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# Characterization of *Acanthosicyos horridus* and *Citrullus lanatus* seed oils: two melon seed oils from Namibia used in food and cosmetics applications

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**Abstract** The physicochemical characteristics, fatty acid, tocopherol, stigmaterol,  $\beta$ -sitosterol, and <sup>1</sup>H NMR profiles of *Citrullus lanatus* and *Acanthosicyos horridus* melon seed oils were determined and compared among different extraction methods (cold pressing, traditional, and Soxhlet). The oil content was  $40.2 \pm 3.45$  and  $37.8 \pm 7.26\%$  for *C. lanatus* and *A. horridus*, respectively. Significant differences ( $p < 0.05$ ) were observed among the different extraction methods in the characteristics studied. Physicochemical characteristics of the melon seed oils were saponification value, 180.48–189.86 mg KOH/g oil; iodine value, 108.27–118.62 g I<sub>2</sub>/100 g oil; acid value, 0.643–1.63 mg KOH/g oil; peroxide value; 1.69–2.98 mequiv/kg oil; specific gravity, 0.901–0.922; and refractive indices, 1.4676–1.4726. The dominant tocopherol was  $\gamma$ -tocopherol with total tocopherol in the range 27.61–74.39 mg/100 g. The dominant fatty acid was linoleic acid in the range 52.57–56.96%. The favorable oil yield, physicochemical characteristics, tocopherol, and

fatty acid composition have the potential to replace or improve major commercial vegetable oils and to be used for various applications in the food industry and nutritive medicines.

**Keywords** Namibia · Melon seed oils · *Acanthosicyos horridus* · *Citrullus lanatus* · Physicochemical characterization

## Introduction

*Citrullus lanatus* and *Acanthosicyos horridus* are two melon varieties belonging to the Cucurbitaceae family, which are commonly used by indigenous people for various food and cosmetics applications. The *Citrullus* genus is a member of the Cucurbitaceae family and is generally called the Cucurbits or the gourd family (Alnadif et al. 2017). The family consists of several melon varieties that are of great economic importance (Jeffrey 1990). The fruit and seeds of the *C. lanatus* and the *C. colocynthis* have been classified as being great food sources in many parts of Africa (Mabaleha et al. 2007). The *C. lanatus* (Thunb.) Matsum & Nakai is commonly called the Tsamma melon, wild watermelon or the Kalahari melon, and is widespread throughout Namibia. There are three categories of local melons existing in Namibia: the seed melons for oil production, cooking melons for porridge, and watermelons for consumption of fresh fruit (Maggs-Kölling and Christiansen 2003). In northern Namibia, the melon is also used for intercropping purposes on pearl millet fields (Maggs-Kölling and Christiansen 2003).

The *C. lanatus* melon seed oil is produced commercially in Kavango, Zambezi, and the north-central regions of Namibia (MCA-N 2012). A variety of European cosmetics

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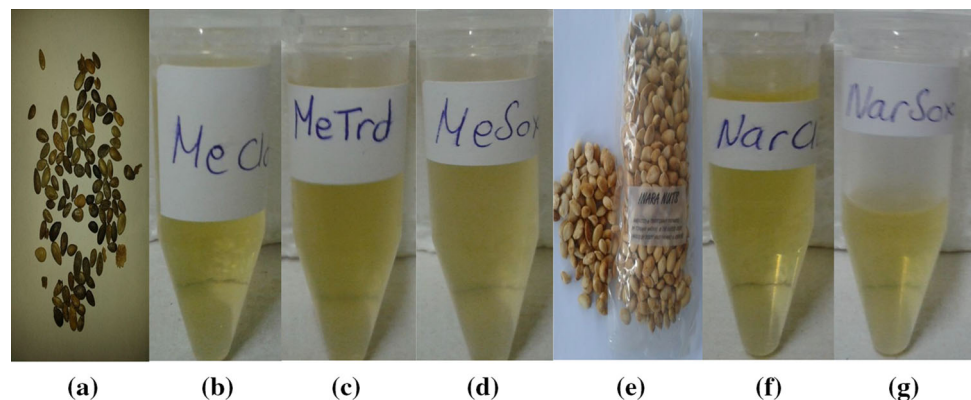
industries use the *C. lanatus* melon seed oil for product development for use as moisturizers, skin regeneration, and restructuring formulations (Nyam et al. 2009). Rural women of Namibia produce the oil in their homesteads, and is then used for healing applications, massages, cooking oil, and as a moisturizer (Lendelvo et al. 2012). Cold pressed *C. lanatus* melon seed oil is produced commercially by various small to medium enterprises. Traditionally, *C. lanatus* melon seed oil is prepared by first pounding the sun-dried seeds (Fig. 1a) to a fine powder and then soaking them in water. This mixture is then filtered through a mesh of straw into a three-legged iron-cast pot. The mixture is then boiled for several hours until the oil separates out from the boiling mixture. The layer of oil that forms on top of the mixture is decanted over time as the boiling process continues. The oil is then stored in 200 mL glass bottles and sold on the traditional markets and for household use (Personal communications).

*Acanthosicyos horridus* Welw. Ex Hook.f. is commonly called the !Nara plant and is a member of the Family Cucurbitaceae. The plant occurs sporadically throughout the Namib Desert to which it is also endemic. The greatest numbers are found around the Kuiseb River Delta and some around the Sossusvlei (Maggs-Kölling et al. 2014) of Namibia. The !Nara plant is a leafless, thorny shrub, and is an important food source for the local Topnaar people and their livestock (Ito 2003; Mizuno and Yamagata 2005). The Topnaar people, a Nama tribe, are one of the oldest indigenous people of Namibia, living in the Lower Kuiseb Valley in the Namib (Henschel et al. 2004). The use of the !Nara plant has been recorded to be utilized by indigenous people along the Namibian coast as far back as 1677 (NBRI 2017). To the Topnaar communities, the !Nara plant is of fundamental cultural value, based on a long tradition of harvesting and gathering. The practice provides an income in addition to that obtained from goat farming (Henschel et al. 2004). The word !Nara is derived from !Naranin, the alternate name for the word Topnaar,

