measuring the amount of iodine formed by the reaction of peroxide (formed in oil) with the iodide ion. Ester value (EV), heat of combustion (HC) and free fatty acid (FFA) values were determined by the following equations: EV = SV- AV; HC= 11380- (IV) - 9.15(SV); FFA = 0.503(AV) [17, 18, 19].

3. Thermal Stability Studies

Peroxide, acid and iodine values are primary detectors of oxidation in oils and thus changes in these values are used to check the thermal stability of an oil. The stability of an oil indicates how resistant it is to changes in acid, peroxide and iodine values on heating and exposure to heat and air over a long period of time. Therefore, the thermal stability study was carried out by determining the peroxide, acid and iodine values of the oil hourly for 8 hours, at 50 °C, 70 °C and 90 °C.

4. Statistical Analysis

All experiments were carried out in triplicate and a one way analysis of variance (ANOVA) was carried out to assess the significance differences for each set of data obtained. The mean of the data was compared using SPSS (Statistical Package for Social Scientists). A significance level of $p < 0.05$ indicates that the data did not differ significantly.

RESULTS AND DISCUSSION

1. The Physicochemical Properties of MOSO

The physicochemical properties of MOSO are presented in Table 1. The extract was a pleasant-smelling liquid at room temperature, thus falling into the classification of oils and also indicating that it has some level of unsaturation. Generally, the edibility of oils is determined by their physical appearance and smell [20]. Edible oils are usually canary yellow or yellow in colour [21]. The golden yellow colour and pleasant smell makes it desirable as an edible oil. Similar studies [11, 22] have also recorded the same colour.

Oil content is a key determinant of the viability of an oil source, which is also used in its classification as oil-bearing. MOSO had a yield of 40.00 ± 0.20 % in this study, which makes it a viable oil source. The yield obtained is higher than that of many oil-bearing seeds such as *M. concanensis* (38.82 % yield) [23] and oil palm seeds (28 - 33.6 % yield) [24;25]. The yield is also within the range for coconut (31.28 – 42.37 %) [26]). A lower yield (34.86 %) for a different variety of moringa seeds (*Moringa ovalifolia*) was obtained in a similar study [11]. However, higher values have been reported elsewhere, e.g., 49.8 % for *M. peregrina* seeds [27], 41.47 % for MOSO [12] and 50.1 % for peanuts [28]. This variation in oil yield could be attributed to differences in crop type, seed species and extraction methods or solvent.

The specific gravity of MOSO was 0.89. This value is lower than those reported for some vegetable oils [29] and below the 0.919-0.925 range, as advised by FAO/WHO for edibility [30]. However, the specific gravity was comparable to values (0.889 - 0.899) obtained for hayat oil [31]. The low specific gravity obtained for MOSO could be attributed to the presence of low linoleic acid content, because previous studies associated high specific gravity levels to the presence of linoleic acid [31]. Linoleic acid consumption improves insulin sensitivity and supports a healthy heart [32]. Therefore, the low specific gravity value obtained in this study suggests that MOSO oil has a lower tendency to improve insulin sensitivity and support heart health.

The acid value of an oil gives an indication of its deterioration, rancidity, or edibility. It measures the amount of free fatty acids present in the oil and the quantity of KOH required to neutralize the Free Fatty Acids (FFAs) in a sample. The acid value obtained for MOSO (17.54 ± 0.15 mg KOH/g) in this study was very high compared to the 2.12 ± 0.15 mg KOH/ g [11] and the 3.80 ± 0.28 mg KOH/g [12] recorded in similar studies. This value is also higher than the acceptable standard (< 4.0 mg KOH/g) for edible oils [33]. FFA values, like the acid value, followed the same trend. The FFA value (8.77 %) obtained in this study is higher than the accepted standard of 5 % for edible oils [34; 35]. A high FFA value indicates a higher tendency for the oil to become rancid [36]. A high acid value may be due to a hydrolytic reaction during processing, or to enzymatic action. However, alkali refining can can be used to achieve the desired acid content [37; 38], otherwise the oil is unsuitable for edible applications.

