BNR Online ISSN: 2709-7374

Received: 18 July 2023 | Revised: 19 March 2024 | Accepted: 11 July 2024

ARTICLE

Quality Parameters of Seed Oil of *Moringa oleifera* Lam. Grown in Gaborone, Botswana

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Abstract

This study was conducted to characterise the physicochemical properties of the seed oil of *Moringa oleifera* grown in Gaborone, Botswana. The Moringa seeds used for oil extraction were collected from the backyards of different households in Gaborone city. The cold press technique was used to extract oil from Moringa seeds. Quality parameters of Moringa seed oil were compared with a commercial virgin olive oil using the student's T-test. The Moringa seed oil had an average refractive index, density, acid value, peroxide value and saponification value of 1.4675 ± 0.000, 0.9085 ± 0.001(g/cm^3), 0.670 ± 0.313 (mg KOH/g), 0.15 ± 0.710 (mEq O_2/kg) and 134.08 ± 7.140 (mg KOH/g), respectively. The Moringa oil had comparable physicochemical characteristics with virgin olive oil except for refractive index, peroxide value and colour. The results showed that the values for the physicochemical parameters of *M. oleifera* seed oil fall within the recommended limits for edible oils. This suggests that *M. oleifera* seed oil grown in Botswana could potentially be used as edible oil for human consumption.

Keywords: *Moringa oleifera*; Moringa oil; Gaborone; quality parameters; physicochemical properties; potential application

Introduction

The genus Moringa consists of 13 species of which *Moringa oleifera* Lam (hereafter referred to as Moringa) is the most widely distributed and utilised species (NRC 2006). Moringa belongs to the family Moringaceae and is a native plant from India, south of the Himalayan Mountain (Fuglie 1999). Moringa is a multipurpose and fast-growing tree that tolerates a wide range of soils and rainfall conditions. It is now extensively cultivated and has become naturalised in many locations in the tropics (Fahey 2005) and worldwide. Moringa tree is the most popular underutilised tropical crop being one of the most useful trees in the world, with a huge potential and benefits. Various parts of the plant namely the leaves, roots, stems and the seeds are used for different purposes. Some of the benefits of Moringa include its medicinal value, also consumed as vegetable, and used as forage for livestock. The seeds are used as a source of oil, which can be used for cooking, illumination and lubrication of delicate mechanisms. They are also used for water purification, as well as in pharmaceutical and cosmetic industries (HDRA- the organic organisation 2002).

Almost all parts of the Moringa tree have been utilised for various purposes. The leaf, flower, fruit, bark and roots have been used as traditional medicine for treatments of various aliments (HDRA 2002). The leaf is consumed as green vegetable and a good source of essential minerals and vitamins. The seed powder has a strong coagulation activity and used for purification of wastewater (HDRA 2002). Also, its seed extract is reported to exhibit antimicrobial properties (Eilert *et al.* 1981).

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The oil extracted from Moringa seeds is highly edible and used for cooking and in salad dressings. Moreover, the oil is used for lubricating watches and other delicate machinery as well as in the manufacture of perfumes and cosmetics (HDRA 2002). Other uses of Moringa include its use as cleaning agent, fertilizer, gum, honey clarifier, natural pesticide, and pulp for paper. It is also used in alley cropping, honey production, fodder for livestock, soil conservation, making rope and tannin for tanning hides (Fuglie 1999; HDRA 2002).

Although the exact date of introduction of Moringa to Botswana is not known, it is generally believed that it was introduced recently (2003) to the country (Kwaambwa *et al*. 2012; Seifu and Teketay 2020). Despite its recent introduction, Moringa is widespread throughout the country, including Gaborone areas. It is now common to see Moringa plant in the backyards of many households in the city (Kwaambwa *et al*. 2012; Seifu and Teketay 2020). It was reported that Moringa was introduced into Botswana from different places, in particular, from India, Netherlands, Malawi, Tanzania and Kenya (Nduwayezu *et al*. 2007).