underpinning the cultural importance of this plant to the Topnaar (Dentlinger 1977; Van den Eynden et al. 1992). The harvesting period for the !Nara fruit is between December and March (Mizuno and Yamagata 2005). The ripe fruits are spiny and pale-green, and can weigh between 1.0 and 2.5 kg (Maggs-Kölling et al. 2014). Harvesting around 1 kg of the !Nara fruit seeds takes about 3 h with harvesters moving among plants by foot and/or donkey cart. It is essential to use the correct harvesting techniques to ensure that the plant is not damaged, therefore ensuring sustainable harvesting practices of indigenous plants. Fruits are removed from bushes according to a traditional method by employing a long wooden stick or with modern ways, using an iron hook (Maggs-Kölling et al. 2014). The fruit flesh is then removed and boiled to extract the seeds or pips (Henschel et al. 2004). Seeds are then dried to be sold to customers for oil processing or to make the so-called healthy “butterpips snack”. The boiled fruit flesh or pulp is also mixed with mealie meal porridge (Wyk and Gericke 2000) or dried to make the so-called “!Nara-chocolate” (Maggs-Kölling et al. 2014). Each !Nara bush may yield 20–500 melons with each fruit containing between 50 and 200 seeds. One requires 10–20 melons to yield 1 kg of seed at which yields depend greatly on the annual climate conditions and predation activities (Maggs-Kölling et al. 2014) in the area of growth. In Namibia, *A. horridus* melon seed oil is not produced traditionally by rural communities and a cold pressed version is produced by the Desert Hills Company in Swakopmund, Namibia. The company purchases the !Nara seeds from the rural communities. A great variety of food and cosmetic products are then produced from the !Nara seed oil and sold in the Namibian market.

Oils produced from the seeds of the species of the Cucurbitaceae family are not used on an industrial scale, although they are commonly used in several African and Middle Eastern countries as cooking oil (Al-Khalifa 1996) and as moisturizer. To date, to the best of our knowledge, no scientific data towards the characterization of the *A.*

**Fig. 1** Seeds of *C. lanatus* (a), cold pressed (b), traditionally extracted (c) and Soxhlet extracted (d) *C. lanatus* melon seed oil, seeds of *A. horridus* (e) cold pressed (f) and Soxhlet extracted (g) *A. horridus* melon seed oil



*horridus* melon seed oil from Namibia have been published. Data of the *C. lanatus* melon seed oil are scattered throughout literature and this study compiled physicochemical characteristics of the seed oil of *C. lanatus* specifically from Namibia. This study reports on the physicochemical characterization of seed oils obtained by different extraction methods from *C. lanatus* and *A. horridus* with the intent of strengthening the importance of promoting their use at industrial scale and towards the improvement of income generation of local communities in Namibia.

## Materials and methods

### Materials

Cold pressed *A. horridus* melon seed oil (NarCld) and seeds were obtained from Desert Hills Company in Swakopmund, Namibia. Cold pressed *C. lanatus* melon seed oil (MeCld) was obtained from the Eudafano Women's Cooperative in Ondangwa, Namibia. Traditionally produced *C. lanatus* melon seed oil (MeTrd) and seeds were obtained from a homestead located near Tsandi, northern Namibia. Seeds were ground using a coffee grinder and hexane-extracted in triplicates with a Soxhlet apparatus. The solvent was removed under vacuum at 40 °C using a rotary evaporator (Heidolph, Germany). The oil yield was determined, and the samples obtained using Soxhlet extraction of *A. horridus* melon seed oil (NarSox) and *C. lanatus* melon seed oil (MeSox) were stored briefly in dark at 4 °C until further analysis.

### Determination of physicochemical characteristics

The characteristics, such as saponification value, acid value, and iodine value of the melon seed oils, were determined according to the AOAC official methods (1998). The *p*-anisidine value was determined according to the AOCS official method Cd 18–90 (1993). Peroxide values were determined using the ferric thiocyanate method according to Uluata and Özdemir (2012). The refractive indices of the oil samples were determined using an ABBE refractometer (K7135, MRC Labs) at 25 °C and the specific gravity was determined according to the AOAC official method No. 40.1.08 (1990).

### Proton NMR spectral analysis

<sup>1</sup>H NMR spectra of the melon seed oils were recorded using the Bruker Avance 400 MHz spectrometer (Germany). Samples were dissolved in CDCl<sub>3</sub> at 25 °C. Data were reported as “chemical shifts (δ)” in ppm.

### GC–MS analysis of fatty acid composition

Fatty acids were esterified to methyl esters according to Yang et al. (2013). The analysis was carried out by gas chromatography, model 7820A coupled with mass spectrometry unit, model 5977E MSD and Agilent ChemStation Software (Agilent Technologies, Palo Alto, CA). Samples (1 μL) after injection were separated using an Agilent capillary column HP5 MS (30 m × 0.25 mm, 0.25 μm). Helium was used as carrier gas with a flow rate of 1.50 mL/min and with a split ratio of 20:1. The injection temperature was 250 °C. The initial temperature was 40 °C, held for 8 min, ramp 1: 10–220 °C for 5 min, and ramp 2: 20–300 °C for 10 min. The mass spectrometer was set to scan in the range of *m/z* 30–600.

