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Characterization of *Schinziophyton rautanenii* (Manketti) nut oil from Namibia rich in conjugated fatty acids and tocopherol

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ABSTRACT

The *Schinziophyton rautanenii* tree is an important food source for many communities of the African continent. Oil extracted from the Manketti nut is of great economic value, due to its unique composition and properties. In this study, the physical and chemical characteristics of the oil obtained from three extraction methods – cold pressed, traditional and Soxhlet extraction – were investigated and compared. Oil yield of the nut was found to be 42.6 ± 0.84%. Good quality characteristics, including saponification values (184–189 mg KOH/g), iodine values (120–131 g/100 g), acid values (0.959–2.44 mg KOH/g) and peroxide values (1.80–3.98 meqO_2/kg) were found for the Manketti nut oil. The total tocopherol content was in the range of 144–206 mg/100 g of oil, with γ-tocopherol as the dominant tocopherol. The oil was rich in conjugated fatty acids, α-eleostearic (9*Z*,11*E*,13*E*-octadecatrienoic acid) (24–36%) and linoleic acid (9*Z*,12*Z*-octadeca-9,12-dienoic acid) (31–32%), making it a potential candidate in the nutraceutical and cosmetics industry.

1. Introduction

Schinziophyton rautanenii (Schinz), formerly known as *Ricinodendron rautanenii* Schinz (Vermaak et al., 2011), belonging to family Euphorbiaceae, is a large spreading dioecious tree of 15 to 20 m in height (Fig. 1a & b), which commonly grows wild on plains, among dunes, wooded hills and the floodplain islands of the eastern Zambezi (Palgrave, 1983; Curtis and Mannheimer, 2005). The *S. rautanenii* (Schinz) tree is commonly found in the north-eastern part of Namibia (Fig. 1c) and is an important food source to the local communities (European Commission, 1998; Graz, 2002). Other countries where the tree grows are Angola, South Africa, Botswana and Zambia (Atabani et al., 2014). In Namibia, the *S. rautanenii* tree is locally known as Omunkete (Oshiwambo), Omungete (Otjiherero), Ngongo (Kavango) and Mungongo (Zambezi) (Curtis and Mannheimer, 2005). The yellow flowers, about 10 mm in diameter and 12 cm in length (Palgrave, 1983), appear from November to February (Curtis and Mannheimer, 2005). Egg-shaped, light grey-green fruits appear from February (Palgrave, 1983) and between April to May, the fruit fall to the ground, at which time the ripening process starts, softening the fruit flesh (Vermaak et al., 2011). The different fruit parts have various uses, but the inner nut of the Manketti seed kernel is of most value (European Commission, 1998), as it is highly nutritious and contains high amounts of a bright yellow edible oil (Palgrave, 1983). Traditionally, the inner edible nut is used for oil extraction after removal from the fruit with an axe (European Commission, 1998), or it may be crushed between two rocks (Vermaak et al., 2011), opening the hard Manketti shell (Fig. 1d) to reveal the inner edible nut (Fig. 1e). The inner nut is eaten raw or roasted (Curtis and Mannheimer, 2005) and is commonly pounded and cooked to be eaten with Mahangu porridge, whilst the oil (Fig. 2a–c) is eaten with vegetables (Namibia Tourism Board, 2014), chicken and spinach. In Namibia, Manketti nut oil is made traditionally in households and a very limited number of small enterprises produce the cold pressed Manketti nut oil. Apart from being used in food preparations

Abbreviations: AV, acid value; AMW, average molecular weight; meq, milliequivalents; mg/kg, milligram per kilogram; mg/100 g, milligram per hundred gram; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; O_2/kg, oxygen per kilogram; SV, saponification value

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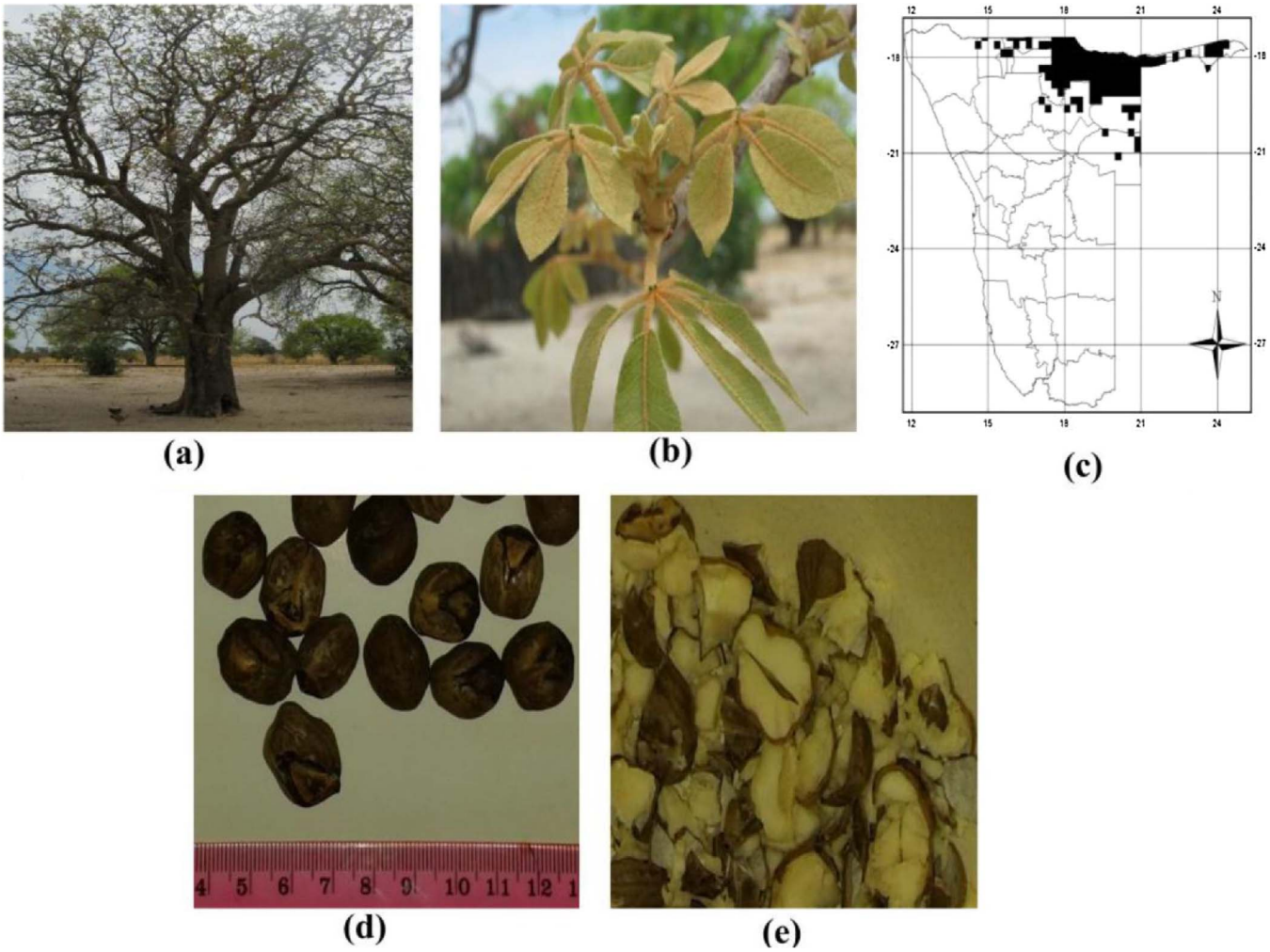