As aforementioned, edible oil can be extracted from moringa seeds (HDRA 2002). Edible oils are prone to physicochemical changes that alter the quality and subsequently influence the nutritional value and acceptability of the oil (Zhang *et al.* 2021). Factors such as high temperature, light, oxygen and metals influence the quality of oils and may alter the quality of oils and result in reduced shelf-life of oils, production off-flavours and toxins that may be harmful to the consumers (Zhang *et al.* 2021; Flores *et al.* 2021. Several analytical methods are available to measure, monitor and assess the quality and stability of oils such as peroxide value, iodine value, acid value, density and refractive index (Flores *et al.* 2021). The acid value (AV) is a common parameter in the specification of fats and oils and defined as the weight of potassium hydroxide (KOH) in mg needed to neutralise the organic acids present in 1g of fat and is a measure of the free fatty acids (FFA) present in the fat or oil (Cuppett 2001; Flores *et al.* 2021). The acid value indicates how much the oil has been broken down by lipases or other factors and it is hastened by heat and light (Flores *et al.* 2021). The higher the AV, the higher the level of FFAs, which translates into decreased oil quality (Kabutey *et al*. 2022).

High levels of FFAs cause rancidity as well as changes in the taste and colour of the oil (Kabutey *et al*. 2022). The peroxide value (PV) of a fat reflects the degree of its oxidation taking place (Bockisch 1998). A high peroxide value is an indicator of oxidation level, and the greater the peroxide value, the more oxidized the oil is (Kabutey et al. 2022).Oxidation of oils may cause undesirable flavours and taste, decomposing the nutritional quality and leading to production of toxic compounds (Flores *et al.* 2021; Zhang *et al.* 2021). Oxidation of oils may be influenced by different factors, such as the degree of unsaturation, heat, light, oil processing, antioxidants and transition metals (Halvorsen and Blomhoff 2011). The saponification value is the amount of alkali required to saponify a definite quantity of fat or oil (Bockisch 1998) and provides evidence on the relative chain lengths of the fatty acids in the system (Cuppett 2001; Flores *et al.* 2021). It indicates the mean molecular weight of the fatty acids in a lipid system. Refractive index provides a relationship between the speed of light in a vacuum and the light in oil at specific temperature (20 to 25°C for oils and 40°C for fats) which serves as an indicator for oils and fats purity and quality (Flores *et al.* 2021). Relative density measures the molecular weight of oils at given temperature as the ratio of the mass in air of a given volume of the oil or fat at a specific temperature to that of the same volume of water (Flores *et al.* 2021). Water has a density of 1,000 kg/m^3. Since the density of oils is always smaller than that of water, they all float on the surface (Ichu and Nwakanma 2019).

Despite the multipurpose uses of *M. oleifera* elsewhere in the tropics, it seems that people in Botswana are not fully aware about the potential uses of Moringa, in particular, the use of Moringa seeds for oil production. To date, no research has been conducted on the oil production potential and characteristics of the seed oil of Moringa grown in Botswana. The

objective of this study was therefore, to characterise the physicochemical properties of the seed oil of Moringa grown in Gaborone Botswana.

Materials and Methods

Collection of moringa seed samples

Moringa seeds were collected from households in Gaborone which grow Moringa trees in their backyards, and the seeds were transported to the laboratory of the Department of Food Science and Technology at Botswana University of Agriculture and Natural Resources (BUAN).

Only seeds in dry fruits were used for the study. The seeds were removed from the fruits, sorted and dried at room temperature until extraction. Only seeds that had not been damaged were chosen and stored under cool dry storage conditions until used.

Oil extraction

The oil was extracted using an electric/hydraulic oil press (made by John Marshall Company of South Africa) at the National Food Technology Research Centre (NFTRC) in Kanye, Botswana. Moringa kernels weighing 3.1 kilograms were placed into the receiving cage of the hydraulic presser until half full and then, pressed at a pressure of 550/ 600 bar. The oil was allowed to drip from the cage onto the tray and collected through the collection pipe into a transparent glass jar giving an oil yield of 211 ml.

The oil extracted was stored in a refrigerator at 4˚C until analysis. Quality parameters of the Moringa seed oil, namely refractive index, density, acid value, saponification value, peroxide value and colour, were compared with virgin olive oil (Santa Bianca, product of Spain), which was used as a control.