### GC–MS analysis of tocopherol and major sterol composition

Tocopherol and major sterol compositions were determined according to Du and Ahn (2002). Oil, about 100 mg, was extracted with 10 mL of the saponification reagent [ethanol:33% KOH (w/v):20% ascorbic acid (94:6:0.5)]. Then 100 μL of 5α-cholestane (10 ppm) was added as the internal standard. The mixture was vortexed and incubated for 60 min at 50 °C, and then cooled for 10 min in ice. Deionized water (5 mL) and hexane (5 mL) were then added. The mixture was then vortexed to allow for phase separation and left for 15 h. Then 1000 μL of the supernatant was dried, after which the dry samples were reconstituted with 200 μL pyridine followed by 100 μL BSTFA with 1% TMCS, vortexed, and then derivatized by incubating for 1 h at 50 °C. The injection volume used was 1 μL and injected into the GC, Agilent 6890 N (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5975 MS detector, using a Zebron AB-MultiResidue (30 m, 0.25 mm ID, 0.25 μm film thickness) column (Part No. 7HG-G016-11) for a runtime of 40 min. 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 was used as carrier gas at a flow rate of 1.2 mL/min, and the injector temperature was maintained at 200 °C and operated in a split-less mode. The mass spectral data were recorded on a MSD operated in full scan mode (35–600 *m/z*) with both the ion source and quadrupole temperatures maintained at 240 and 150 °C, respectively. The transfer line temperature was maintained at 200 °C. Solvent delay was held at 5 min. The relative retention times of the samples were compared with authentic standards and their calibration curves. Data were reported as mg of tocopherols or major sterols in 100 g of oil.

## Statistical analysis

All experiments were carried out in triplicates, and means were compared with an analysis of variance (ANOVA) followed by analysis with the Tukey's HSD Test using IBM® SPSS® Statistics Version 24 Software to determine significant differences ( $\alpha = 0.05$ ). Values with different letters within rows indicated significant differences ( $p < 0.05$ ).

## Results and discussion

### Oil sources and extraction

The extractions of the two melon seed oils yielded edible yellow oils with a nutty flavor and odour, which are both liquid at room temperature (Fig. 1). The melon seed oils extracted from *C. lanatus* and *A. horridus* seeds resulted in a yield of  $40.16 \pm 3.45$  and  $37.79 \pm 7.26\%$  of oil, respectively, which is higher than that reported for melon seeds (24.80%) from Botswana (Mabaleha et al. 2007) and *C. colocynthis* (23.16%) from Saudi Arabia (Nehdi et al. 2013). The seeds of the *C. lanatus* melon are easily removed from the fruit and the plant is fast growing. In addition, its high oil yield makes this melon species a sustainable resource for exploitation commercially and is a sustainable food source for indigenous people during times of food scarcity.

### Physicochemical characteristics

The physicochemical characteristics of *C. lanatus* and *A. horridus* melon seed oils obtained from different extraction methods were determined and compared, and presented in Table 1. Most of the physicochemical characteristics showed significant differences ( $p < 0.05$ ) among the oils extracted. The saponification value for *C. lanatus* melon seed oil ranged from 181.12 to 189.26 mg KOH/g of oil, with the MeSox being significantly lower. Saponification values for *C. lanatus* melon seed oils have been reported to range from 173 to 185 mg KOH/g of oil (Mabaleha et al. 2007; Nyam et al. 2009). The saponification value of the *C. lanatus* melon seed oil is comparable to some common vegetable oils, such as canola (182–193) oil, rice bran (181–189) oil, and castor (176–187) oil, with MeCId and MeTrd comparing specifically to that of corn (187–195) oil, groundnut (187–196) oil, and sesame (187–195) oil (Gunstone et al. 2007). The *A. horridus* melon seed oil had saponification values ranging between 180.48 and 186.19 mg KOH/g of oil with the NarSox being the significantly lower. The saponification value for the *A. horridus* melon seed oil is comparable to major edible oils,

such as canola (182–193) oil and rice bran (181–189) oil (Gunstone et al. 2007). The saponification values of *C. lanatus* and *A. horridus* melon seed oils would suggest the presence of mainly the medium-chain fatty acids such as C16 and C18 (Mabaleha et al. 2007), which was confirmed with the GC–MS compositional analysis of fatty acids (Table 4).

Iodine values are a measure of the degree of unsaturation (Khatab and Zeitoun 2013). The iodine values of *C. lanatus* melon seed oil ranged from 110.28 to 118.61 g of  $I_2/100$  g of oil with the MeSox being significantly lower (110.28 g of  $I_2/100$  g of oil). Iodine values for *Citrullus* seed oils across Africa have been reported to range from 95.0 to 125 g of  $I_2/100$  g of oil (Mabaleha et al. 2007; Gbogouri et al. 2011; Nyam et al. 2009; Mariod et al. 2009). The iodine values for *C. lanatus* melon seed oil are compared to major edible oils, such as canola (110–126) oil, corn (107–128) oil, and sesame (104–120) oil (Gunstone et al. 2007). The iodine values for *A. horridus* melon seed oil were lower than that observed for the *C. lanatus* melon seed oil, and among NarCId (111.03 g of  $I_2/100$  g of oil) and NarSox (108.27 g of  $I_2/100$  g of oil), the values were significantly different. The iodine values for *A. horridus* melon seed oil were compared to major edible oils, such as rice bran (99–108) oil, canola (110–126) oil, and corn (107–128) oil (Gunstone et al. 2007).

The acid values of *C. lanatus* melon seed oil ranged from 0.95 to 1.63 mg KOH/g of oil with the MeTrd being significantly higher. The acid values of *A. horridus* melon seed oil ranged between 0.643 and 0.708 mg KOH/g of oil. The peroxide values differed significantly among the oils of *C. lanatus* melon seed oil ranging from 1.69 to 2.98 mequiv/kg of oil with the MeTrd being significantly higher, whilst the peroxide value of the *A. horridus* melon seed oil ranged between 2.51 and 2.77 mequiv/kg, with no significant differences between the two extraction methods. Values observed for acid and peroxide values of *C. lanatus* and *A. horridus* melon seed oils are within the acceptable levels of the standards for edible oils of cold pressed origin (CODEX STAN 210-1999 2011), and are within reported values (2.3–9.8 mequiv/kg of oil) of melon seed oils (Mabaleha et al. 2007; Gbogouri et al. 2011; Nyam et al. 2009; Mariod et al. 2009). MeTrd was observed to have the highest *p*-anisidine value (1.69), and was significantly higher compared to MeCId and MeSox, but lower than the value (2.2) reported from melon seed oil from Botswana (Mabaleha et al. 2007). No significant differences in *p*-anisidine values between NarCId and NarSox were observed, and were comparatively lower than those observed for the *C. lanatus* melon seed oil. The measurement of the *p*-anisidine value is directly proportional to the measurement of the free fatty acids (Khatab and Zeitoun 2013), reported as the acid value in this study. The reason