Fig. 1. (a-e). Tree (a) and leaves (b), wild tree distribution of *S. rautanenii* throughout Namibia (Adopted from Graz, 2002) (c), dried kernels (d) and crushed nuts (e).

and cooking, the Manketti nut oil can also be used in skincare formulations for cleansing and moisturizing (Kivevele and Huan, 2015), since the oil is easily absorbed into the skin (Zimba et al., 2005).

The seed kernel of *S. rautanenii* generally contains between 41 and 53% oil (Chisholm and Hopkins, 1966; Chivandi et al., 2008; Mitei et al., 2008; Gwatidzo et al., 2017a), allowing this oil to be exploited commercially (Chivandi et al., 2008). The Manketti nut oil is reported to be highly stable to oxidation, with a long shelf life (Zimba et al., 2005). Interestingly, Peters (1987) reported that dried kernels stored under proper conditions for about 6 years can still be edible and palatable. This provides a continuous food source for rural communities throughout the year, especially during food scarcity periods (Curtis and Mannheimer, 2005), such as droughts and during winter periods. The aim of the study was to analyze the physical and chemical properties, fatty acid profile, tocopherol composition of the Manketti nut oil currently produced locally in Namibia in its cold pressed and traditionally extracted form and to compare data inclusive of a Soxhlet extraction. The characterization data is intended to assist rural communities and small upcoming enterprises to produce value-added products from their indigenous seed oil resources, in order to uplift livelihoods of rural communities and assist in the economic improvement of a developing country such as Namibia.

2. Experimental

2.1. Sources of manketti kernels and oil

The mature kernels of *S. rautanenii* (Manketti) were purchased from the village of Oshikulufitu in the Omusati region of Namibia. The cold pressed Manketti nut oil was purchased from Mungongo Trading Enterprise, Zambezi region of Namibia. The traditionally extracted Manketti nut oil was prepared in a rural homestead near Outapi, Namibia. The traditionally extracted Manketti nut oil (Fig. 3a–g) was produced by first roasting the Manketti nuts using hot coals with the aims of improving the flavor of the oil and oil extraction from Manketti nuts (Fig. 3a). The roasted Manketti nuts were then pounded, producing a sticky nut paste (Fig. 3b & c), which was then mixed with some water (Fig. 3d) and boiled for a time, eventually expressing the oil (Fig. 3e & f). Oil extracted was scooped off (Fig. 3g) using a spoon-shaped calabash and then stored in 200-mL glass bottles.

2.2. Solvent extraction of oil from manketti nuts

The oil-containing nuts of *S. rautanenii* (Manketti) kernels were manually removed from their shells and crushed to a fine powder using a mortar and pestle. The crushed nuts were extracted with *n*-hexane (Merck, Darmstadt, Germany) in a Soxhlet apparatus for 6 h. The solvent was then removed under vacuum at 40 °C using a vacuum rotary evaporator (Heidolph, Schwabach, Germany). The oil yield was determined and samples were stored in the dark at 4 °C for further

