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Proximate composition and determination of the physicochemical characteristics of Mmilo (*Vangueria infausta*) oil from Botswana

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ABSTRACT

Mmilo (*Vangueria infausta)* is an indigenous fruit that is available in parts of Botswana and is well consumed in several countries in Southern Africa. This study investigated the physicochemical properties of Mmilo oil and conducted a comparative proximate composition analysis of Mmilo pulp from Mochudi and Gabane areas in Kweneng District, Botswana. The results suggested that there was a significant difference ($P < 0.05$) between the moisture content, crude protein, total carbohydrate and total energy content of Mochudi and Gabane Mmilo pulp while for ash, fiber and crude fat, no significant difference was observed. A high oil yield of 53% was obtained from a mixed sample of Mmilo and the iodine value was calculated to be 90.3 gI_2/100g, while the specific gravity was 0.87. The saponification, acid and peroxide values respectively were 6.73 mg KOH/g, 0.45 mg KOH/g and 0.03 meq/kg. These values indicated that Mmilo oil has the potential of being categorised as an oil of good quality.

INTRODUCTION

Wild medlar *Vangueria infausta* subsp. *infausta* is a deciduous shrub or small tree growing up to 3 m to 8 m in height with a short multi-stemmed trunk supporting hanging branchlets (Behr, K., 2004). The fruit is almost round, glossy dark green at young stage and changes to a light brown colour when ripe. The ripe fruit is soft and fleshy with a leathery skin that encloses 3-5 seeds surrounded in soft pulp. The seeds are normally brown in colour having a bean like shape. Its stems have prominent triangular stipules between each pair of leaves. The bark is pale grey-brown, which peels in untidy flakes and the dull green leaves are opposite, medium to large, shape varying from ovate to round. Its leaf apices are either obtuse with a elongated base that has an entire margin and a 3-10 mm long petiole. It has short leaf stalks that are about

5-10 mm long. Small gall-like growths may be noticed on the leaf surfaces which are proposed to be caused by a species of fungus, which often attacks these trees. The tree has soft, velvety, acorn-shaped buds that appear either before or simultaneously with the new leaves in spring, around September to October. These open into small flowers, greenish white to yellowish in colour that occur in clusters along the short lateral branches.

The fruit is usually borne singly or in pairs on twigs below the leaves. The *Vangueria Infausta* fruits are almost round, glossy dark green when young and changing to a light brown when ripe. The ripe fruits are soft and fleshy with a leathery skin that encloses 3-5 hard-coated seeds, 2-3 cm long, 1-5 cm wide that are embedded in soft pulp. The fruit is edible and has a pleasant sweet sour, mealy taste. It tastes like an apple. In southern Africa, the tree flowers from September to November and the fruits can be found on the plant during summer from November to April (Setshogo, M.P. and Venter, F. 2003). The fruit bears a characteristic star-shaped scar from the remains of the calyx.

Vangueria infausta has been traditionally used as a medicinal plant for centuries in many cultures around the world to cure a variety of diseases. It is a typical nutraceutical, and the phytochemicals present in the species may be responsible for a wide range of therapeutic effects against a number of diseases such as abdominal pains, asthma, blood pressure, chest pains, cold, cough, diabetes, epilepsy, heartbeat problems, nervous system disorders, pneumonia and stomach ulcers (Maroyi, 2018). The leaf, fruit, stem bark and root bark are used as a remedy for many ailments. In Tanzania, different parts of the species have been traditionally used for the treatment of malaria, wounds, menstrual and uterine problems (Ramalingum. N and Mahomoodally. F.M, 2014). Traditional healers use the roots for a variety of illnesses such as malaria and pneumonia. An infusion made from the roots is also used to treat cough, other chest troubles and to treat ringworms.

It is well known that plant seeds are good sources of vegetable oil. The difference among the oils is their composition in relation to the fatty acids, the number of carbon molecules in the fatty acid chain is what determines the type of oil in the material (Emmanuel, 2012).

Although Mmilo fruits have been used to formulate various products overtime in Botswana, which some have made it to the market shelfs, no particular use has been deployed concerning their seeds. They are, therefore, thrown away during processing because they are of no significant value. This project, thus, has aimed to investigate the proximate composition, oil yield and determining the quality of the oil extracted from Mmilo.

MATERIALS AND METHODS

Sample collection

Mmilo fruits were collected from Gabane (24,664^{o} S, 25.7836^{o} E) and Mochudi (24.399 ^{o} 5 S, 26.1478 ^{o} E) areas in Botswana and then transported to Botswana University of Agriculture and Natural Resources, where they were stored at chilled temperatures in a refrigerator in the Food Science and Technology department laboratory. Thereafter, the seeds were separated from the pulp for the instigation of the experiment.