**Table 1** Physico-chemical characteristics of *C. lanatus* and *A. horridus* melon seed oils

Extraction method	<i>C. lanatus</i>			<i>A. horridus</i>		Sunflower oil <sup>a</sup>	Olive oil <sup>a</sup>
	Cold pressed Liquid	Traditional Liquid	Soxhlet Liquid	Cold pressed Liquid	Soxhlet Liquid		
Saponification value (mg KOH/g oil)	187.86 ± 2.42 <sup>a</sup>	189.26 ± 1.40 <sup>a</sup>	181.12 ± 1.85 <sup>b</sup>	186.19 ± 2.21 <sup>a</sup>	180.48 ± 0.85 <sup>b</sup>	188–194	184–196
Acid value (mg KOH/g oil)	1.63 ± 0.059 <sup>a</sup>	1.09 ± 0.029 <sup>b</sup>	0.947 ± 0.13 <sup>b</sup>	0.643 ± 0.041 <sup>a</sup>	0.708 ± 0.098 <sup>a</sup>		
Peroxide value (mequiv/kg)	2.98 ± 0.20 <sup>a</sup>	2.12 ± 0.052 <sup>b</sup>	1.69 ± 0.092 <sup>c</sup>	2.77 ± 0.22 <sup>a</sup>	2.51 ± 0.12 <sup>a</sup>		
<i>p</i> -Anisidine value	1.69 ± 0.17 <sup>a</sup>	1.18 ± 0.11 <sup>b</sup>	0.79 ± 0.21 <sup>b</sup>	0.427 ± 0.14 <sup>a</sup>	0.616 ± 0.18 <sup>a</sup>		
Iodine value (g of I <sub>2</sub> /100 g oil)	118.61 ± 1.03 <sup>a</sup>	117.64 ± 1.96 <sup>a</sup>	110.28 ± 2.60 <sup>b</sup>	111.03 ± 1.70 <sup>a</sup>	108.27 ± 0.94 <sup>b</sup>	118–145	75–94
Specific gravity (20 °C)	0.920 ± 0.001 <sup>a</sup>	0.922 ± 0.001 <sup>a</sup>	0.910 ± 0.011 <sup>b</sup>	0.921 ± 0.001 <sup>a</sup>	0.901 ± 0.008 <sup>b</sup>	0.918–0.923	0.910–0.916
Refractive index (25 °C)	1.4720 ± 0.001 <sup>a</sup>	1.4726 ± 0.001 <sup>b</sup>	1.4665 ± 0.001 <sup>c</sup>	1.4724 ± 0.001 <sup>a</sup>	1.4676 ± 0.001 <sup>b</sup>	1.467–1.469 (40)	1.468–1.470 (20)

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

<sup>a</sup> Gunstone et al. (2007)

is that the *p*-anisidine value reflects the composition of secondary products as generated from free fatty acids due to oxidation and decomposition (Frankel 1985; Khattab and Zeitoun 2013).

The specific gravity of *C. lanatus* melon seed oil ranged from 0.910 to 0.922, with the MeSox being significantly lower, whilst the specific gravity for *A. horridus* melon seed oil ranged between 0.881 and 0.921. Specific gravities of melon seed oils have been reported to range from 0.886 to 0.950 (Mabaleha et al. 2007; Mariod et al. 2009; Gbogouri et al. 2011). The *C. lanatus* melon seed oil was observed to have refractive indices (1.4665–1.4720) which were significantly different among the three extraction methods investigated. The same was observed for the *A. horridus* melon seed oil (1.4676–1.4724). Refractive indices of melon seed oils have been reported to range from 1.429 to 1.469 (Mabaleha et al. 2007; Mariod et al. 2009).

### Tocopherol and major sterol composition

The tocopherol and major sterol content of *C. lanatus* and *A. horridus* melon seed oils as determined by GC-MS are presented in Table 2. The MeTrd was observed to have the lowest total tocopherol content (27.6 mg/100 g oil) compared to MeCld and MeSox, of which the MeCld had the highest total tocopherol content (74.4 mg/100 g oil). The low value was affected by the lower presence of the  $\gamma$ -tocopherol (16.3 mg/100 g of oil) found in the MeTrd. The *A. horridus* melon seed oil had a total tocopherol

content range 44.3–46.1 mg/100 g of oil, which was lower when compared to MeCld and MeSox. The dominant tocopherol for *C. lanatus* and *A. horridus* melon seed oils was the  $\gamma$ -tocopherol, with the highest concentration (59.99 mg/100 g of oil) found in MeCld. The most effective antioxidant among the tocopherols is the  $\gamma$ -tocopherol (O'Brian 2009). Tocopherols are considered to be potent natural antioxidants and efficiently prevent lipid peroxidation (Nasri et al. 2012) by imparting stability on free radicals and improving the quality of the oil (O'Brian 2009). The total tocopherol content of the *C. lanatus* and *A. horridus* melon seed oil is higher than that found in groundnut (37 mg/100 g oil) oil, olive (22 mg/100 g oil) oil, sunflower (55 mg/100 g oil) oil, almond (28 mg/100 g oil) oil, and peanut (19 mg/100 g oil) oil (Gunstone et al. 2007).

The MeCld exhibited the highest stigmaterol content (44.11 mg/100 g of oil) with the lowest content found in the NarCld (20.53 mg/100 g of oil). The stigmaterol content of MeCld and NarCld is higher than that of coconut (12.5 mg/100 g oil) oil, cotton seed (5.0 mg/100 g oil) oil, and sunflower (33.7 mg/100 g oil) oil (Gunstone et al. 2007). The MeSox was observed to contain the highest amount of  $\beta$ -sitosterol (58.1 mg/100 g of oil), with the NarSox containing the lowest amount of  $\beta$ -sitosterol at 19.84 mg/100 g of oil. The presence of  $\beta$ -sitosterol has been shown to exhibit antiviral, anti-inflammatory, and antifungal properties (Malini and Vanithakumari 1990), which adds to the potential use of these melon seed oils as nutritive and medicinal commodities.