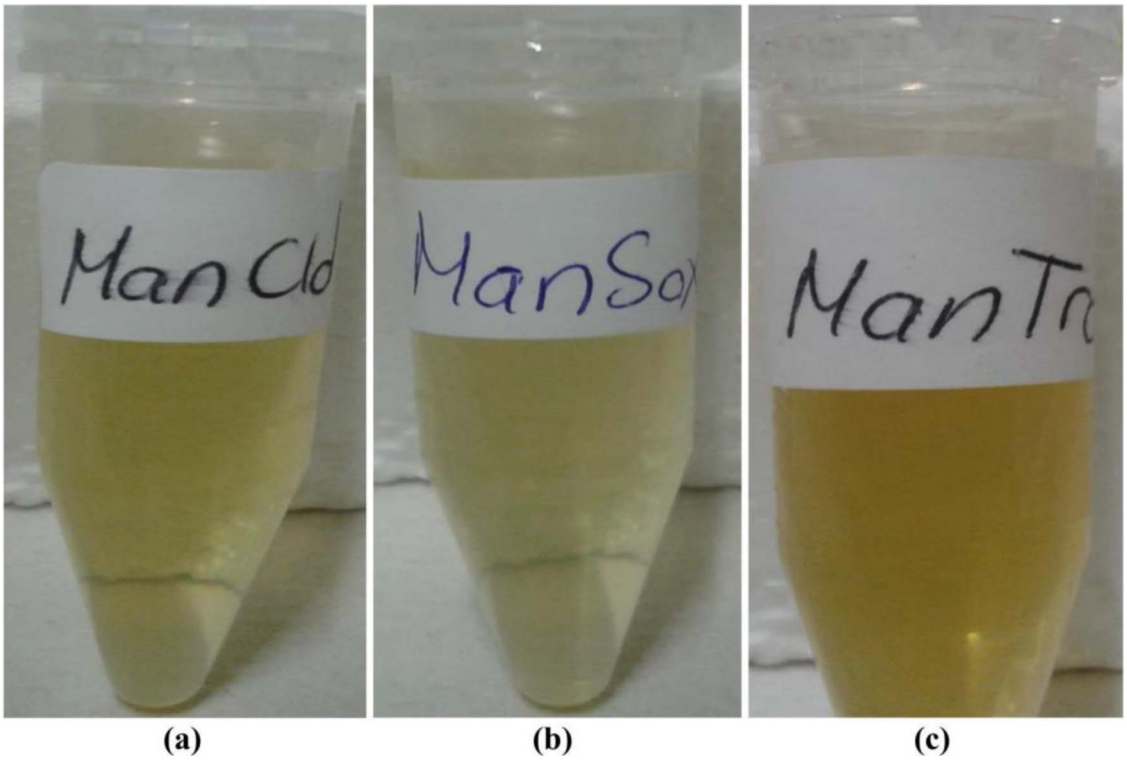

Fig. 2. Cold pressed (a), Soxhlet extracted (b) and traditionally extracted (c) *S. rautanenii* (Manketti) nut oil.

analysis. The oil was extracted from Manketti nuts in triplicate.

2.3. Determination of chemical and physical characteristics

The chemical characteristics of Manketti nut oil, such as saponification value (SV), acid value (AV) and iodine value, were determined according to the AOAC official methods, 920.160, 940.28 and 920.158, respectively (AOAC, 1998). The *p*-anisidine values were determined according to the AOCS official method Cd 18–90 (AOCS, 1993). The peroxide values were determined according to Uluata and Özdemir (2012), using the ferric thiocyanate method and data were expressed as milliequivalents of active oxygen per kilogram of oil (meqO_2/kg). The physical characteristics of the Manketti nut oil, such as the refractive index and the specific gravity, were determined using an ABBE

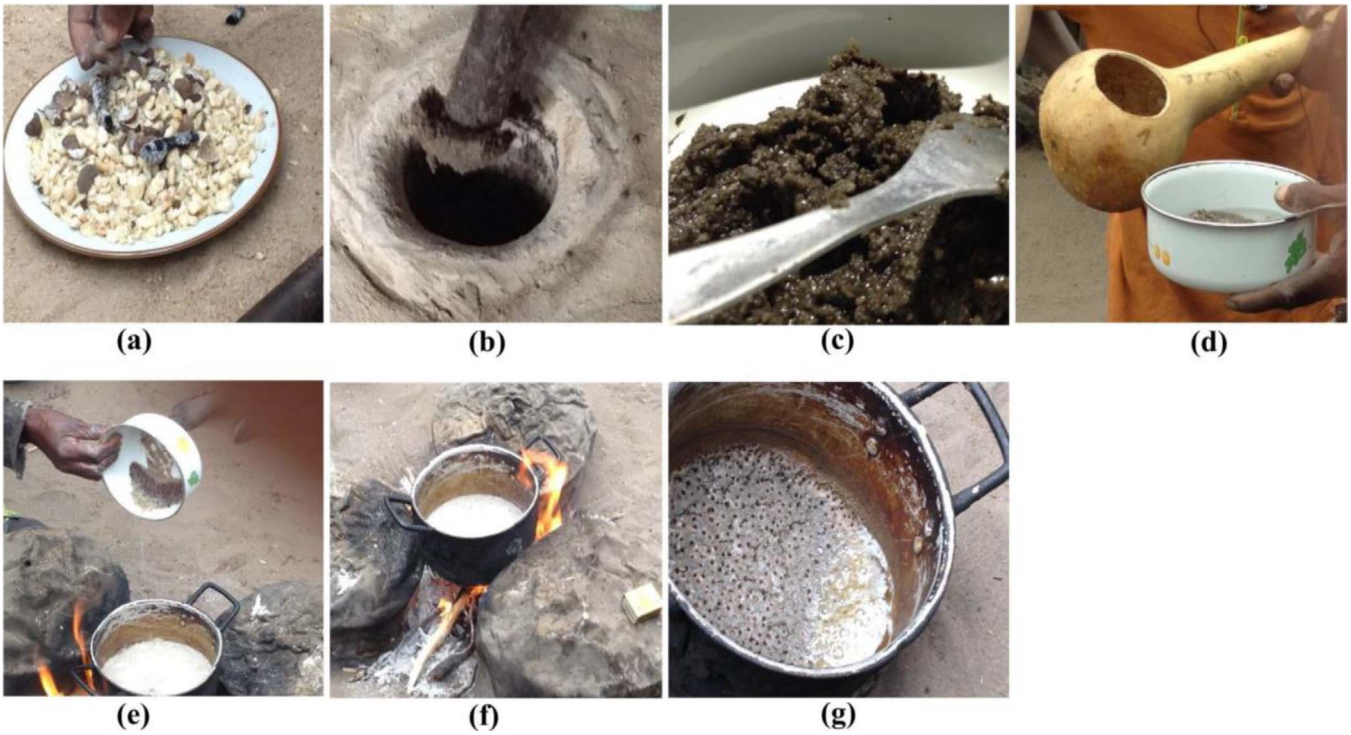

Fig. 3. Traditional extraction process of Manketti nut oil.

refractometer (K7135; MRC, Holon, Israel) at 25 °C and according to the AOAC official method 40.1.08 (AOAC, 1990), respectively. The ester value was calculated from the difference between the determined SV and the AV of the oil. The average molecular weight (AMW) of the oil was calculated according to Yang et al. (2013) with the following formula:

AMW = (3*56.1*1000)/SV of oil

2.4. Proton nuclear magnetic resonance analysis

^{1}H NMR spectra of the Manketti nut oil were recorded using the Bruker Avance 400 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany). Samples were dissolved in $CDCl_3$ (Merck, Darmstadt, Germany) at 25 °C. $CDCl_3$ was used as reference with a signal at 7.24 ppm. Data were reported as "chemical shifts (δ)" in ppm.