Solvent extraction method

The seeds were cracked with mortar and pestle. The kernels were removed by trampling, then weighed and oven dried before being crushed, ground and kneaded to form a paste. Afterwards the paste was stored in a labelled airtight container inside a cupboard and later used to extract oil. The paste was then weighed into filter papers and a volume of 150ml of n-hexane (solvent) was poured into a round bottom flask, then connected to the soxhlet extractor for commencement of oil extraction.

Mmilo pulp proximate composition analysis procedure

Determination of Moisture content

Moisture content analyser was used to determine the moisture content of Mmilo pulp.

Determination of Crude protein content

About 0.5g of Mmilo pulp sample was weighed in to Kjeldahl digestion flask and a few boiling chips were added (catalyst- carborundum stones) to each digestion flask along with the concentrated sulphuric acid (12 ml). The digestion flask was placed on the digester in which they were digested at a temperature of 420^{o}C for 1 hour. The flasks were removed from the digester and allowed to cool. Digestion was rendered complete when the samples became colourless. After cooling, 30m L of distilled water was added into digestion flask and mixed well, followed by addition of 25mL of 32% NaOH. The contents were then distilled by inserting the digestion tube in the receiver flask that contains 50mL of 4% boric acid solution. About 150mL of the distillate was collected and titrated by standard acid (0.1N HCl). The end point of the titration was marked by a colour change from green to steel-blue when a drop of acid is added. The volume of HCl consumed was used to calculate the %N and %protein as follows:

$$Nitrogen\ \% = \frac{(V1 - V2)x\ N\ x\ 14.00}{sample\ weight\ in\ g\ on\ dry\ basis}\ x\ 100$$

Where: V1= volume of HCl in L consumed to the end point of titration for the sample V2 = volume of HCl in L consumed to the end point of titration for the blank

N = normality of HCl used usually about 0.1N
14.00 = molecular weight of nitrogen

% nitrogen was converted to % protein using the following formula:

$$Protein\ \% = F\ xN$$

Where F is conversion factor of 6.25

Determination Crude fat content

About 3g of sample was accurately weighed into thimble lined with a circle of filter paper. The thimble with the contents was placed into a 50mL beaker and dried in an oven for 2 hours at a temperature of 110^{o}C. The thimble and the contents were then transferred to the extraction apparatus, which was connected to a round bottom flask, filled with n-Hexane. The sample contained in the thimble was extracted with the n-Hexane in the Soxhlet extraction apparatus for 6 hours at a condensation rate of 3-6 drops per second. After extraction, n-hexane was evaporated from the fat extracts in the round bottom flask. The flask and contents were dried in an oven for 30 minutes at 100^{O}C. The flask was then removed from the oven, cooled in a desiccator, and weighed as flask plus contents (mf). The % crude fat was calculated as follows:

$$F = \frac{mf - mi}{mass\ of\ sample}\ x\ 100$$

Determination of Fibre content

The samples were digested with boiled (1.25%) sulphuric acid, filtered (75 μm) and washed. They were boiled again with dilute alkali (1.25% NaOH), filtered (75 μm), washed and dried. The extracted fiber was dried for 1 h at 100^{O}C. The dried residue was ignited for 2 hours in a muffle furnace at 600ºC and crude fibre was estimated as the loss in mass on ignition of the dried residue as follows:

$$Fibre = \frac{residue - ash}{sample} * 10$$

Determination of Ash content

A clean porcelain sample dish was dried at 120^{O}C and ignited at 550^{O}C in a muffle furnace for 1 h. The dish was removed and cooled in a desiccator. The mass of the crucible (m1) was weighed on an analytical balance and recorded. Mmilo pulp of (about 3 g) was weighed in to a porcelain dish and the mass (m2) was recorded. The sample was carbonized over a blue bunsen burner flame and then ashed at 550^{O}C until grey ash is obtained (about 12 h). The sample was then cooled for a moment and placed in a desiccator until temperature assumed ambient temperatures and mass (m3) was recorded. The percentage of ash on dry matter basis was calculated as follows:

$$Ash\ \% = \frac{m3 - m1}{m2 - m1} x\ 100$$

Where: m3 – m1=mass of ash in g and m2-m1= sample mass in g before ashing.