**Table 2** Tocopherol and sterol compositions (mg/100 g of oil) of *C. lanatus* and *A. horridus* melon seed oils

Extraction method	<i>C. lanatus</i>			<i>A. horridus</i>		Sunflower oil <sup>a</sup>	Olive oil <sup>a</sup>
	Traditional	Cold pressed	Soxhlet	Cold pressed	Soxhlet	Bleached	Cold pressed
$\alpha$ -Tocopherol	6.22	9.23	7.07	4.66	6.31	49	20
$\beta$ -Tocopherol	0.78	0.74	0.73	0.52	0.79	–	–
$\gamma$ -Tocopherol	16.33	59.99	44.81	35.54	45.13	5	1
$\delta$ -Tocopherol	4.28	4.43	4.54	3.62	4.14	1	–
Total Tocopherol	27.61	74.39	57.15	44.51	46.10	55	22
Stigmasterol	37.13	44.11	28.30	20.53	24.63	33.7	–
$\beta$ -Sitosterol	39.66	42.08	58.05	19.84	26.80	265.3	130.3

<sup>a</sup> Gunstone et al. (2007)

### NMR analysis

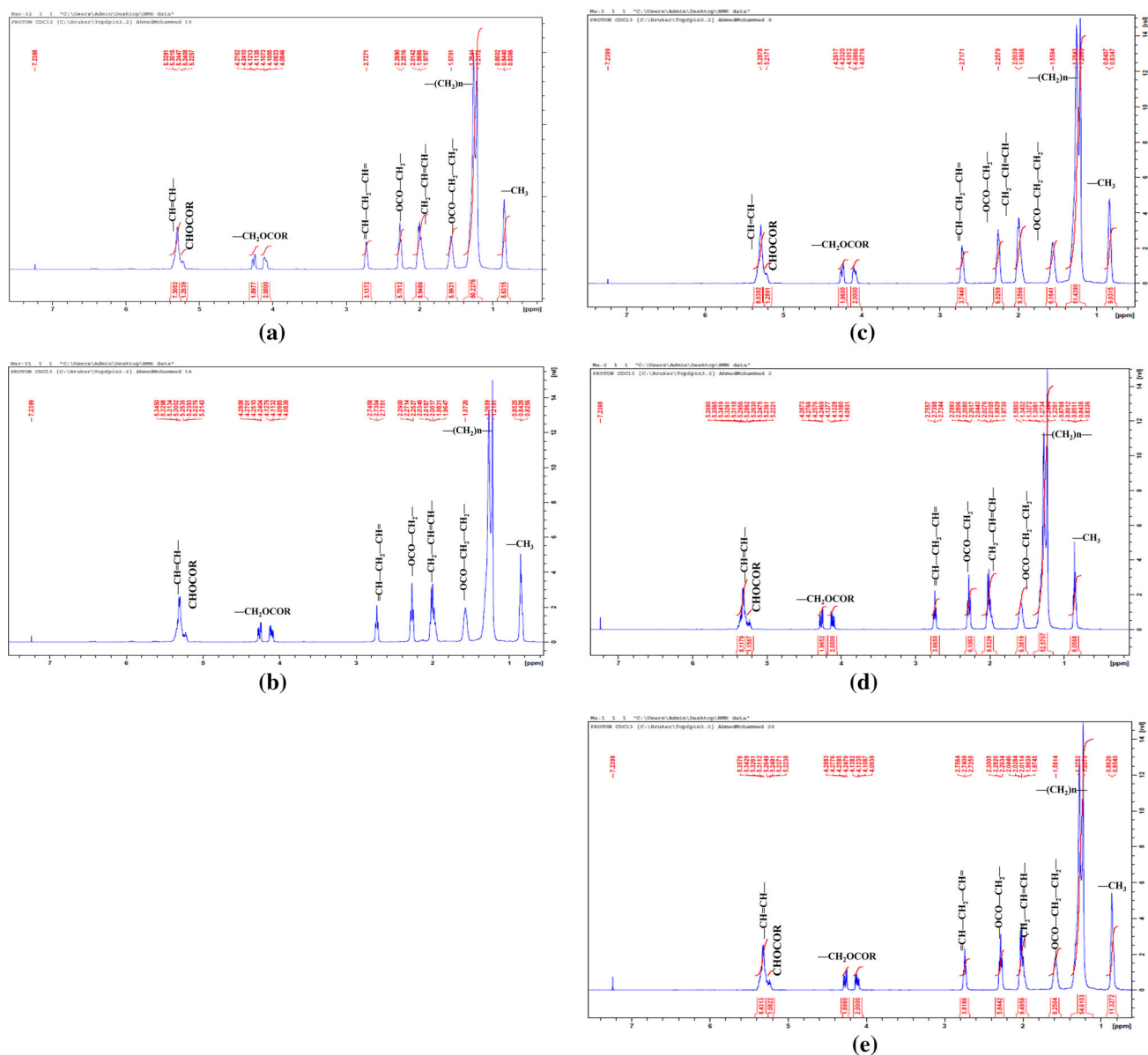
The assignments of the main resonances in the <sup>1</sup>H NMR spectra (Fig. 2) of *C. lanatus* and *A. horridus* melon seed oils are shown in Table 3, and were assigned according to Nehdi et al. (2013), Zhao and Zhang (2013), and Siyanbola et al. (2015). The <sup>1</sup>H NMR spectroscopy allowed for the study of the molecular structure of the melon seed oils (Bhutada et al. 2016) and gave a comparative insight on oil composition among the different extraction methods. The <sup>1</sup>H NMR profiles of cold pressed, traditionally, and Soxhlet extracted melon seed oils were similar among the different extraction methods, indicating minor variation in oil composition and quality. The presence of protons in the main components of the melon seed oils, such as the triacylglycerides (Zhao and Zhang 2013), resulted in a total of nine spectral signals, which has also been reported for *C. colocynthis* melon seed oil from Saudi Arabia (Nehdi et al. 2013). The  $-\text{CH}_2-$  protons of the glycerol appeared in the region 4.08–4.29 ppm. Signals observed at 5.51–5.36 ppm accounted for the  $-\text{CH}$  of the glycerol backbone and  $=\text{CH}$  of the unsaturated carbons. Internal protons, namely,  $-\text{CH}_2$ , within fatty acids, were recognized in the region 1.22–1.28 ppm.