2.5. Determination of the fatty acid profile

Methyl esters of fatty acids were obtained by esterification according to Yang et al. (2013). The composition of fatty acid methyl esters was determined by gas chromatography, model 7820A coupled with a mass spectrometer, model 5977E MSD (Agilent Technologies, Santa Clara, CA). An Agilent capillary column HP5 MS (30 m × 0.25 mm, 0.25 μm) was used to separate the fatty acid methyl esters after injection of 1 μL of sample. Helium at a flow rate of 1.50 mL/min and with a split ratio of 20:1 was used as carrier gas. Injection temperature was 250 °C. Initial temperature was 40 °C, held for 8 min, ramp 1: 10 °C to 220 °C for 5 min and ramp 2: 20 °C to 300 °C for 10 min. The mass spectrometer was set to scan in the range of *m/z* 30–600. Data were expressed as relative percentages of the total peak areas of methyl esters contained in the sample.

2.6. Tocopherols and major phytosterols analysis

Tocopherols and major sterols compositions were analyzed according to Du and Ahn (2002). A 100-mg aliquot of oil was extracted with 10 mL of the saponification reagent. The internal standard, 100 μL 5α-cholestane (10 ppm), was added, then vortexed and incubated for 60 min at 50 °C, then cooled. Deionized water and hexane, each 5 mL, were added then vortexed and then left to rest for 15 h. The supernatant (1000 μL) was dried, after which the dry samples were reconstituted with 200 μL pyridine and with addition of 100 μL BSTFA with 1% TMCS were then derivatized by incubation at 50 °C for 1 h.

Derivatized compounds were then analyzed using a gas chromatograph, Agilent 6890N, coupled to an Agilent 5975 MS detector (both Agilent Technologies, Santa Clara, CA) using a Zebron™ ZB-MultiResidue (30 m × 0.25 mm ID, 0.25 μm film thickness) column (Part No. 7HG-G016-11). The oven temperature program was maintained at 100 °C for 2 min, ramped at 15 °C/min to 180 °C, held for 0 min, ramped at 5 °C/min to 250 °C and held for 3 min and finally at 20 °C/min to 320 °C, held for 12 min. Helium at a flow rate of 1.2 mL/min was used as the carrier gas and the injector temperature was maintained at 200 °C in splitless mode. Mass spectral data was recorded on an MSD operated in full scan mode (*m/z* 35–600) with the ion source and quadrupole temperatures maintained at 240 °C and 150 °C, respectively. Transfer line temperature was maintained at 200 °C.

2.7. Statistical analysis

All experiments unless otherwise stated were carried out in triplicate and means were compared with analysis of variance (ANOVA) followed by analysis with the Tukey's HSD Test using IBM® SPSS® Version 24 software. Values with different letters within rows indicated significant differences ($p < 0.05$).

3. Results and discussion

3.1. Oil yield and color

The oil yield of Manketti nut oil after solvent extraction was determined to be 42.6 ± 0.84%. The oil yield was comparable to the Manketti nut oil from Botswana (41.5%) (Mitei et al., 2008) but slightly lower than the oil yield (53.3%, 45.3%) reported by Chivandi et al. (2008) and Gwatidzo et al. (2017a) for Manketti oil from Zimbabwe and the Okavango region of Namibia, respectively. The resultant Manketti nut oil extracted by the three different methods studied is shown in Fig. 2. The different extractions resulted in a yellow oil, in liquid form at 25 °C, with the cold pressed (Fig. 2a) and Soxhlet extracted (Fig. 2b) Manketti nut oil being lighter in color, compared to the traditionally extracted oil (Fig. 2c). This could be because the nuts were roasted before the traditional extraction of the oil to improve flavor and the oil extraction process (Personal communications). Roasting temperature and time affect color development of oil, due to the development of browning agents, as reported by Kim et al. (2002) for rice germ oil.

3.2. Physical and chemical characteristics

A summary of reported characteristics and chemical compositions of *S. rautanenii* (Manketti) nut oil from different origins is presented in Table 1. It is evident that variations in fatty acid compositions among different regions exist, with some reporting the presence of the α-eleostearic acid and linolenic acid and others not having detected or reported the presence of these fatty acids. The profile of fatty acids in an oil depends not only on the source of the plant species, but also on plant physiology, genetic makeup, geographical and climate conditions among others (O'Brian, 2009). The physicochemical characteristics of Manketti nut oil obtained through cold pressed, traditional and Soxhlet extraction methods were determined and are presented in Table 2. Significant differences ($p < 0.05$) among the three extraction methods were found for the characteristics of acid value, *p*-anisidine value, iodine value and refractive index. The Soxhlet extracted Manketti nut oil was significantly different ($p < 0.05$) from the traditionally extracted and the cold pressed Manketti nut oil for the characteristics of saponification value, average molecular weight, ester value and specific gravity. Traditionally extracted Manketti nut oil was significantly different ($p < 0.05$) in terms of its peroxide value from the cold pressed and the Soxhlet extracted Manketti nut oil, with a higher peroxide value.

Evidence as to the lengths of relative chains of fatty acids present in the triglyceride can be obtained by the determination of the saponification value (Wrolstad et al., 2005). The saponification value for Manketti nut oil ranged between 184 mg KOH/g and 189 mg KOH/g of oil with the Soxhlet extracted Manketti nut oil being significantly different ($p < 0.05$) from the cold pressed and the traditionally extracted oil. The saponification values obtained for Manketti oil suggest the presence of mainly medium-chain fatty acids (C16 and C18) (Mabaleha et al., 2007), which was confirmed with the GC–MS compositional analysis of the fatty acids (Table 3). The saponification value for the Manketti nut oil was comparable to the saponification value (185 mg KOH/g) reported by Mitei et al. (2008) for Manketti oil from Botswana and to those of major vegetable oils such as olive oil (184–196 mg KOH/g), rice bran oil (181–189 mg KOH/g) and canola oil (182–193 mg KOH/g) (Gunstone et al., 2007). The saponification values reported in this study were within the range (183–193 mg KOH/g) reported by Gwatidzo et al. (2017b) for nut oil extracted using four different methods from Manketti nuts from the Okavango region of Namibia.