Table 1. Physicochemical properties of the MOSO

Characteristics	*Moringa olifera* oil
State	Liquid
Colour	Golden yellow
Odour	Pleasant
Percentage yield (%)	40.00 ± 0.20
Specific gravity	0.89 ± 0.01
Acid value (AV, mg KOH/g)	17.54 ± 0.15
Free Fatty Acid (FFA, % oleic acid)	8.77 ± 0.30
Iodine value (IV, gI_2/100g oil)	48.22 ± 0.11
Peroxide value (PV, meqO_2/kg)	6.00 ± 0.01
Saponification value (SV, mg KOH/g)	257.36 ± 0.13
Ester value (EV, mg KOH/g)	239.82 ± 0.37
Heat of Combustion (HC, gcal/g)	8976.94 ± 1.19

Results are expressed as the mean of triplicate determinations

The iodine value (IV) is an indication of the degree of unsaturation of an oil. It quantifies the amount of iodine in grams that will saturate 100 grams of the oil or fat. A high iodine value is attributed to high unsaturation. IV also measures the drying properties of an oil, and is used to classify oils as drying (IV > 150 g I_2/100g), semi-drying (IV within 100 - 150 g I_2/100g) and non-drying (IV < 100 g I_2/100g) [18, 19, 39]. This information determines the ability of an oil to form a solid film on exposure to air. MOSO recorded a low iodine value of 48.22 ± 0.11 g I_2/100 g, suggesting it is a non-drying oil with low levels of unsaturated fatty acids. The low iodine value of MOSO indicates few unsaturated bonds, which in turn means that the oil is more stable and less prone to oxidative rancidity. The iodine value recorded for MOSO in this study was significantly lower than the 65.58-69.45 g I_2/100 g [22] and 65.70 g I_2/100 g [40] reported in similar studies of MOSO. The low iodine value of MOSO suggests that the oil is not suitable for ink and paint production due to its non-drying properties, but could be useful for other applications such as in the manufacture of soaps, body creams, cosmetics, plasticizers and lubricants.

The peroxide value (PV) is used to determine the extent to which an oil can go rancid during storage, heating or oxidation. The PV (6.00 ± 0.01 mEq/kg) of MOSO obtained in this study indicates that the oil was fresh and resistant to oxidation. The Standard Organization of Nigeria (SON) recommends peroxide values of less than 10 mEq O_2/kg for edible oils [41] and that a rancid oil has peroxide values of 10 - 20 mEq O_2/kg oil. The peroxide value for MOSO, being below 10 mEq O_2/kg, makes it desirable as an edible oil and less prone to rancidity [19]. Elsewhere, lower PVs have been recorded for MOSO, such as 0.24 mEq/kg, 1.15 mEq/kg [42] and 1.27 mEq/kg [43]. The differences between these values could be attributed to variations in the extraction method and geological origin of the seeds.

The saponification value (SV) gives an indication of the wholesomeness, or the contamination level of an oil [24]. It also measures the molecular weight of the fatty acids in the oil. Low SVs indicates high molecular weight fatty acids in the glycerides or a low number of ester bonds [31]. The high SVs (257.36 ± 0.13 mg KOH/g) obtained in this study suggest that the oil had a low molecular weight fatty acid content with little impurities and would be suitable for making cosmetics and hard soaps with good lathering properties.

The ester value (EV) quantifies the mass of potassium hydroxide required to saponify the esters contained in 1 g of oil. In this study, the EV obtained (239.82 ± 0.37 mg KOH/g) was high. Similar studies on vegetable oil recorded lower EVs, e.g., castor oil (174.09 mg KOH/g), avocado pear oil (172.8 mg KOH/g) [35], groundnut oil (173.90 mg KOH/g) [44] and soybean oil (188.02 mg KOH/g) [45]. The high EV of MOSO implies that it contains more esters than fatty acids.

Heat of combustion (HC) is the heat released when a compound undergoes complete combustion in air under standard conditions. It is calculated based on energy released per unit mass. A high heating value is an essential property of fuel because it relates to fuel economics and efficiency. A fuel with a higher energy density generates more energy per volume [46]. The heat of combustion value (HC) (8976.94 ± 1.19 gcal/g) for MOSO obtained in this study was high and within the range (8904.25 – 11303.35 gcal/g) reported for edible oils [18] with potential as feedstock for producing fuel [22]. The thermal stability properties of MOSO in terms of acid and peroxide values are recorded in Tables 2 - 4 and Figures 1 and 2, while iodine values are presented in Figure 3.