Determination of physicochemical characteristics of the oil

Refractive index

Refractive index of the oil was determined according to Olaleye *et al*. (2018) as follows. A refractometer (J257 Refractometer, Hackettstown, USA) was used for the determination of the refractive index of the oil.

The refractometer was calibrated with distilled water which has refractive index of 1.3330 at 20^0C. A drop of Moringa seed oil was placed on the prism. The prism was closed firmly, and the instrument was allowed to stand for a few minutes after which the reading was taken.

Relative density

The relative density of the oil was determined according to the method of Food Safety and Standards Authority of India (FSSAI) (2015). A dry, empty pycnometer was weighed using an analytical scale, after which the pycnometer was filled with freshly distilled water. A stopper was inserted in such a way that the capillary was completely filled with water and was immersed in a water bath at 100°C for one hour. The pycnometer was removed from the bath, dried outside and then weighed.

The same procedure was repeated with a test portion (oil) and the weight recorded. The relative density was calculated using the following formula:

$$RD = A - C \,/\, B - C$$

where, RD = relative density; A, B and C are weights in grams of specific pycnometer bottle with oil, water and empty pycnometer, respectively.

Acid value

The acid value was determined according to FSSAI (2015). A 10 gram (g) of Moringa seed oil was weighed in a 250 millilitre (ml) conical flask, and a 150 ml of ethanol was added to it along with about 1 ml of phenolphthalein indicator solution. The solution was put to boil for about five minutes and titrated whilst hot against standard alkali solution (0.1N sodium hydroxide), shaking vigorously during the titration. The acid value was calculated using the following formula:

$$\text{Acid value} = \frac{56.1xVxN}{W}$$

where, V = volume in ml of standard sodium hydroxide used, N = normality of the sodium hydroxide solution; and W = weight (g) of the sample.

Saponification value

The saponification value was determined according to FSSAI (2015). Moringa seed oil was thoroughly mixed, and 2 g of sample was weighed into a 250 ml flask. 25 ml of alcoholic potassium hydroxide solution (0.4N ethanolic potassium hydroxide) was poured into the flask. The sample flasks and the blank flask were put under reflux for an hour using a Soxhlet extraction unit, steadily heating until saponification was complete, as indicated by absence of any oily matter and appearance of clear solution. After the flask and condenser had cooled, 1 ml of phenolphthalein indicator was added, and the excess potassium hydroxide was titrated with 0.5N hydrochloric acid. The saponification value was calculated using the following formula:

$$\text{Saponification Value} = \frac{56.1(B-S)N}{W}$$

where B = volume (in ml) of standard hydrochloric acid required for the blank, S = volume (in ml) of standard hydrochloric acid required for the sample, N = normality of the standard hydrochloric acid and W = weight (g) of the sample.

Peroxide value

The peroxide value of the oil samples was determined according to Bockisch (1998). Approximately 2 ml of oil sample was added to a conical flask in which 1 g of potassium iodide was added along with 10 ml of chloroform and 10 ml glacial acetic acid. The mixture was shaken for about a minute then placed in a dark cupboard for 5 minutes at room temperature. After 5 minutes, 0.5 ml starch indicator was added. Then, 25 ml of deionised water was added, and the contents were titrated against 0.01 N sodium thiosulphate until the yellow colour almost disappeared. A blank test was run. The same procedure was used for olive oil. The peroxide value was calculated using the following formula:

$$PV = \frac{[(S-B)\ x\ N\ x\ 100]}{m}$$

where, S = volume (ml) of standard sodium thiosulphate required for the sample, B = volume (ml) of standard sodium thiosulphate required for the blank, N = normality of the sodium thiosulphate solution and m = weight (g) of the sample.

Colour

Ten ml of Moringa oil was poured into a small beaker. The beaker was placed on a white paper and a Minolta colorimeter (CR-400, Japan) was used to measure the colour, taking the readings in terms of (L*a*b*). The same procedure was followed for olive oil. Colour was expressed as follows: L* (dark-light), a* (green-red) and b*(blue-yellow).