The acid value reflecting the total acidity of the oil sample (Wrolstad et al., 2005) of the traditionally extracted Manketti nut oil was 2.44 mg KOH/g, which was higher than the acid value reported for

Table 1
Reported characteristics and compositions of *S. rautanenii* (Manketti) nut oil from different origins.

S. rautanenii (Manketti) oil origin	Zambia[a]	Zambia	Zambia[d]	Zimbabwe[a]	Botswana[a]	Namibia
saponification value (mg KOH/g)	NR	NR	NR	NR	185	183–193
iodine value (g/100 g)	NR	NR	NR	NR	122	128–129
specific gravity	NR	NR	NR	NR	0.907[b]	0.908-0.914[b]
refractive index	1.48	NR	1.48	NR	1.48 (25 °C)	1.49
palmitic acid (16:0)	NR	9.8	8	10.8	12.0	8.74[a]
linolenic acid (18:3*n*–3)	NR	16.7	ND	ND	ND	0.042
linoleic acid (18:2*n*–6)	NR	39.0	37	49.5	51.9	37.8[a]
oleic acid (18:1*n*–9)	NR	19.2	15	15.2	24.4	17.5[a]
stearic acid (18:0)	NR	7.7	9	3.04	11.8	6.78[a]
erucic acid (22:1 *n*–9)	NR	ND	ND	21.5	ND	ND
α-eleostearic acid (9c,11t,13t-18:3)	23.8	ND	25	ND	ND	26.3[a]
β-tocopherol (mg/100 g)	NR	NR	NR	NR	ND	NR
γ-tocopherol (mg/100 g)	NR	NR	NR	NR	223	NR
α-tocopherol (mg/100 g)	NR	NR	NR	NR	0.564	NR
δ-tocopherol (mg/100 g)	NR	NR	NR	NR	ND	NR
stigmasterol (mg/100 g)	NR	NR	NR	NR	3.62	98.3[a]
β-sitosterol (mg/100 g)	NR	NR	NR	NR	133	1733[a]
reference	Chisholm and Hopkins (1966)	Zimba et al. (2005)	Juliani et al. (2007)	Chivandi et al. (2008)	Mitei et al. (2008, 2009)	Gwatidzo et al. (2014, 2017a, 2017b)

Note: [a]Extraction method = Organic solvent(s); [b]Reported as density (g/ml); [c]bleached; [d]cold pressed; ND = not detected, NR = not reported.

Table 2
Physicochemical characteristics of S. rautanenii (Manketti) nut oil from Namibia.

characteristic	traditional	cold-pressing	Soxhlet
oil appearance	yellow	light yellow	light yellow
state of oil at 25 °C	liquid	liquid	liquid
saponification value (mg KOH/g)	189 ± 1.28[a]	188 ± 1.52[a]	184 ± 1.17[b]
average molecular weight (g/mol)	890 ± 6.03[a]	897 ± 7.30[a]	913 ± 6.44[b]
acid value (mg KOH/g)	2.44 ± 0.17[a]	2.06 ± 0.03[b]	0.959 ± 0.15[c]
ester value	187 ± 1.12[a]	186 ± 1.55[a]	183 ± 1.29[b]
peroxide value ($meqO_2$/kg)	3.98 ± 0.26[a]	2.19 ± 0.27[b]	1.80 ± 0.021[b]
p-anisidine value	2.51 ± 0.18[a]	0.877 ± 0.08[b]	0.245 ± 0.07[c]
iodine value (g/100 g)	120 ± 0.11[a]	131 ± 1.37[b]	126 ± 2.43[c]
specific gravity (20 °C)	0.922 ± 0.002[a]	0.923 ± 0.003[a]	0.903 ± 0.006[b]
refractive index (25 °C)	1.48 ± 0.001[a]	1.48 ± 0.001[b]	1.48 ± 0.001[c]

Data shown as means with ± SD of three replicates. Means with different letters (a, b and c) in the same row are significantly different ($p < 0.05$) as determined with the Tukey's HSD test.

Table 3
Tocopherol phytosterols and fatty acid composition (%) of S. rautanenii (Manketti) nut oil from Namibia.

	traditional	cold pressing	Soxhlet
tocopherols (mg/100 g oil)			
β-tocopherol	ND	0.73	0.77
γ-tocopherol	164	123	189
α-tocopherol	14.8	9.55	11.8
δ-tocopherol	3.84	4.22	4.45
total-tocopherol	183	137	206
phytosterols (mg/100 g oil)			
stigmasterol	44.3	45.3	42.3
β-sitosterol	587	668	682
fatty acids (%)			
palmitic acid (16:0)	11.2 (1.1)	14.3(1.4)	10.4(1)
stearic acid (18:0)	8.59 (1)	16.3 (1.9)	9.29 (1.1)
arachidic acid (20:0)	0.43 (1)	0.44 (1.02)	0.48 (1.1)
α-eleostearic acid (9c,11t,13t-18:3)	34.0 (1.4)	24.2 (1)	35.7 (1.5)
oleic acid (18:1 *n*–9)	12.9 (1.2)	13.0 (1.2)	11.2 (1)
linoleic acid (18:2 *n*–6)	32.2 (1)	31.2 (1)	32.1(1)
11-eicosenoic acid (11–20:1)	0.74 (1.2)	0.62 (1)	0.82 (1.3)
Total unsaturated	79.8 (1.2)	69.0 (1)	79.9 (1.2)
Total MUFA	13.6 (1.1)	13.6 (1.1)	12.1 (1)
Total PUFA	66.2 (1.2)	55.4 (1)	67.8 (1.2)
Total saturated	20.2 (1)	31.0 (1.5)	20.1 (1)

Note: Ratios of fatty acid contents are presented in brackets.

