PROFILING STUDIES OF FIVE NAMIBIAN INDIGENOUS SEED OILS OBTAINED USING THREE DIFFERENT EXTRACTION METHODS

A DISSERTATION SUBMITTED IN FULFILLMENT

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ABSTRACT

Namibia has a rich biodiversity of plant species of which a great variety is used for food, cosmetics and medicinal applications. Seed oil production from indigenous sources has economic importance for Namibia and is an asset for improving the livelihoods of local communities. Knowledge of the quality and composition of these oils can be used towards value-added product development strategies and improved marketing of indigenous resources. A comprehensive profile of indigenous seed oils currently produced in Namibia at traditional and commercial level was developed. This profile included a presentation of the physico-chemical properties of the oils and their quality characteristics among three different extraction techniques, namely the traditional extraction method, machine-operated cold pressing and Soxhlet extraction. The oils studied were *Citrullus lanatus* (Kalahari melon) oil, *Schinziophyton rautanenii* (Schinz) Radcl.-Sm. (Manketti) nut oil, *Sclerocarya birrea* (A. Rich) Hochst. (Marula) nut oil, *Ximenia americana* (Blue sour plum) nut oil and *Acanthosicyos horridus* Welw. ex Hook.f. (!Nara) oil. Significant differences ($p<0.05$) among the three extraction methods for Manketti nut oil were found for the characteristics of AV, *p*-AV, IV and RI. Cold pressed Manketti nut oil was significantly different ($p<0.05$) in terms of its PV from the traditionally and the Soxhlet extracted Manketti nut oil, with a higher PV. Significant differences ($p<0.05$) were found for the characteristics of AV, PV and IV for Marula nut oil. No significant differences ($p\geq0.05$) were found for the *p*-AV among the three extraction techniques. Significant differences ($p<0.05$) were found for the characteristic of the RI for *Ximenia* nut oil. The traditionally extracted *Ximenia* nut oil was significantly different ($p<0.05$) from the cold pressed and the Soxhlet extracted *Ximenia* nut oil for the characteristics

of PV and *p*-AV, with higher values. Significant differences ($p<0.05$) among the two extraction techniques for the !Nara seed oil were found for the characteristics of SV, AMW, EV, IV, SG and RI and non-significiant differences ($p\geq0.05$) for the characteristics of AV, PV and *p*-AV. Major fatty acids found were linoleic (31.2-32.2%) acid, α-eleostearic (24.2-35.7%) acid (Manketti), oleic (66-6-67.6%) acid (Marula), oleic (46.3-44.1%) acid, ximenynic (6.5-12.0%) acid (*Ximenia*), linoleic (52.6-57.0%) acid, oleic (10.5-17.7%) acid (Melon) and linoleic (53.1-54.5%) acid, oleic (12.8-13.9%) acid (!Nara). Highest total tocopherol content found was 205.64 mg/100g (Manketti; Soxhlet extracted), 29.72 mg/100g (Marula; traditionally extracted), 10.75 mg/100g (*Ximenia*; traditionally extracted), 46.10 mg/100g (!Nara, Soxhlet extracted) and 74.39 mg/100g (Melon; cold pressed). The highest stigmasterol (53.11 mg/100g) was found in traditionally extracted Marula nut oil and the highest β-sitosterol content (682.43 mg/100g) was found in Soxhlet extracted Manketti nut oil. The hydrolysis of Marula and Manketti nut oils for fatty acid production with *Candida rugosa* lipase was studied using a 2^3 full factorial design was applied to study the interactions of experimental factors such as pH, temperature, oil concentration and *C. rugosa* lipase concentration. The degree of hydrolysis ranged from 49.5% to 77.0% and 56.3% to 75.7% for Manketti oil and Marula oil, respectively. The effect of initial pH, temperature, oil and enzyme concentration and their interaction had significant effect on the degree of hydrolysis of Manketti oil. For the Marula oil hydrolysis, oil concentration, enzyme conentration and the interaction between temperature and oil concentration were observed to be significant. The optimal conditions for achieving a %H of 76.5% for Manketti oil was initial pH of 6.0, a temperature of 30 °C, an oil concentration of 30% and an enzyme concentration of 10 mg/g of oil. The optimal condition for achieving %H of 62.5% for Marula oil hydrolysis, was temperature of

36.8 °C, oil concentration of 11.3% and enzyme concentration of 23.6 mg/g of oil. The present study revealed that the indigenous seed and nut oils obtained from three different extraction techniques have the potential for promotion in the food, in particular as functional foods, cosmetics and the pharmaceutical industry.