Determination of Total carbohydrate content by difference

The total carbohydrate content was determined by difference. The other constituents in the food (protein, fat, moisture, fiber, and ash) were determined individually, summed and subtracted from the total weight of the food as follows.
Total carbohydrate = 100 - [%protein + %fat + %moisture + %ash + % fiber]

Energy

The total energy of the pulp was calculated using the protein, fat and carbohydrate content.
Total energy = (available protein x 4) + (available fat x 9) + (available carbohydrate x 4).

Physicochemical characteristics of mmilo oil

Saponification value

Ethanolic potassium hydroxide (0.4N) solution was prepared by dissolving 16g of potassium Hydroxide in 1000ml of ethanol. The solution was left to stand for 24hrs before use. An approximate amount of 2ml of sample was transferred into a 250ml round bottomed flask thereafter, 0.4N ethanolic KOH was added to the sample then boiled under reflux for 1 hour. Phenolphthalein indicator (2-3 drops) were added to the mixture followed by titration with 0.4N HCL while the mixture was still hot. The end was indicated by a pinkish to clear colour. Saponification value was calculated as follows:

SV = 56.1 x M x (B-V) / m

Where
M = Molarity of HCL
V = Volume of HCL (ml) used in titration of sample
B= Volume of HCL (ml) used in titration of blank
m =mass (g) of oil sample

Acid value

Fifty ml of ethanol containing 0.5ml of phenolphthalein indicator was boiled and whilst its temperature was still over 70℃, it was neutralised using 0.1 mol/l sodium hydroxide. The neutralized ethanol was added to the test portion in the flask and mixed thoroughly. After boiling the contents, a titration with sodium hydroxide solution followed. The contents were vigorously agitated during titration to thoroughly mix them. Acid value was calculated as follows:

Acid value = (56.1 x v x c) / m

V = volume in ml of standard volumetric sodium hydroxide solution used
c = the exact concentration in moles per liter of the standard volumetric sodium hydroxide solution used
m = the mass in grams of the test portion

Iodine value

Oil sample mass of 0.2g was dissolved in ethanol solvent in a conical flask and 25ml of the hanus reagent (0.2 N ICI) was added. The flask was sealed and shaked well then placed in a darkened area for 30 minutes. This was followed by addition of 10ml of 10% KI that was mixed with the solution. Titration of the sample using thiosulphate was started immediately. The sample was titrated to a yellow straw colour, followed by addition of 5ml of 1% starch solution. The solution changed to a purple-black colour and it was then titrated releasing a drop at a time from the burette until it became transparent/ clear. This marked the end point of the titration. The volume of thiosulphate was recorded, and the titration was repeated 3 times.

Peroxide value

A mass of 1.5g of the sample was added into a conical flask together with 10ml of chloroform plus 10ml of Glacial Acetic Acid. This was followed by addition of 10ml saturated Potassium Iodine then the flask was closed using a stopper. The contents were mixed by shaking for a minute. The flask was placed in a dark cupboard for 5 minutes at a room temperature. After 5 minutes, 75ml of deionised water was added to the flask and titrated with 0.01N Sodium Thiosulphate using starch as an indicator. The blank was also runned. Peroxide value was calculated as follows:

PV = ((V-B) X N X100)) / m

Where N = Normality of Sodium Thiosulphate
V = Volume (ml) of Sodium Thiosulphate used in titration of sample
B = Volume (ml) of Sodium Thiosulphate used in titration of blank
m = mass (g) of oil sample

RESULTS AND DISCUSSION

Proximate composition of Mmilo

The parameters that exhibited a significant difference (P< 0.05) during the comparative proximate composition of Mochudi and Gabane Mmilo pulp were the moisture content, protein content, carbohydrate and energy content (Table 1). Proximate composition is an important criterion used to determine the nutritional values and quality of food. It helps to estimate and determine how much of the major food components, which are moisture, carbohydrates, fats, proteins, ash and crude fibre, exist in a given food. Moisture content in food can have a substantial influence on factors such as the product's taste, texture, appearance, shape, and weight. Deviations from the optimal moisture content can severely affect numerous qualities of the food product, which can have implications not only on product quality but also on its safety. The findings of this study as depicted on Table 1, show the mean value for moisture content of Mochudi and Gabane Mmilo pulp as 45.62% and 51.34%, respectively.