Statistical analyses

Quality parameters of Moringa seed oil were compared with that of virgin olive oil using student's T-test using Statistical Package for Social Sciences (SPSS) and significant difference was declared at 5% significance level ($p < 0.05$).

Results and Discussion

Physicochemical parameters

There was no significant difference ($p>0.05$) in acid value between Moringa seed oil and olive oil (Table 1). The acid value of Moringa seed oil observed in the present study is lower than values reported by Tsaknis *et al*. (1999) (1.01 mg KOH/g oil), Anwar and Rashid (2007) (0.81 mg KOH/g oil) and Rahman *et al*. (2009) (0.98 mg KOH/g oil). However, it is higher than the value (0.40 mg KOH/g oil) reported by Anwar and Bhanger (2003). According to Ramadan (2019), high acid value indicates oil with reduced quality and, thus, the acid value is considered as an important indicator of the quality of vegetable oils. Anwar and Rashid (2007) also indicated that oils with lower values of acidity are more acceptable for edible applications. The present results showed that the Moringa seed oil had an acid value within the recommended limit. According to CODEX STAN 19-1981 (Rev. 2-1999), the maximum level of acid value for virgin oils and cold pressed fats and oils is up to 4.0 mg KOH/g fat or oil. The acid value observed in the present study for Moringa seed oil conforms to this standard.

The acid value (AV) is defined as the weight of KOH in mg needed to neutralise the organic acids present in 1 g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil (Kabutey *et al*. 2022). An increment in the amount of FFA in a sample of oil or fat indicates enzymatic hydrolysis of triglycerides and it indicates the quality and age of the fat, with higher AV showing more hydrolysis of triglycerides over time from factors like heat or moisture (Kabutey *et al*. 2022). Free fatty acids or acid value measures the amount of free acid groups existing in fat/oil systems. It can reflect total acidity (acid value) or the level of fatty acids that are free, i.e. not attached to a glycerol backbone (Cuppett 2001). Free fatty acids are not desirable in edible oils because when oils with high FFA content are used in foods, they reduce the oxidative stability of the product, increase acidity and lead to off flavour formation (Dunford 2016).

The FFA content of the oil has implications during processing of foods. In deep-fat frying, the FFA content of the oil is critical for maintaining final product quality and shelf life. In addition, as the FFA content increases, the oil's smoke and flash points drop, and without proper monitoring, the oil could produce excessive smoke and eventually ignite (flash) into flames in the processing arena (Cuppett 2001). When people consume foods that contain high levels of free fatty acids it will result in increased levels of low-density lipoproteins and lower blood levels of high-density lipoproteins, reducing the body's ability to control blood sugar because it can reduce the response to the hormone insulin (Febrianto *et al*. 2019). Ideally frying oil FFA content should not be greater than 2% (Dunford 2016).

Table 1: Physicochemical properties of *Moringa oleifera* seed oil in comparison to virgin olive oil

Parameters	Moringa oil	Virgin olive oil
Acid value (mg KOH/g)	0.670 ± 0.313	0.785 ± 0.448
Peroxide value (mEq O_2/kg)	0.15^a ± 0.710	1.58^b ± 0.106
Saponification (mg KOH/g)	134.08 ± 7.140	140.81 ± 0.793
Refractive index (20°C)	1.4675^a ± 0.000	1.4688^b ± 0.000
Relative density (g/cm^3)	0.9085 ± 0.001	0.9090 ± 0.001
Color (b* values)	23.44^b ± 0.346	15.96^a ± 0.120

Values in the table are expressed as means ± standard deviations; ^{ab}Values with different superscript letters in a row are significantly different ($p \leq 0.05$).