LIST OF PUBLICATIONS

- Cheikhyoussef, N., Kandawa-Schulz, M. & Böck, R. (2015). *Status of Namibian indigenous seed oils and potential applications***.** Paper presented at the Faculty of Science 2nd Annual Science Research Conference, 30-31 October 2014, University of Namibia.

- Cheikhyoussef, N., Kandawa-Schulz, M. & Böck, R., Cheikhyoussef, A. & Hussein, A.A. (2016) *Characterization of Namibian Sclerocarya birrea (marula/ondjove) oil.* Poster presented at the 1st Food Chemistry Conference, Amsterdam, Netherlands. doi: 10.13140/RG.2.2.23916.97928

- Ndala, A.M., Kahaka, G., Cheikhyoussef, N. (2016). *Detection and partial characterization of the TPS1 gene encoding trehalose-6-phosphate in the Namibian Myrothamnus flabellifolius*. Book of Abstracts: Faculty of Science, 3rd Annual Science Research Conference, 18-19 November 2015, University of Namibia.

- Cheikhyoussef, N., Kandawa-Schulz, M., Böck, R., de Koning, C., Cheikhyoussef, A. & Hussein, A.A. (2017). *Comparative analysis of nutritional components of selected indigenous plant oils of Namibia*. Poster presented at the 31st EFFoST Conference, Sitges, Spain, doi: 10.13140/RG.2.2.18974.02889

- Cheikhyoussef, N., Kandawa-Schulz, M., Böck, R., de Koning, C., Cheikhyoussef, A. & Hussein, A.A. (2017). Characterization of *Acanthosicyos horridus* and *Citrullus lanatus* seed oils: two melon seed oils from Namibia used in food and cosmetics applications. *3 Biotech*, 7, 297.

- Cheikhyoussef, N., Kandawa-Schulz, M., Böck, R., de Koning, C., Cheikhyoussef, A. & Hussein, A.A. (2018). Characterization of *Schinziophyton rautanenii* (Manketti) nut oil from Namibia rich in conjugated fatty acids and tocopherol. *Journal of Food Composition and Analysis*, *66,* 152-159.

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ACRONYMS

ANOVA	Analysis of Variance
AOCS	American Oil Chemists' Society
AMW	Average molecular weight
AV	Acid value
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CAF	Central Analytical Facility
^{13}C NMR	Carbon 13 Nuclear Magnetic Resonance
CV	Coefficent of Variation
CRL	*Candida rugosa* lipase
EWC	Eudafano Women's Cooperative
EV	Ester value
α-ESA	Alpha eleostearic acid
FA	Fatty acid
FAMEs	Fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nations
FFA	Free fatty acid
^{1}H NMR	Proton Nuclear Magnetic Resonance

GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
%H	Percentage hydrolysis/Degree of hydrolysis
Mequiv/Meq	milliequivalent
MHETI	Ministry of Higher Education, Training and Innovation
MT	Metric tonnes
IV	Iodine value
NCRST	National Commission on Research, Science and Technology
nd	not detected
NMR	Nuclear Magnetic Resonance
p-AV	*para*-Anisidine value
PV	Peroxide value
R^2	Coeffcient of determination
RI	Refractive index
SG	Specific gravity
SV	Saponification value

TAG	Triacylglycerides
TMCS	Trimethylchlorosilane
TTC	Tulongeni Twahangana Cooperative Ltd.
TUSFA	Total unsaturated fatty acid
TSFA	Total saturated fatty acid
WIPO	World Intellectual Property Organization

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DEDICATION

TO MY FAMILY AHMAD, MALIK, SAFIA, FATHER GERT,

MOTHER HEDWIG AND OPA HEINZ

DECLARATIONS

- I, Natascha Cheikhyoussef, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Natascha Cheikhyoussef Date

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Oils and fats make up a great percentage of the international commerce as agricultural products and are thus of huge economic importance (Christie & Han, 2012). Over the last 30 years, the production of seed oils has been increasing steadily in order to keep up with the demand for food and non-food or industrial applications (Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008). Worldwide, interests are targeted towards finding new sources of oils originating from natural plant resources (Mariod et al., 2009; Nasri et al., 2012), with potential applications in nutraceuticals, aromatherapies, lubricants, pharmaceutical, cosmetics, food and oleo-chemical industries (Kurki et al., 2008; Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008).