Manketti nut oil (0.36 mg KOH/g) from Botswana (Mitei et al., 2008) The cold pressed Manketti nut oil (2.06 mg KOH/g) was similar to the value (2.08 mg KOH/g) reported by Atabani et al. (2014). The Soxhlet extracted Manketti nut oil (0.959 mg KOH/g) had the lowest acid value. Acid values reported for Manketti nut oil extracted from the nuts obtained from the Okavango region of Namibia had acid values of 1.03–1.23 mg KOH/g (Gwatidzo et al., 2017b) which are similar to the Soxhlet extracted Manketti nut oil.

The presence of primary and secondary oxidation products in the Manketti nut oil samples was investigated through the determination of the peroxide and *p*-anisidine values, respectively. The oil obtained from the three extraction methods was found to have low peroxide values, with the traditional extracted oil (3.98 $meqO_2$/kg) being significantly different ($p < 0.05$) from the cold pressed (2.19 $meqO_2$/kg) and the Soxhlet extracted (1.80 $meqO_2$/kg) Manketti nut oil. The peroxide value for the traditional extracted oil, which had the highest peroxide value, is higher than the value (2.51 $meqO_2$/kg) reported by Mitei et al. (2008) for Manketti nut oil from Botswana. The results obtained for the acid value and peroxide value of Manketti nut oil are within the acceptable levels of the standards for edible oils as being reported to be < 4.0 mg KOH/g and the peroxide value to be < 13 $meqO_2$/kg (FAO, 2017). Low *p*-anisidine values were observed for Manketti nut oil among the three extraction techniques but were found to be significantly different ($p < 0.05$), ranging between 0.88 (cold pressed oil) and 2.51 (traditionally extracted oil). The traditionally extracted Manketti nut oil had the highest *p*-anisidine value among the three extraction methods used but was lower when compared to the *p*-anisidine value reported by Mitei et al. (2008) for Manketti nut oil from Botswana, and that reported by Gwatidzo et al. (2017b) for Manketti nut oil extracted from the nuts obtained from the Okavango region of Namibia. The low levels of peroxide and *p*-anisidine values are an indication of the good oil quality of the Manketti nut oil obtained using the three different extraction methods. In particular, the traditionally extracted Manketti nut oil could be commercially exploited towards value-added products, thereby assisting rural communities with income

generating initiatives.

The level of unsaturation can be represented by the iodine value (Wrolstad et al., 2005). The iodine values of the oil from the three different extraction methods were significantly different ($p < 0.05$) and ranged between 120 and 131 g/100 g oil. The high iodine value recorded is due to the high content of unsaturated fatty acids (Lianhe et al., 2012) present in the Manketti nut oil, as confirmed by the GC–MS compositional analysis (Table 3). The iodine value of the traditionally extracted (120 g/100 g) Manketti nut oil is comparable to the iodine value (122 g/100 g) reported by Mitei et al. (2008) for Manketti nut oil from Botswana, with the cold pressed (131 g/100 g) and Soxhlet extracted (126 g/100 g) Manketti nut oil having higher iodine values. The iodine values observed for Manketti nut oil are within the ranges of major vegetable oils such as sunflower oil (118–145 g/100 g) and soybean oil (124–139 g/100 g) (Gunstone et al., 2007). The iodine values of the traditionally and the Soxhlet extracted Manketti nut oil are lower than the values (128–129 g/100 g) reported by Gwatidzo et al. (2017b) for oil extracted using four different methods from the Manketti nuts obtained from the Okavango region of Namibia.

The specific gravity at 20 °C of the traditionally (0.922) extracted and the cold pressed (0.923) extracted oil was significantly different ($p < 0.05$) from the Soxhlet extracted (0.903) Manketti nut oil. Mitei et al. (2008) reported a density of 0.907 g/cm^3, which is similar to the Soxhlet extracted Manketti nut oil and lower when compared to the traditional extracted and cold pressed Manketti nut oil. Atabani et al. (2014) reported a density of 0.943 g/cm^3 at 15 °C and 0.925 g/cm^3 at 40 °C, which are higher than the observed values for the Namibian Manketti nut oil. Significant differences ($p < 0.05$) were observed in the refractive index (RI) at 25 °C among the three different extraction methods. The refractive index reported by Mitei et al. (2008) at 25 °C was 1.48, which is lower than the traditionally extracted (1.48) and cold pressed oil (1.48) and higher than the Soxhlet extracted (1.48) Manketti nut oil. Atabani et al. (2014) reported a refractive index of 1.49, which is higher than the observed values for the Manketti nut oil. Increases in the degree of saturation, in particular polyunsaturation, as revealed by higher iodine values, increase the refractive index of the seed oil (Mitei et al., 2008). This was observed from data of the iodine values (Table 1) and the GC–MS fatty acid compositional analysis (Table 3) for Manketti nut oil. Significant differences among characteristics of oils obtained through different extraction techniques have been reported. Yang et al. (2013) have reported significant differences among different extraction methods in chemical parameters, such as acid value, iodine value, saponification value and average molecular weight for the seed oil from Chinese vegetable tallow and stillingia oil after supercritical carbon dioxide extraction and Soxhlet extraction. Janporn et al. (2015) also reported significant differences among different extraction methods in fatty acid composition and some chemical and physical parameters for *Terminalia catappa* seed oil after using extraction methods such as Soxhlet and maceration extraction.