The oxidative stability of the oil in the present study was determined by measuring the peroxide value. Rancidity of food items can be the result of auto and photo-oxidation, which are natural oxidation and chemical degradation processes of edible oils, where fatty acid esters of oils are converted into free fatty acids giving off odour observed in many vegetable oils (Anwar and Bhanger 2003). A rancid taste is often noticeable when the PV is between 20 and 40 mEq O_2kg^{-1} of oil (Ekwenye 2006) and high peroxide value, usually, means poor flavour ratings (O'Brien 2008). The peroxide value of Moringa seed oil observed in the current study was significantly lower ($p<0.05$) than that of olive oil (Table 1). The observed Moringa oil peroxide value is similar to the value (0.15 meq O_2kg^{-1}) reported by Rahman *et al*. (2009). However, it is lower than the values reported by Tsaknis *et al*. (1999) (0.36 mEq O_2kg^{-1}), Anwar and Rashid (2007) (1.27 mEq O_2kg^{-1}) and Anwar and Bhanger (2003) (0.59 mEq O_2kg^{1}) for Moringa seed oil.

According to CODEX STAN 19-1981 (Rev. 2-1999), the maximum peroxide value for virgin oils and cold pressed fats and oils is up to 15 milli equivalents of active oxygen/kg oil. So, the calculated peroxide value observed in the present study conforms to this standard. The lower peroxide value of the Moringa seed oil observed in the present study shows its high resistance to oxidation. The high oxidative stability of Moringa oil exhibited in the present study, as witnessed by the low peroxide value, could be attributed to a significantly higher level of monoenoic fatty acids, particularly oleic acid, which is less prone to oxidation than polyonics as suggested by Anwar and Bhanger (2003). It could also be attributed to high contents of α-, γ- and δ-tocopherols (Anwar and Bhanger 2003).

The saponification value of Moringa oil examined in the current study was lower than that of olive oil although the difference was not statistically significant ($p>0.05$) (Table 1). The saponification value of Moringa oil observed in the current study is lower than values reported by Tsaknis *et al*. (1999) (179.80 mg of KOH/g of oil), Anwar and Rashid (2007) (181.4 mg of KOH/g of oil), Anwar and Bhanger (2003) (186.67 mg of KOH/g of oil) and Rahman *et al*. (2009) (187 mg of KOH/g of oil). This difference may be due to factors, such as differences in chemical composition and/or experimental procedures. The smaller the saponification value, the longer the fatty acids on the glycerol backbone while a high value indicates shorter fatty acids (Flores *et al.* 2021; Cuppett 2001). As the chain length increases there is a decrease in saponification value (Flores *et al.* 2021, Cuppett 2001). Aurand *et al*. (1987) reported that high saponification values of fats and oils are due to the predominantly high proportions of shorter carbon chain lengths of the fatty acids. This assertion was confirmed by Nielson (1994) who reported that the smaller the saponification value, the longer the average fatty acid chain. If the fatty acids present in the glycerides are of low molecular weight (short-chain acids), there will be more glyceride molecules per gram of fat than if the acids are higher in molecular weight (long-chain acids). Thus, since each glyceride molecule requires three potassium hydroxide

molecules for saponification, fats containing glycerides of low molecular weight correspondingly have higher saponification values.

The refractive index of Moringa seed oil was significantly lower ($p<0.05$) than that of virgin olive oil (Table 1). However, its refractive index is higher than the values reported by Anwar and Bhanger (2003) (1.4608), Anwar and Rashid (2007) (1.4571), Tsaknis *et al*. (1999) (1.4591) and Rahman *et al*. (2009) (1.459). According to Ariponnammal (2012), refractive index is an important optical parameter used to determine adulteration of oil. The refractive index of vegetable oils was one of the most used specifications for quality of oil. It provides a useful guide to the degree of unsaturation of the oil and is often accompanied by the chemically determined iodine value or iodine number (Simpson and Hamilton 1982). It was further stated that the refractive index of a substance is a physical property, which can be related to structure, and the main structural difference in vegetable oils is the number of double bonds in the fatty acids of the triglycerides. Additional double bonds and particularly, conjugated double bonds increase the refractive index. If there is oxidation and polymerisation during storage, the refractive index rises (Simpson and Hamilton 1982).