Economically, seed oil, also called vegetable oil, is extracted from seeds or nuts, for a number of food and non-food or industrial applications from major economic seed oils such as cotton, flax, sunflower, rape, soybean, palm kernel, sesame among others. Such seeds are commonly referred to as oilseed crops (Blair & Regenstein, 2015). Resins, surfactants, plastics, solvents, lubricants, emulsifiers and plasticizers are non-food or industrial applications where vegetable oils are used in their production (Erhan, 2005). The fact that such oils are derived from a renewable resource, have lower toxicity and are biodegradable and hence environmentally friendly, has led to research being done towards the development of new products such as polyols, biodiesel, engine oils and others (Erhan, 2005).

Rural communities have used seed oils for centuries in applications such as cosmetics, fuel and food, whereby they collect and process seeds to provide food and income

(Vermaak, Kamatou, Komane-Mofokeng, Viljoen, & Beckett, 2011). Therefore, a rich indigenous knowledge exists among African rural communities on the methods of producing the seed oils and their applications in health and food.

In order for any seed oil to realize its commercial significance, its physico-chemical properties need to be identified in order to establish possible applications. In order to characterize a seed oil, the triglyceride and fatty acid composition is analysed through analytical methods such as gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectrometry (NMR spectroscopy). Other analytical methods such as infrared (IR) spectroscopy and ultraviolet (UV) spectroscopy have also been applied. The study of fats and oils has been revolutionized with the application of GC to determine their composition of fatty acids as fatty acid methyl esters (FAME's) in a short period of time (Christie, 1989; 1993), and is thus the preferred analytical method of analysing FAME's (Khan, 2013).

Other chemical parameters, such as the saponification value (SV), the iodine value (IV), the acid value (AV), peroxide value and anisidine value are also determined and can be used to identify and monitor the quality of the seed oil. The physical parameters such as the refractive index, the specific gravity, and antioxidant properties, in terms of tocopherols content can also be determined. Knowledge of the biochemical profile of indigenous natural products is critical in the development of value-added products from such a resource base.

The hydrolysis of oils also commonly known as lipolysis, is generally carried out to produce concentrated free fatty acids, which have significant industrial applications in producing high-value products by industries such as waste processing, oleochemical,

petroleum, cosmetics, soap and detergents, pharmaceuticals, coatings, adhesives, lubricating oils, shampoos and food industries (Albasi, Bertrand & Riba, 1999; Murty, Bhat & Muniswaran, 2002; Beuve & Morison, 2010). The natural fatty acid's global market was $7.2 billion in 2011 and $6.8 billion in 2012 of which the market is expected to reach $13 billion by 2017, with a 13.6% compound annual growth rate (CAGR) from 2012 to 2017 (BCC Research, 2013), which emphasizes the potential of finding ways to enter this market with available indigenous resources, towards increased revenue from applications of natural products.

1.2. Statement of the research problem

The search for novel products by industry continues as growth in the global population is putting pressures on food and non-food resources. However, mankind continues to utilize the same resources, while there are countries such as Namibia whose rich biodiversity is widely untapped and can, if investigated, provide novel products for niche markets, in particular for the biochemical and food industry. Information on African seed oils is scattered in literature and often published in obscure and outdated manuscripts (Vermaak et al., 2011). Namibia currently produces Manketti, *Ximenia* and Marula nut oil, Melon and !Nara seed oil, either traditionally or commercially (cold pressed) at varying market levels. To the best of my knowledge, after a literature review of published reports on the physico-chemical characteristics of theses indigenous seed and nut oils of Namibia, it was found that limited or no data is available on their composition and physico-chemical properties including enzymatic hydrolysis studies. This limited data on seed oils inhibits innovators and product developers from assessing the potential of these seed oils and the development of novel and innovative products. Rural communities do not have the capacity to conduct

scientific research, which could allow them to gain from the economic potential of the natural products, such as seed oils, which they utilize daily.