### Fatty acid composition

The fatty acid compositions determined by GC-MS of *C. lanatus* and *A. horridus* melon seed oils are presented in Table 4. The fatty acid content of MeCld and MeTrd was similar and varied slightly from the MeSox. The individual fatty acid content in MeSox was lower when compared to the MeCld and MeTrd oils, except for the fatty acid, oleic acid. The *C. lanatus* melon seed oil contained arachidic acid (1.03–1.38%), which was not detected in the *A. horridus* melon seed oil. On the other hand, *A. horridus* was found to contain eicosenoic acid (4.37–4.43%) and  $\alpha$ -eleostearic acid (1.0%). The  $\alpha$ -eleostearic acid, a linolenic acid isomer, has been reported to have potent antioxidant and anti-tumor

activity (Tsuzuki et al. 2004; Zhang et al. 2012). The dominant fatty acid in both oils was linoleic acid, an  $\omega$ -6 polyunsaturated fatty acid, in the range 52–57%. Linoleic as the dominant fatty acid is a characteristic property of melon seed oils (Mabaleha et al. 2007; Gbogouri et al. 2011; Mariod et al. 2009). Linoleic acid, other than palmitic, oleic, and stearic acids, is not synthesized in the body. A lack of intake will cause a deficiency (Vermaak et al. 2011), while it is necessary for the skin's normal growth (Choi et al. 2014), such as cell membrane synthesis and tissue regeneration (Górnaś and Rudzińska 2016). The  $\omega$ -6 polyunsaturated fatty acids have various medicinal uses such as decreasing total cholesterol in plasma (Kris-Etherton and Yu 1997) and improving the body's immune response (Ntambi et al. 2002). The linoleic acid content also allows the two melon seed oils to be used for various seasoning applications in the food industry (Nehdi et al. 2013). The two melon seed oils from Namibia are a good source of linoleic acid, since more than half of the fatty acids are made up of this essential fatty acid and have thus many applications in the food industry and in nutritive medicine developments. Palmitic acid content was in the range 14.8–16.6%, which was slightly higher than those reported (9.94–12.4%) for melon oils (Mabaleha et al. 2007; Gbogouri et al. 2011; Mariod et al. 2009). The oleic acid content ranged between 11.44 and 17.73%, which is comparable to reported (14.43–17.10%) values (Mabaleha et al. 2007; Gbogouri et al. 2011; Mariod et al. 2009). The oleic acid content of the two melon seed oils is higher than that of coconut (5.0–10.0%) oil and comparable to watermelon seed (13–19%) oil and hemp seed (8–15%) oil. The linoleic acid content is comparable to that of hemp seed (53–60%) oil, raspberry seed (55%) oil, rose hip seed (54%) oil, corn (34.0–65.6%) oil, cottonseed (46.7–58.2%) oil, and soybean (48.0–59.0%) oil, but higher than that of argan (31–37%) oil and Nigella seed (45%) oil (Gunstone et al. 2007).

The total unsaturated fatty acid (TUSFA) of *C. lanatus* melon seed oil made up 67.5–70.3% and the total saturated fatty acid (TSFA) made up 31.8–32.5%. The TUSFA of *A. horridus* melon seed oil made up 72.5–72.7% and the TSFA



**Fig. 2**  $^1\text{H}$  NMR spectra for cold pressed (a), Soxhlet extracted (b) *A. horridus* (!Nara) melon seed oil, cold pressed (c), Soxhlet extracted (d) and traditionally extracted (e) *C. lanatus* (Kalahari) melon seed oil

27.3–27.5%. The TSFA of the melon seed oils allows these to be highly resistant to oxidation (Choi et al. 2014), which has beneficial effects towards the improved shelf-life of products. The TUSFA content of the two melon seed oils was found to be higher than that of coconut (6.0–10.4%) oil and palm (48.5–53.7%) oil (Firestone 2006).

## Conclusions

In this study, the physicochemical characteristics, fatty acid, tocopherol, stigmasterol,  $\beta$ -sitosterol, and  $^1\text{H}$  NMR profiles of *C. lanatus* and *A. horridus* melon seed oils were

determined among different extraction methods (cold pressing, traditional, and Soxhlet). The oil content of the melon seeds (37–40%) allows these oils to be exploited at industrial scale production. The physicochemical characteristics determined conform to acceptable standards and could replace or improve common commercial vegetable oils, commonly consumed in Namibia. MeClId had the highest tocopherol content (74 mg/100 g), with MeSox having the highest oleic acid content (18%). The highest linoleic acid content (57%) was found in MeTrd and MeClId. In all of the oils, linoleic acid made up more than half of the total fatty acid content. The !Nara melon seed oil contained eicosenoic acid (4.4%) and  $\alpha$ -eleostearic acid



**Table 3** Chemical shifts and assignments of main resonances in ppm of the  $^1\text{H}$  NMR spectra of *C. lanatus* and *A. horridus* melon seed oils