Table 2. Effect of heating time on acid and peroxide values on MOSO at 50 °C

Time (hrs)	Acid value (mg KOH/g)	% increase in Acid value	Peroxide value (mEq/kg)	% increase in Peroxide value
0	17.54 ± 0.30 [a]		6.00 ± 0.10 [a]	
1	18.17 ± 0.12 [b]	3.59	7.00 ± 0.00 [b]	16.67
2	18.35 ± 0.05 [b]	4.62	8.00 ± 0.12 [c]	33.33
3	19.00 ± 0.33 [c]	8.32	9.12 ± 0.02 [d]	52.00
4	19.52 ± 0.02 [d]	11.26	10.00 ± 0.03 [e]	66.67
5	20.18 ± 0.21 [e]	15.05	10.50 ± 0.05 [f]	75.00
6	20.35 ± 0.04 [e]	16.02	10.90 ± 0.10 [g]	81.67
7	20.95 ± 0.05 [f]	19.44	11.30 ± 0.15 [h]	88.33
8	21.21 ± 0.10 [f]	20.92	12.00 ± 0.76 [i]	100

Results are expressed as the mean of triplicate determinations. The superscripts a, b and c represent statistical significance. Values with the same superscript letters on the same column do not differ significantly at $p < 0.05$.

2. The Effect of Heating Time on the Acid and Peroxide Values of MOSO

Table 2 shows the effect of heating time on the acid and peroxide values of MOSO at 50 °C. From these results, it is clear that the acidity of the oil increased slowly with heating time by 3.59 % in one hour to 20.92 % after eight hours. The increase in AV as heating progressed is also a sign of deterioration. Previous studies indicated that high acidity in oils was related to off-flavours and a short shelf life [47]. The slow rate at which AV increased with heating time suggests this oil is relatively stable in terms of acid value.

The peroxide value of the oil increased rapidly with heating time, from an initial value of 6.00 ± 0.10 to 12.00 ± 0.76 (a 100 % increase) after 8 hours of heating. The rapid increase in PV could be attributed to the fact that the heating temperature was less than 150 °C. Only minor increases in PVs were observed during frying at elevated temperatures because the hydroperoxides of lipids decompose rapidly in these conditions [48].

Table 3. Effect of heating time on the acid and peroxide values of MOSO at 70 °C.

Time (hrs)	Acid value (mg KOH/g)	% increase in acid value	Peroxide value (mEq/kg)	% increase in peroxide value
0	17.54 ± 0.30 [a]		6.00 ± 0.01 [a]	
1	18.43 ± 0.02 [b]	5.07	7.50 ± 0.02 [b]	25.00
2	18.55 ± 0.01 [bc]	5.76	9.32 ± 0.01 [c]	55.33
3	18.68 ± 0.02 [c]	6.50	10.00 ± 0.01 [d]	66.67
4	19.19 ± 0.03 [e]	9.41	11.00 ± 0.00 [e]	83.33
5	20.12 ± 0.21 [e]	14.71	12.00 ± 0.00 [f]	100.00
6	21.71 ± 0.01 [f]	23.77	13.00 ± 0.12 [g]	116.67
7	22.22 ± 0.02 [g]	26.68	14.00 ± 0.76 [h]	133.33
8	22.74 ± 0.01 [h]	29.65	16.00 ± 0.20 [i]	166.67

Results are expressed as the mean of triplicate determinations. The superscripts a, b and c represent statistical significance. Values with the same superscript letters in the same column do not differ significantly at $p < 0.05$.

Table 4. Effect of heating time on the acid and peroxide values of MOSO at 90 °C

Time (hrs)	Acid value (mg KOH/g)	% change in acid value	Peroxide value (mEq/kg)	% change in peroxide value
0	17.54 ± 0.30 [a]		6.00 ± 0.01 [a]	
1	19.19 ± 0.01 [b]	9.41	9.00 ± 0.00 [b]	50.00
2	19.57 ± 0.02 [c]	11.57	10.00 ± 0.02 [c]	66.67
3	20.45 ± 0.05 [d]	16.59	15.00 ± 0.05 [d]	150.00
4	20.83 ± 0.03 [e]	18.76	16.00 ± 0.02 [e]	166.67
5	21.96 ± 0.02 [f]	25.20	16.45 ± 0.01 [f]	175.00
6	22.21 ± 0.03 [g]	26.62	17.00 ± 0.01 [g]	183.33
7	22.72 ± 0.02 [h]	29.53	18.00 ± 0.06 [h]	200.00
8	22.97 ± 0.07 [i]	30.96	20.00 ± 0.02 [i]	233.33

Results are expressed as the mean of triplicate determinations. The superscripts a, b and c represent statistical significance. Values with the same superscript letters on the same column do not differ significantly at $p < 0.05$.