Table 1. Proximate composition of Mochudi Mmilo pulp and Gabane Mmilo pulp

Proximate analysis of Mmilo	Sample (Mean ± SD)	
	Mochudi	Gabane
Moisture (%)	45.62 ± 0.94[a]	51.34 ± 3.07[b]
Ash (%)	3.73 ± 0.01 [a]	3.86 ± 0.02 [a]
Fiber (%)	9.69 ± 1.54 [a]	11.71 ± 0.09 [a]
Protein (%)	2.65 ± 2.76 [a]	6.77 ± 2.28 [b]
Fat (%)	15.59 ± 0.78 [a]	13.43 ± 2.01 [a]
Carbohydrate (%)	22.73 ± 1.04 [a]	12.72 ± 3.66 [b]
Energy (Kcal)	241.83 [a]	198.83 [b]

Means not followed by the same superscript letters for individual parameter analysis are significantly different (P< 0.05)

The results further reveal that, there was a significant difference between the protein contents of Mochudi and Gabane Mmilo pulp. Only protein content of 2.65% was recorded for Mochudi Mmilo pulp and this is far less than the 6.77% which was reported for Gabane Mmilo pulp. These values were slightly closer to the findings of Legwaila (2011) who reported crude protein values of 3.0% for other Mmilo pulp. According

to Nwosuogwa (2009), the actual protein content depends on, among other composition of the substrate, the size of the fruits and harvesting time. Therefore, the variation observed between the protein contents of Mochudi and Gabane could be correlated to the size and the harvesting time.

The fiber and crude fat content of Mmilo pulp did not have any significant differences. Mochudi Mmilo pulp had a fiber content of 9.69% while 11.71% was recorded for Gabane. While comparing these results with a 6.7% fiber content reported by Chamsama (2006), the fiber content values of the present samples were slightly higher. Maroyi (2018) reported that, Mmilo fruit is an important source of fiber and could be utilised if a high fiber fruit is required. Crude fiber is also useful in the chemical determination of succulence of fresh vegetables and fruits as over mature products have increased levels of crude fiber. Thus, the high fiber content exhibited by Gabane Mmilo pulp in this study shows that it was at the peak of its maturity stage. Furthermore, a crude fat value of 15.59% was obtained for Mochudi Mmilo pulp whereas 13.43% was obtained for Gabane Mmilo pulp. Crude fat content analysis is a quality determinant factor in food which in respect to commercial regulations, it is imperative for food producers to be able to closely monitor and report in their products since it affects the quality and value of food. A high crude fat value is indicative of a fruit's ability to be a good source of energy and possibly a source of fat-soluble vitamins and proteins (Chamsama, 2006). Therefore, the high crude fat values obtained for Mmilo pulp in this study implicates that it as good source of energy and proteins.

The results also indicated that, Mochudi Mmilo pulp had an ash content of 3.73% whereas Gabane Mmilo pulp had an ash content of 3.86%, which was almost similar to the 3.9% recorded by Legwaila (2011). Statistically, the ash content of the two samples were not significantly different. Haque et al., (2008) suggested that, ash content reflects the amount of minerals present in samples. When compared to other indigenous fruits, Mmilo pulp has a lower ash content which is reflective of its low amount of minerals**.**

Carbohydrates are one of the most important constituents of many foods as they immensely contribute to a variety of physicochemical properties of foods such as sweetness, appearance, stability and texture. Hence, it is important to determine their concentration in foods. The 22.73% carbohydrate content recorded for Mochudi Mmilo pulp was higher than the 12.72% recorded for Gabane Mmilo pulp ($P< 0.05$). The overall carbohydrate content in this study was rather not in alignment with the 78.1% reported by Kalenga Saka and Jerome D. Msonthi, 1994 for Mmilo pulp in Malawi. This indicates that Botswana Mmilo pulp has a lower carbohydrate content. The results indicated that there was a significant difference marked between the total energy contents of Mochudi Mmilo pulp and Gabane Mmilo pulp. Total Energy value of 241.83% was obtained for Mochudi Mmilo pulp while 198.83% was obtained for Gabane Mmilo pulp.

Oil physico-chemical properties

A combined sample of Mmilo from Mochudi and Gabane was used for this experiment. The oil extracted using the Soxhlet extraction method is shown in Figure 1.

Fig 1. Oil extracted from Mmilo Seeds in Botswana

The results in Table 2 indicate that, an average oil yield of 53% was obtained during oil extraction from Mmilo seeds. Oil yield refers to the amount of oil that can be derived from an oil seed and it varies according to an oil seed's plant species. Mmilo oil was analysed to determine the different physicochemical properties including, iodine value, peroxide value, acid value, saponification value and specific gravity.