Functional group	<i>C. lanatus</i>	<i>A. horridus</i>
–CH <sub>3</sub> (methyl proton)	0.85–0.86	0.83–0.85
–(CH <sub>2</sub> ) <sub>n</sub> –(acyl groups)	1.23–1.28	1.22–1.27
–OCO–CH <sub>2</sub> –CH <sub>2</sub> –(acyl groups)	1.58	1.57
–CH <sub>2</sub> –CH=CH–(allylic protons)	1.97–2.04	1.96–2.03
–OCO–CH <sub>2</sub> –(acyl groups)	2.26–2.30	2.25–2.29
–CH=CHCH <sub>2</sub> –(linoleyl chains)	2.73–2.76	2.72–2.75
–CH <sub>2</sub> OCOR (glycerol group)	4.09–4.29	4.08–4.28
>CHOCOR (glycerol group)	5.22–5.26	5.21–5.25
–CH=CH–(olefinic proton)	5.29–5.37	5.30–5.35

**Table 4** Fatty acid percent compositions (%) of *C. lanatus* and *A. horridus* melon seed oils

Extraction method	<i>C. lanatus</i>			<i>A. horridus</i>		Sunflower oil <sup>a</sup>	Olive oil <sup>b</sup>
	Cold pressed	Traditional	Soxhlet	Cold pressed	Soxhlet		
16:0	16.34	16.58	14.84	15.62	15.62	5.0–7.6	11.9
18:0	14.11	14.58	13.83	11.91	11.66	2.7–6.5	2.8
20:0	1.30	1.38	1.03	nd	nd	0.1–0.5	
18:1n-9	11.44	10.51	17.73	13.93	12.84	14.0–39.4	80.1
18:2n-6	56.81	56.94	52.57	53.12	54.54	48.3–74.0	3.3
11-20:1	nd	nd	nd	4.43	4.37		
9,11,13-18:3	nd	nd	nd	0.99	0.97	nd–0.3	
TUSFA	68.25	67.45	70.30	72.47	72.72		
TSFA	31.75	32.54	29.70	27.53	27.28		

nd not detected, TUSFA total unsaturated fatty acids, TSFA total saturated fatty acids

<sup>a</sup> Gunstone et al. (2007)

<sup>b</sup> Zimba et al. (2005)

(1%), whilst the *C. lanatus* contained arachidic acid (1–1.4%). MeCld and MeSox had the highest stigmaterol (44 mg/100 g) and  $\beta$ -sitosterol content (58 mg/100 g), respectively. The utilization of the seeds from indigenous sources into value-added products such as oils and products, thereof, allows for the improvement of better living standards for the rural communities who are the suppliers of such a unique resource.

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**Compliance with ethical standards**

**Conflict of interest** Authors report no conflict of interest.

## References

- Alnadif AM, Mirghani MES, Hussein I (eds) (2017) Cucurbits, Cucurbita species as new oil sources. In: Unconventional Oilseeds and Oil Sources. Elsevier, Netherlands, pp 81–84
- Al-Khalifa AS (1996) Physicochemical characteristics, fatty acid composition, and lipoxygenase activity of crude pumpkin and melon seed oils. J Agric Food Chem 44:964–966. doi:10.1021/jf950519s
- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington D.C
- AOAC (1998) Official methods of analysis of AOAC international (16th edition), 4th revision. Association of Official Analytical Chemists, Gaithersburg
- AOCS (1993) Official methods and recommended practices of the American Oil Chemists' Society, Method Cd 18-90. In: Firestone D (ed) 4th edn. American Oil Chemists' Society, Champaign
- Bhutada PR, Jadhav AJ, Pinjari DV, Nemade PR (2016) Solvent assisted extraction of oil from *Moringa oleifera* Lam. seeds. Ind Crops Prod 82:74–80. doi:10.1016/j.indcrop.2015.12.004
- Choi D, Lim G-S, Piao YL, Choi O-Y, Cho K-A, Park C-B, Chang Y-C, Song Y-II, Lee M-K, Cho H (2014) Characterization,