1.3 Objectives of the study

The objectives of this study were to 1) compile information on the types of oils, the types of extraction methods applied and uses of the oils by Namibian communities, through literature searches of published reports and/or conducting interviews with selected target participants; 2) assess the effects of extraction methods of oils from seeds on the oils physico-chemical characteristics; 3) compile data such as fatty acid composition, tocopherol and major sterol composition of the seed oils using techniques such as GC-MS, ^{1}H and ^{13}C NMR spectroscopy; 4) establish the effects of enzymatic hydrolysis by *Candida rugosa* lipase under different operating conditions on Manketti and Marula nut oil.

1.4. Significance of the study

The significance of the study was to compile information of the usage of the oils currently produced and used by the Namibian communities and to conduct an in-depth profiling of the oils, which can assess the economic importance of these oils in food and non-food uses or as potential health enhancers. The study was also based on assessing the parameters of these oils based on current production methods (traditional process and cold pressing). This data was also compared with an organic solvent extraction method (Soxhlet extraction) allowing to form a basis for comparison to other reported literature having mostly used organic solvent extraction.

In order for Namibia to join the race in satisfying the demand by industry for seed oils and benefitting economically from it, it needs to establish what the potential is of its indigenous seed oil resource. There is a need for more research to establish data on seed oils specific for Namibia. To the best of my knowledge, to date, no literature is available on enzymatic hydrolysis studies that have been conducted using Namibian indigenous seed oils. It is a growing trend to search for new seed oils, in particular of indigenous origin, to be used for industrial applications, of which hydrolysis studies can provide data for further applications.

CHAPTER 2: LITERATURE REVIEW

2.1. Oil and fat chemistry

Oils and fats are composed of triacylglycerides (TAG), being esters of glycerol containing three molecules of fatty acid (FA). Figure 1 illustrates the structure of a triacylglycerol. The R_1, R_2, R_3, are the side chains being fatty acids (FAs). Table 1 provides a general summary of fatty acids commonly found in seed oils. Saturated and unsaturated fatty acids are straight-chain compounds with most common chain lengths of C_{12} to C_{22} (Gunstone & Padley, 1997). The olefinic compounds with *cis* (Z) configuration, are usually the acids with one unsaturated center (Gunstone & Padley, 1997).

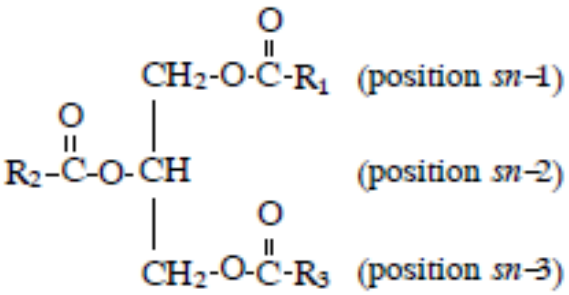
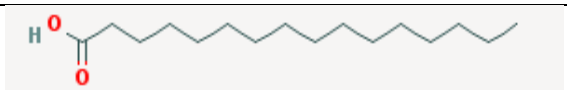
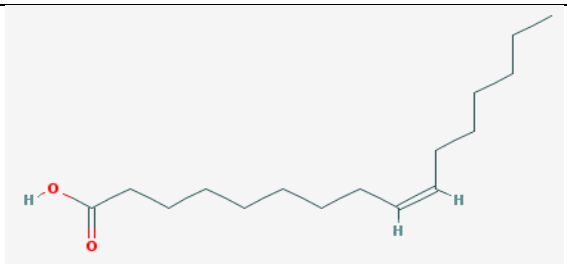
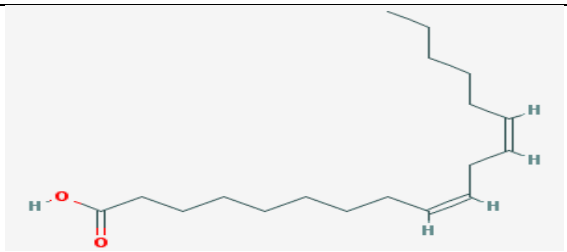
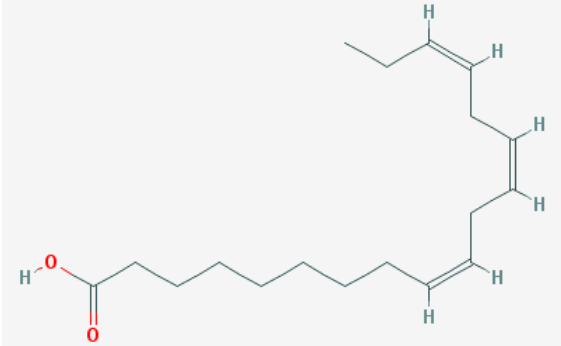