Iodine value measures the extent of unsaturation in the oil and it is a useful indicator in quantifying the amount of double bonds present in the oil, which in turn reflects its susceptibility to oxidation. Lower iodine value indicates reduced number of unsaturated bonds and reduced susceptibility to oxidative rancidity (Ajai et al 2018). The iodine value obtained for Mmilo oil was 90.3 gI_2/100g, which indicates that the oil is highly unsaturated, hence its

increased susceptibility to oxidation. According to Ajai et al., (2018), oils with iodine values less than 100gI_2/100g are known as non-drying oils, above 100gI_2/g but less than 130 gI_2/100g as semi drying oils while above 130 gI_2/100g as drying oils. The author further elaborated that non-drying oils are not suitable for ink and paint due to their non-drying characteristics but may be useful in the manufacture of soaps and can be regarded as liquid oil. Therefore, Mmilo oil can be considered as a non-drying oil because its iodine value is less than 100 gI_2/100g and can hence be used for soap making.

Table 2. Physico-chemical properties of Mmilo Oil (Composite sample of Mmilo collected from Mochudi and Gabane areas)

Parameters	Value
Oil yield (%)	53
Iodine value (gI_2/100g)	90.3
Perioxide value (meq/kg)	0.03
Acid value (mg KOH/g)	0.45
Saponification value (mg KOH/g)	6.73
Specific gravity	0.87

From the results, the acid value obtained for Mmilo oil was 0.45 mg KOH/g. Acid value quantifies the degree of free fatty acids in oils, which can further be used to check the level of oxidative deterioration of the oil due to enzymatic or chemical oxidation. The higher the acid value, the higher the level of free fatty acids, which translates into decreased oil quality. According to Ayoade et al., (2015), the acid value of oil is suitable for edible purposes and should not exceed 4mg KOH/g. With that being said, the acid value for Mmilo oil was lower than the recommended limit for edible oils hence, it could be suitable for consumption purposes. The low acid value also suggests that the oil will be less susceptible to lipase action.

The peroxide value is a determinant of rancidity in oils, accordingly a high peroxide value indicates poor resistance of the oil to peroxidation during storage. The low numbers of peroxide value are indicative of low levels of oxidative rancidity of oils and suggest strong presence or high levels of antioxidants (Sabinus Oscar, 2012). Codex Alimentarius Commission has set a maximum limit of Peroxide value for nuts and seed oils to be 10 meq/Kg. Thus, Mmilo oil being considered a seed oil, its peroxide value was far less than the maximum limit, as it was recorded to be 0.03 meq/kg and hence the oil is very much stable to oxidative rancidity.

The saponification value of Mmilo oil was found to be 6.73 mg KOH/g, which was very low compared to the 175.34 mg KOH/g recorded by Ajai et al., (2018) for *Blighia sapida* seed oil. High saponification value indicates that the oil contains fatty acids with higher number of carbon atoms, hence this verifies that Mmilo oil has fewer fatty acids with lower number of carbon atoms. Our results also show that the oil had a specific gravity value of of 0.87. Specific gravity is an index used to measure the density of a liquid, so any liquid with a density greater than water has a specific gravity greater than 1. Therefore, the 0.87 specific gravity obtained for Mmilo oil indicates that it is less dense than water.

CONCLUSION

Mmilo *(Vangueria infausta)* is a fruit that has vast nutritional components. The results suggested that there was a significant difference ($P < 0.05$) between the moisture content, crude protein, total carbohydrate and total energy content of Mochudi and Gabane Mmilo pulp whereas for ash, fiber and crude fat, no significant difference was observed. Furthermore, Mmilo seeds contain oil, with a higher yield when compared to other oil seeds. The low acid value obtained for Mmilo oil indicated that it has fewer free fatty acids and will be less susceptible to lipase action. This is supported by the low saponification value obtained, which also suggest that the oil has fewer free fatty acids and number of carbon atoms. Therefore, it can be of good quality oil. The low numbers of peroxide value recorded are indicative of the oil's low levels of oxidative rancidity, which means that it will be stable to oxidative rancidity during storage. However, the high iodine value recorded for the oil suggest that it is highly unsaturated hence susceptible to oxidation. Nonetheless, the high iodine value qualifies Mmilo oil to be considered as a non-drying oil and can further be used for soap making.

RECOMMENDATIONS

Further studies should be conducted on Mmilo (*Vangueria infausta)* fruits from various locations in Botswana, to gather more information and draw a clear conclusion on their proximate composition. In addition, we recommend further analysis on Mmilo oil for it's mineral, vitamin and phytochemicals as literature indicates that the plant exhibits several medicinal values.

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