3.3. Composition of alpha (α)-, beta (β)-, gamma (γ) −, and delta (δ)-tocopherol

The α-, β-, γ- and δ-tocopherol compositions of the Manketti nut oil obtained through three different extraction methods are presented in Table 3. The cold pressed Manketti oil contained the lowest amount of total tocopherol of 137 mg/100 g of oil, with the Soxhlet extracted Manketti nut oil having the highest total tocopherol content of 206 mg/100 g oil. The dominant tocopherol detected was γ-tocopherol. In various seeds, the major form of the vitamin E is γ-tocopherol and has been reported to have various potential medicinal properties (Jiang et al., 2001). β-Tocopherol was not detected in the traditionally extracted Manketti nut oil but was detected in the cold pressed and Soxhlet extracted Manketti nut oil, although at very low levels. In the Manketti nut oil from Botswana, β- and δ-tocopherol was not detected, while it contained α- (0.564 mg/100 g) and γ- (223.3 mg/100 g) tocopherol (Mitei et al., 2009). The content of total tocopherol of the Manketti nut oil is comparable to that found in hemp seed oil (150 mg/100 g oil) and redcurrant seed oil (145 mg/100 g oil), but higher than compared to soybean (96 mg/100 g oil), sunflower (55 mg/100 g oil), coconut (1 mg/100 g oil) and olive (22 mg/100 g oil) oil (Gunstone et al., 2007). The presence of high amounts of tocopherol improves the quality of oil (O'Brian, 2009) as tocopherols are potent natural antioxidants and efficiently prevent lipid peroxidation (Nasri et al., 2012). In particular, γ-tocopherol, which is an effective free-radical remover, has been reported by Ju et al. (2010) to possess strong anti-inflammatory mechanisms with inhibition abilities towards colon, prostate, mammary and lung tumorigenesis. It has been reported that increased resistance to oxidation inside the seed of *Jatropha curcas* is due to a high content of γ-tocopherol and its presence in the oil protects it from oxidation (Rodrigues et al., 2015). This could explain why the kernels could be stored up to 6 years and still be edible and palatable as reported by Peters (1987).

3.4. Composition of stigmasterol and β-sitosterol

The stigmasterol and the β-sitosterol content of Manketti nut oil obtained through the three different extraction methods are presented in Table 3 and ranged from 42.3–45.3 mg/100 g of oil and 587–682 mg/100 g of oil, respectively. The Soxhlet extracted Manketti nut oil had the lowest content of stigmasterol (42.3 mg/100 g oil) and the highest content of β-sitosterol (682 mg/100 g oil), while the traditionally extracted Manketti nut oil had the lowest content of β-sitosterol (587 mg/100 g oil). Mitei et al. (2009) reported a stigmasterol content of 3.62 mg/100 g of oil and a β-sitosterol content of 133 mg/100 g of oil for Manketti nut oil from Botswana. The ranges for stigmasterol and the β-sitosterol content for Manketti nut oil are lower than values reported by Gwatidzo et al. (2014) for Soxhlet extracted and screw press/cold press Manketti nut oil from the Okavango region of Namibia (Table 1). Gwatidzo et al. (2014) reported significant differences among four studied extraction methods in terms of the concentrations of stigmasterol and β-sitosterol in Manketti nut oil. The stigmasterol content of the Manketti nut oil is higher than the values reported for sunflower (33.7 mg/100 g), cotton (5 mg/100 g) and coconut (12.5 mg/100 g) oil and lower than soybean (57.7 mg/100 g) and corn (67.7 mg/100 g) oil (Gunstone et al., 2007). The β-sitosterol content of Manketti nut oil was comparable to that of corn oil (646 mg/100 g oil), higher than coconut (48.6 mg/100 g), olive (130.3 mg/100 g oil), soybean (173 mg/100 g) and sunflower (265 mg/100 g) oil (Gunstone et al., 2007). The presence of β-sitosterol has been reported to impart anti-fungal, anti-inflammatory and anti-viral properties (Malini and Vanithakumari, 1990). Generally, phytosterols have been reported to possess antioxidant, anticancer and anti-inflammatory properties (Yoshida and Niki, 2003; Gabay et al., 2010; Suttiarporn et al., 2015) and have a wide range of applications in fortified foods (Duong et al., 2016).

3.5. Compositional analysis of fatty acids

The fatty acids found in Manketti nut oil from Namibia as determined by GC–MS analysis are presented in Table 3. Linoleic and arachidic acid were found to be at the same concentration ratio among the three extraction methods. The linoleic acid content for Manketti nut oil among the three extraction methods was similar to that of argan oil (31–37%), and higher than values reported for some major edible oils such as coconut oil (1.0–2.5%), palm kernel oil (1.0–3.5%) and rapeseed (11.0–23.0%) oil (Gunstone et al., 2007). The linoleic acid content was lower than that reported by Mitei et al. (2008), Chivandi et al. (2008) and Gwatidzo et al. (2017a) for Manketti nut oil (Table 1). Mitei et al. (2008) and Chivandi et al. (2008) did not report the presence of α-eleostearic acid in Manketti nut oil from Botswana and Zimbabwe, respectively, but it was reported in Manketti nut oil by Chisholm and Hopkins (1966), Juliani et al. (2007), Yeboah et al. (2017) and

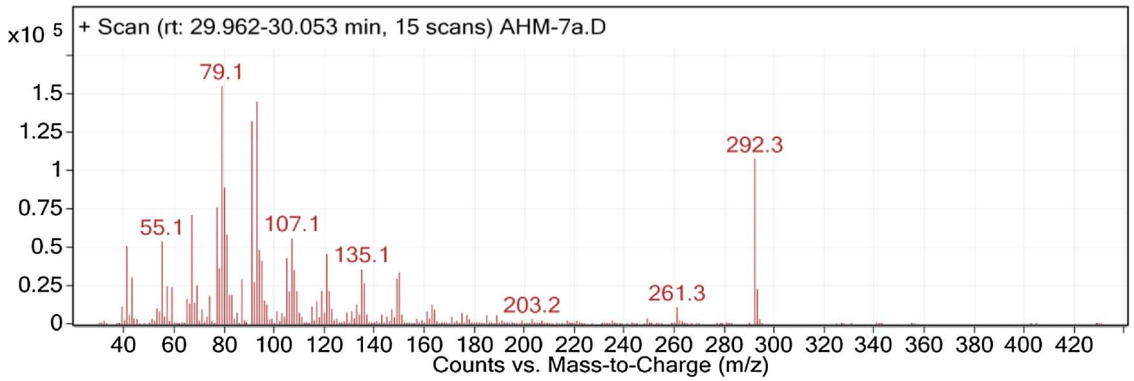

Fig. 4. Mass spectrum of α-eleostearic acid (C18:3 9c,11t,13t).