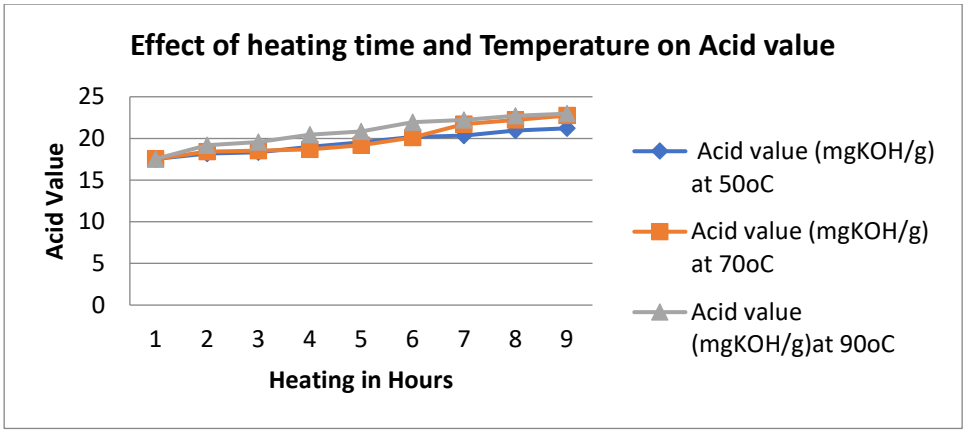

Figure 1. Effect of heating time and temperature on the acid value of MOSO.

Similar trends were also observed in the properties of MOSO at 70 °C and 90 °C for AV and PV. The results show that MOSO was thermally stable in terms of acid value but the peroxide values were significantly different from the control.

3. The Effect of Heating Time and Temperature on the Acid Value of MOSO

Fig. 3.1 shows the effects of heating time and temperature on the AV of MOSO. From the plot it is observed that the AV increased with heating time at each temperature. On heating for the first one hour at all temperatures, the AV increased sharply, after which a slow and steady increase continued for up to eight hours. The rapid rise in AV in the first hour of heating may be due to the oil breaking down into glycerol and free fatty acids at the first instant of a temperature increase. Further, there may be an increase in the mobility of the fatty acids in the oil as it is being heated. The AV test is one method to estimate how susceptible an oil is to thermal changes and oxidation. It is however important to note that the acid value does not directly measure the rate of oxidation; it only measures the by-products of oxidation. In this study, the AV and consequently the Free Fatty Acid (FFA) content of the oil sample generally increased with temperature and heating time.

A very useful way to determine the extent to which oxidation is taking place in an oil sample is by measuring the PV. It is the best test for auto-oxidation. PVs of fresh oils are usually below 10 mEq/kg. PVs between 20 and 40 mEq/kg are usually a pointer that the oil has started to turn rancid [49]. As shown in Figure 2 above, the peroxide value of the MOSO sample increased with heating time and temperature.