- stability, and antioxidant activity of *Salicornia herbacea* seed oil. Korean J Chem Eng 31(12):2221–2228. doi:10.1007/s11814-014-0163-7
- CODEX STAN (2011) Codex standard for named vegetable oils. FAO/WHO, Rome, (CODEX STAN 210-1999)
- Dentlinger U (1977) An ethnobotanical study of the !Nara plant among the Topnaar Hottentots of Namibia. Munger Afr Libr Notes 38:3–37
- Du M, Ahn DU (2002) Simultaneous analysis of tocopherols, cholesterol and phytosterols using gas chromatography. J Food Sci 67:1696–1700. doi:10.1111/j.1365-2621.2002.tb08708.x
- Firestone D (2006) Physical and chemical characteristics of oil, fat, and waxes. American Oil Chemists' Society Press, New York
- Frankel EN (1985) Chemistry of autoxidation: mechanism, products and flavour significance. In: Min DB, Smouse TH (eds) Flavor chemistry of fats and oils. AOCS, Champaign, pp 1–37
- Gbogouri GA, Brou K, Linder M, Tehrani EA, Gnakri D, Zoro Bi IA (2011) Comparative study of physicochemical and thermal properties of the seed oils of three cucurbit species. Int J Biol Chem Sci 5:1165–1177. doi:10.4314/ijbcs.v5i3.72246
- Górnaś P, Rudzińska M (2016) Seeds recovered from industry by-products of nine fruit species with a potential utility as a source of unconventional oil for biodiesel and cosmetic and pharmaceutical sectors. Ind Crops Prod 83:329–338. doi:10.1016/j.indcrop.2016.01.021
- Gunstone FD, Harwood JL, Dijkstra AJ (2007) The lipid handbook with CD-ROM, 3rd edn. CRC Press, Boca Raton
- Henschel J, Dausab R, Moser P, Pallett J (2004) !Nara—fruit for development of the !Khuiseb Topnaar. John Meinert Printing, Windhoek. ISBN 99916-40-33-9
- Ito M (2003) The change of !Nara vegetation and its influence on the people in the lower stream of the Kuiseb River, Namib Desert (in Japanese). In: Proceedings of the 40th annual meeting of the Japanese Association for African Studies, pp 90
- Jeffrey D (1990) Appendix: an outline classification of the Cucurbitaceae. In: Bates DM, Robinson RW, Jeffrey C (eds) Biology and utilization of the Cucurbitaceae. Cornell University, Ithaca, pp 449–463
- Khattab RY, Zeitoun MA (2013) Quality of flaxseed oil obtained by different extraction techniques. LWT Food Sci Technol 53:338–345. doi:10.1016/j.lwt.2013.01.004
- Kris-Etherton PM, Yu S (1997) Individual fatty acids effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 65:1628S–1644S
- Lendelvo S, Munyebvu F, Suich H (2012) Linking women's participation and benefits within the Namibian community based natural resource management program. J Sustain Dev 5:27–39
- Mabaleha MB, Mitei YC, Yeboah SO (2007) A comparative study of the properties of selected melon seed oils as potential candidates for development into commercial edible vegetable oil. J Am Oil Chem Soc 84:31–36. doi:10.1007/s11746-006-1003-7
- Maggs-Kölling GL, Christiansen JL (2003) Variability in Namibian landraces of watermelon (*Citrullus lanatus*). Euphytica 132:251–258. doi:10.1023/A:1025053331528
- Maggs-Kölling G, Iileka R, Gottlieb R, Uushona E (2014) Chapter 1: !Nara (*A. horridus*); in indigenous plant products in Namibia. Venture Publications, Windhoek
- Malini T, Vanithakumari G (1990) Rat toxicity studies with  $\beta$ -sitosterol. J Ethnopharmacol 28:221–234. doi:10.1016/0378-8741(90)90032-O
- Mariod AA, Ahmed YM, Matthaus B, Khaleel G, Siddig A, Gabra AM, Abdelwahab SI (2009) A comparative study of the properties of six Sudanese cucurbit seeds and seed oils. J Am Oil Chem Soc 86:1181–1188. doi:10.1007/s11746-009-1459-3
- Millenium Challenge Account-Namibia (MCA-N) (2012) Kalahari melon seed oil: INP Market Bulletin: Indigenous Natural Products in Namibia, Issue 4, p 3. <http://www.mcanamibia.org/files/files/INP%20Market%20Bulletin%20Issue%20IVRevised.pdf>. Accessed 12 Jan 2017
- Mizuno K, Yamagata K (2005) Vegetation succession and plant use in relation to environmental changes along the Kuiseb River in the Namib Desert. Afr Study Monogr 30:3–4
- Nasri N, Elfalleh W, Nizar T, Hannachi H, Triki S, Khaldi A (2012) Minor lipid components of some Acacia species: potential dietary health benefits of the unexploited seeds. Lipids Health Dis 11:49. doi:10.1186/1476-511X-11-49
- NBRI (2017) National Botanical Research Institute. <http://www.nbri.org.na/sections/economic-botany/INP/sectors/nara>. Accessed 6 April 2017
- Nehdi IA, Sbihi H, Tan CP, Al-Resayes AI (2013) Evaluation and characterisation of *Citrullus colocynthis* (L.) Schrad seed oil: Comparison with *Helianthus annuus* (sunflower) seed oil. Food Chem 136:348–353. doi:10.1016/j.foodchem.2012.09.009
- Ntambi JM, Choi Y, Park Y, Peters JM, Pariza MW (2002) Effects of conjugated linoleic acid (CLA) on immune responses, body composition and stearoyl-CoA desaturase. Can J Appl Physiol 27:617–628
- Nyam KL, Tan CP, Lai OM, Long K, Che Man YB (2009) Physicochemical properties and bioactive compounds of selected seed oils. LWT Food Sci Technol 42:1396–1403. doi:10.1016/j.lwt.2009.03.006
- O'Brian RD (2009) Fats and oils: formulating and processing for applications, 3rd edn. CRC Press, Boca Raton
- Siyambola TO, James OO, Gurunathan T et al (2015) Synthesis, characterization and antimicrobial evaluation of polyesteramide resin from *Moringa oleifera* seed oil (MOSO) for surface coating application. Can J Pure Appl Sci 9:3229–3240. ISSN 1920-3853
- Tsuzuki T, Tokuyama Y, Igarashi M, Miyazawa T (2004) Tumour growth suppression by  $\alpha$ -eleostearic acid, a linoleic acid isomer with a conjugated triene system, via lipid peroxidation. Carcinogenesis 25:1417–1425. doi:10.1093/carcin/bgh109
- Uluata S, Özdemir N (2012) Antioxidant activities and oxidative stabilities of some unconventional oilseeds. J Am Oil Chem Soc 89:551–559. doi:10.1007/s11746-011-1955-0
- Van den Eynden V, Vernemmen P, Van Damme P (1992) The ethnobotany of the Topnaar. Universitet Gent, Belgium
- Vermaak I, Kamatou GPP, Komane-Mofokeng B, Viljoen AM, Beckett K (2011) African seed oils of commercial importance-cosmetic applications. S Afr J Bot 77:920–933. doi:10.1016/j.sajb.2011.07.003
- Wyk BE, Gericke N (2000) People's plants. Briza Publication, Pretoria
- Yang XQ, Pan H, Zeng T, Shupe TF, Hse CY (2013) Extraction and characterization of seed oil from naturally grown Chinese tallow trees. J Am Oil Chem Soc 90:459–466. doi:10.1007/s11746-012-2178-8
- Zhang T, Gao Y, Mao Y, Zhang Q, Lin C, Lin P, Zhang J, Wang X (2012) Growth inhibition and apoptotic effect of alpha-eleostearic acid on human breast cancer cells. J Nat Med 66:77–84. doi:10.1007/s11418-011-0556-4
- Zhao S, Zhang D (2013) A parametric study of supercritical carbon dioxide extraction of oil from *Moringa oleifera* seeds using a response surface methodology. Sep Purif Technol 113:9–17. doi:10.1016/j.seppur.2013.03.041
- Zimba N, Wren S, Stucki A (2005) Three major tree nut oils of southern central Africa: their uses and future as commercial base oils. Int J Aromather 15:177–182. doi:10.1016/j.ijar.2005.10.009