Gwatidzo et al. (2017a) at a level of 23–26%. The mass spectrum for α-eleostearic acid is presented in Fig. 4. The traditional and the Soxhlet extracted Manketti nut oil had a similar concentration ratio of α-eleostearic acid and was found to be at higher levels (34–36%) as compared to reported values. The cold pressed Manketti nut oil (24.2%) was found to be comparable to the reported values of 23–26%. α-Eleostearic acid has been found to possess potent antioxidant and anti-tumor activity (Zhang et al., 2012). The presence of erucic acid was not detected in the Manketti nut oil studied, but this fatty acid was reported by Chivandi et al. (2008) in Manketti nut oil from Zimbabwe (Table 1). The levels of stearic acid reported for Manketti nut oil are in the range of 7–12% (Zimba et al., 2005; Juliani et al., 2007; Mitei et al., 2008; Gwatidzo et al., 2017a), although Chivandi et al. (2008) reported a stearic acid content of 3.04% (Table 1). The traditional extracted (11.2%) and the Soxhlet extracted (10.4%) Manketti nut oil is within this reported range, with the cold pressed Manketti nut oil high in stearic acid (14.3%). The cold pressed Manketti nut oil had the highest concentration ratio of palmitic acid when compared to the other two methods. Lowest content of oleic acid was found in the Soxhlet extracted Manketti nut oil with the other two methods having the same concentration ratio. Traditional and Soxhlet extracted Manketti nut oil had the same concentration ratio of total saturated fatty acids (1:1) while that of the cold pressed oil was slightly higher (1.5). The total content of saturated fatty acids of cold pressed Manketti nut oil (31%) allows the oil to be highly resistant to oxidation (Choi et al., 2014), with the traditional extracted (20.1%) and Soxhlet extracted (20.1%) oils being less resistant to oxidation, due to lower content of saturated fatty acids. The total content of unsaturation of was similar for the traditional and Soxhlet extracted Manketti nut oil, in particular with relation to the polyunsaturation, and slightly higher when compared to the cold pressed oil. Total content of monounsaturation was at the same ratio for traditional and cold pressed Manketti nut oil and slightly higher when compared to the Soxhlet extracted oil. The total unsaturated fatty acids (69.0–79.9%) and total saturated fatty acids (20.1–31%) was comparable to that of the Manketti nut oil from Botswana (Mitei et al., 2008). The Manketti nut oil has potential applications in tissue regeneration, cellular repair and treatment of inflammation due to the significant presence of tocopherol, phytosterol, α-eleostearic and linoleic acid (Zimba et al., 2005).

3.6. ^{1}H NMR spectral analysis

The assignments of the main resonances in the ^{1}H NMR spectra were assigned according to Sacchi et al. (1997), Popescu et al. (2015), Timilsena et al. (2017) and Yeboah et al. (2017) and are presented in Table 4. The presence of protons of the main components of the Manketti nut oil resulted in a total of 11 spectral signal groupings. The signal profiles for the three different extraction methods were similar. The functional groups as assigned and presented in Table 4 were $-CH_3$ (methyl proton) at 0.83–0.88 ppm, $-(CH_2)_n-$(methylene groups) at 1.22–1.33 ppm, $-OCO-CH_2-CH_2-$ (methylene groups) at 1.58 ppm, $-CH_2-CH=CH-$ (allylic protons) at 1.96–2.16 ppm, $-OCO-CH_2-$ (methylene groups) at 2.26–2.30 ppm, $=CHCH_2CH=$ (bisallylic methylene protons) at 2.72–2.75 ppm, $-CH_2OCOR$ (glycerol group) at 4.09–4.29 ppm, $>CHOCOR$ (glycerol group) at 5.21–5.26 ppm and $-CH=CH-$ (olefinic protons) at 5.28–5.38 ppm. The signals observed in the region of 5.63–6.37 ppm account for the presence of α-eleostearic acid (9Z,11E,13E-octadecatrienoic acid) (Seremeta et al., 2015; Yeboah et al., 2017) in the Manketti nut oil.

Table 4
Chemical shifts and assignments of main resonances in ppm (δ) of the ^{1}H NMR spectra of Manketti nut oil obtained from different extraction methods.

assignment (proton)	traditional	cold pressing	Soxhlet
$-CH_2-CH_2-CH_2-CH_3$	0.83–0.88	0.83–0.88	0.84–0.82
$(CH_2)_n$	1.22–1.34	1.23–1.33	1.22–1.26
$-CH_2-CH_2-COOH$	1.58	1.58	1.56
$-CH_2-CH=CH-$	1.97–2.15	1.96–2.16	2.00–2.12
$-CH_2-COOH$	2.26–2.30	2.26–2.30	2.25–2.26
$-CH=CH-CH_2-CH=CH-$	2.75–2.82	2.72–2.75	2.73
$-CH_2-OCO-R$	4.09–4.13	4.09–4.14	4.08–4.12
$-CH_2-O-COR$	4.25–4.29	4.25–4.29	4.24–4.27
$CH-OCO-R$	5.21–5.26	5.21–5.26	5.22–5.24
$-CH=CH-$	5.28–5.38	5.28–5.38	5.30
Protons of α-(ESA)[a]	5.63–6.37	5.63–6.37	5.63–6.34
Solvent ($CDCl_3$)	7.24	7.24	7.24

[a] Yeboah et al. (2017).

4. Conclusions

The physicochemical characterization of Manketti nut oil from Namibia revealed that this oil possesses good quality parameters. The oil, in particular the traditional and the Soxhlet extracted Manketti nut oil contained a significant presence of the conjugated fatty acids, α-eleostearic (9*Z*,11*E*,13*E*-octadecatrienoic acid) and linoleic acid (9*Z*,12*Z*-octadeca-9,12-dienoic acid)., Tocopherols, particular the traditional and Soxhlet extracted Manketti nut oil have been found at significant levels with potential in applications for functional foods production. Phytosterols, in particular the cold pressed and Soxhlet extracted Manketti nut oil were present at significant levels for promoting the further use of this unique oil in nutraceutical developments. The high content of unsaturated fatty acids and γ-tocopherol suggest that this oil can be used for various food applications, such as a frying oil, and could be promoted to be used by local communities as a replacement for the usual imported commercial vegetable oils used in Namibia. This oil is a good candidate for further product development initiatives in the nutraceutical and cosmetics industry.

Conflicts of interest

The authors declare no conflict of interest to disclose.

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