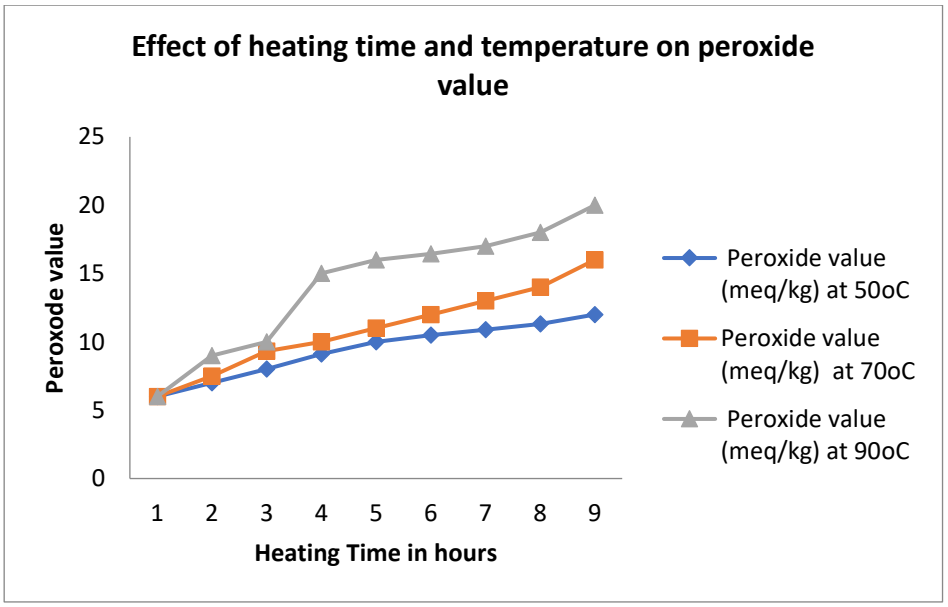

Figure 2. Effect of heating time and temperature on the peroxide value of MOSO

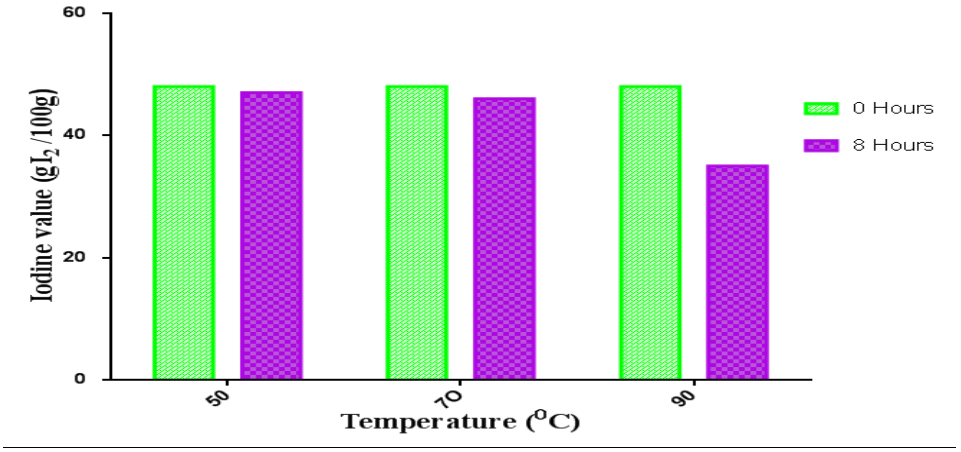

Figure 3. Effect of heating time and temperature on iodine value of MOSO

Fig. 3.3 shows the effect of heating time and temperature on the IV of MOSO. From the graph it is observed that for all the temperatures considered, the IV decreased as the heating time increased. The decrease was more pronounced at 90 °C than at 50 °C and 70 °C.

When the oil was heated at 50 °C for 8 hours, the IV decreased by only 1.31 % (47.59) from its initial value of 48.22. Also, decreases of 3.96 % (46.31) and 26.32 % (35.53) at 70 °C and 90 °C respectively were observed. This observation is consistent with a previous study [51], where the deep frying of different food types reduced their IVs. In that study [51], deep frying at 160 - 190 °C for six hours reduced the IVs of the most stable vegetable oil (peanut oil) by values ranging from 4.9 – 7.4 g I_2/100 g. Reduction in IV is an indication of the deterioration of an oil caused by decreasing unsaturation, attributed to oxidation and hydrolysis occurring concurrently in the oil, as heating progresses [51]. The slight changes in IV recorded in this study suggest that MOSO is stable and less prone to oxidative rancidity.

CONCLUSION

This study determined the physiochemical and thermal stability properties of *Moringa olifera* seed oil. Its physicochemical properties did not all fall within the recommended edibility value range, thus refining is required for the oil to be used in both edible and industrial applications. Thermal stability studies suggest that the oil was stable in terms of acid and iodine values when heated at 50 °C, 70 °C and 90 °C for as long as eight (8) hours. However, the oil was highly prone to auto-oxidation at this temperature range as its peroxide value was found to increase rapidly.

In view of the current global food insecurity, it is recommended that further studies are done on the thermal stability of MOSO at a wider temperature range so as to give more robust information on its edible and industrial potential. More information should also be obtained on the edibility properties of other parts of different species of the plant, to determine whether they contain any toxic components.

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