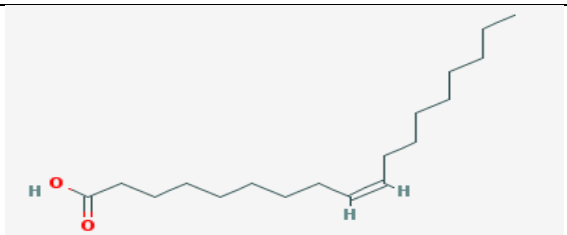
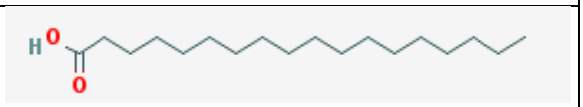
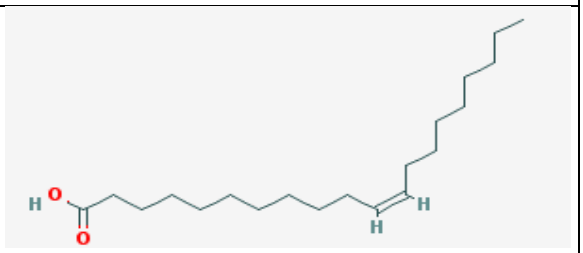
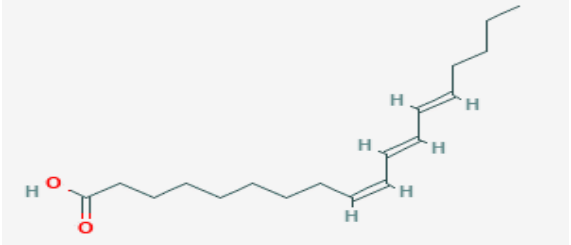
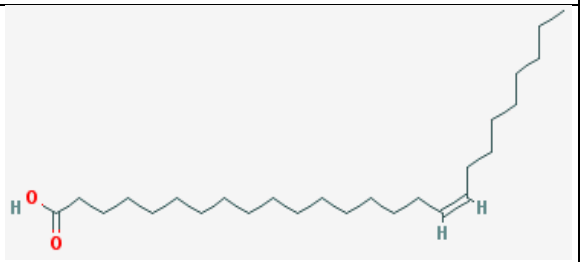
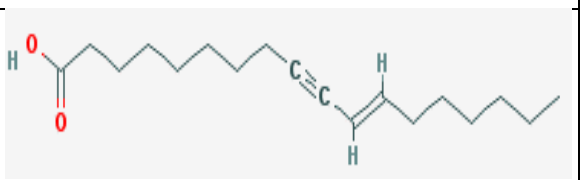
Figure 1: General structure of triacylglycerol (Adopted from Tao & He, 2006).

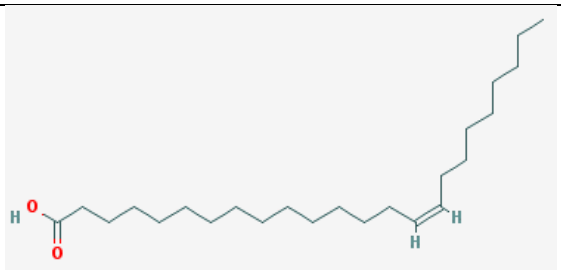
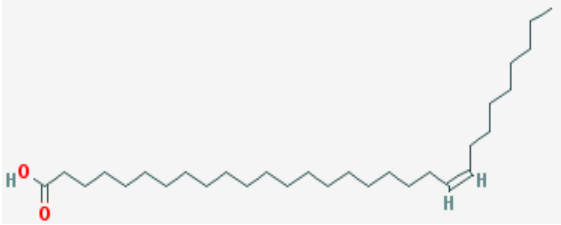
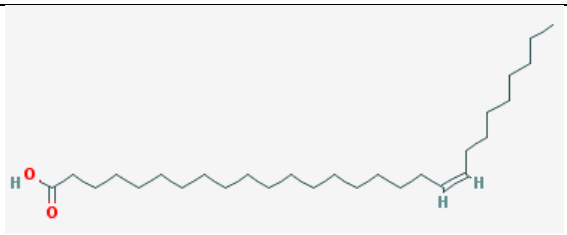
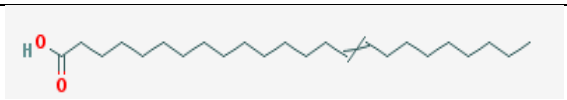
The chemical and physical properties of oils and fats are largely affected by the composition and structure of the fatty acids present. Crude oils and fats also contain nonglyceride components such as tocopherols, carotene and sterols among others, which also have an effect on their chemical and physical properties (O'Brian, 2009). Fatty acids in oils and fats are characterized by the length of fatty acid chains, their locations on the glyceride (R_1, R_2 or R_3) unit and the double bond positions and numbers.