Moringa seed oil and virgin olive oil had similar relative density (Table 1). According to Anwar and Bhanger (2003), the relative density of Moringa oil depends on the method of extraction and can be higher or lower compared with olive oil. The relative density of Moringa seed oil observed in the present study is higher than values by Tsaknis *et al*. (1999) (0.9037 g/cm^3), Anwar and Rashid (2007) (0.9032 g/cm^3) and Anwar and Bhanger (2003) (0.9057 g/cm^3). However, it is lower than that reported by Rahman *et al*. (2009) (0.915 g/cm^3).

Colour

In the present study, the colour of the oil samples was determined by a colorimeter using the CIE *L**, *a**, *b** system of colour determination (Hunter and Harold 1988). In the Hunter/CIE colour scale, L measures lightness where a lower number (0-50) indicates dark, and a higher number (51-100) indicates light. Whereas *a** measures redness when positive and greenness when negative; *b** measures yellowness when positive and blueness when negative. The *L* value for each scale indicates the level of light or dark, the *a* value redness or greenness, and the *b* value yellowness or blueness. The colour scales of the two oil types examined in the present study are indicated in Table 2. The colour (b value) of the Moringa seed oil examined in the present study was significantly higher ($p<0.05$) than that of virgin olive oil (Table 1). This suggest that the Moringa seed oil was light yellow in colour compared with the olive oil, which was darker. This is in agreement with the picture of the two oils shown in Figure 1.

The results of the colour determination observed in the present study are in line with the findings of Tsaknis *et al*. (1998) who reported a red unit of 2.0 and yellow unit of 28 measured using a lovibond tintometer method for *M. oleifera* Malawi seed oil extracted with the cold press system, which translate to a light-yellow colour for the Moringa oil. The results of the colour determination of the present study are also in agreement with the findings of Lalas and Tsaknis (2002) and Tsaknis *et al*. (1999) who reported a colour value (red/yellow units) of 1.9/30 for *M. oleifera* seed oil variety Periyakulam 1 from India and variety Mbololo from Kenya, respectively. Anwar and Bhanger (2003) reported red units of one and yellow units of 29 for a solvent (*n*-hexane) extracted Moringa seed oil grown in temperate regions of Pakistan. The intensity of the colour of vegetable oils depends mainly upon the presence of various pigments, such as chlorophyll, which are effectively removed during the degumming, refining and bleaching steps of oil processing (Anwar and Bhanger 2003). The vegetable oils with minimum values of colour index are more attractive for edible and domestic purposes (Anwar and Bhanger 2003).

Table 2. Colour of Moringa and virgin olive oil as determined by the CIE L*, a*, b* colour scale

Oil type	L* (lightness)	a*(redness)	b*(yellowness)
Moringa oil	43.18	-1.01	23.44
Virgin olive oil	30.01	1.90	15.96

Figure 1: Comparison of the colour of Moringa seed oil extracted in the present study (A) and a commercial virgin olive oil (B)

Conclusion

The results indicated that the Moringa seed oil evaluated has generally comparable physicochemical properties with values reported in the literature for Moringa oil, and the values for the parameters tested fall within the recommended limits of edible oils. The Moringa oil had comparable relative density, acid and saponification values as virgin olive oil. However, it has light yellow colour compared with olive oil which is darker.

The present study indicated that *M. oleifea* grown in Botswana is potentially an important oil seed crop, and the Moringa seed oil has a very good potential for edible and industrial purposes. Moringa is a hardy plant that is well adapted to hot arid environments, such as Botswana, and it grows with little attention and management practices. The trees do not need intensive management, establish easily, adapt well to the arid climate of Botswana and grow with minimal care. This suggests the possibility of growing Moringa on a commercial scale for seed production. Through wide scale cultivation and production of Moringa seed, it would be possible to use Moringa seed oil as a cheaper substitute for conventional expensive oil sources, such as olive and sunflower oils.

In this study, only the physicochemical properties of Moringa seed oil were investigated. Thus, there is a need to analyse the tocopherol content, sterol and fatty acid composition of the oil as well as its nutritional values.

Acknowledgements

The NFTRC in Kanye is acknowledged for allowing us to use their oil extraction machine. We are also very grateful to all households in Gaborone who generously provided the Moringa seed samples used in this study. This study was funded by the Department of Tertiary Education of Botswana.

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