The profile of fatty acids in an oil and fat depends not only on the source of the plant species, but also on plant physiology and genetic makeup, geographical and climate conditions among others (O'Brian, 2009).

Table 1: Common fatty acids found in seed oils.

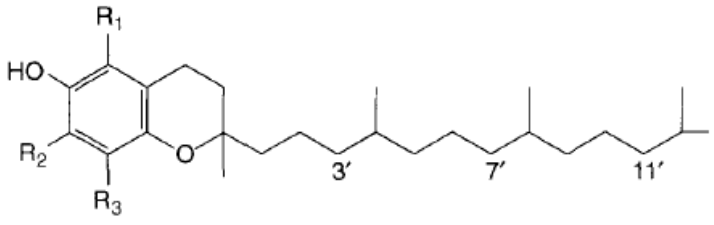
Common name	Chemical name[a]	Structure[a]
Palmitic acid	Hexadecanoic acid	
Palmitoleic acid	*Cis*-9-Hexadecenoic acid	
Linoleic acid	*Cis*-9,12-Octadecadienoic acid	
Linolenic acid	9-*cis*,12-*cis*,15-*cis*-Octadecatrienoic acid	

Oleic acid	*Cis*-9-Octadecenoic acid	
Stearic acid	Octadecanoic acid	
Arachidic acid	Eicosanoic acid	
Gondoic acid	*Cis*-11-Eicosenoic acid	
α-Eleostearic acid	*Cis*-9, *trans*-11, *trans*-13-octadecatrienoic acid	
Ximenic acid	*Cis*-17-hexacosenoic acid	
Ximenynic acid	*Trans*-11-octadecen-9-ynoic acid	

Nervonic acid	*Cis*-15-tetracosenoic acid	
Lumequic acid (Lumepueic acid, Lumequeic acid)	*Cis*-21-triacontenoic acid	
Octacosenoic acid	*Cis*-19-octacosenoic acid	
Tetracosenoic acid	tetracos-15-enoic acid	

[a]National Center for Biotechnology Information (NCBI). PubChem Compound Database (2017)

The richest sources of Vitamin E for nutrition are the vegetable oils and this Vitamin is the most potent antioxidant in the human plasma (Grilo et al., 2014). The components of Vitamin E are the alpha (α)-, beta (β)-, gamma (γ) -, delta (δ)- tocopherols and the alpha (α)-, beta (β), gamma (γ)-, delta (δ)- tocotrienols (Grilo et al., 2014). The major soluble vitamin in extracted oils is a mixture of the tocopherol and their type and concentration of each type varies among different sources of oilseeds (Chen, McClements & Decker, 2011). 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 greatest sources of tocopherol are seed oils, with the delta (δ)-tocopherol being the most active and the α-tocopherol, the least active antioxidant. But the (α)-tocopherol is most active with regard to its Vitamin E activity and function (O'Brian, 2009). Figure 2 represents the general structure of the tocopherols (Wrolstad et al., 2005, Pg 486). The tocopherols contain a chromanol ring attached to a saturated phytyl and vary by the number of methyl groups attached to the chromanol ring (Wrolstad et al., 2005; Traber, 2012).



tocopherol

	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Figure 2: Structure of tocopherol (Adopted from Wrolstad et al., 2005, Pg 486).

Phytosterols are plant sterols present in oils, nuts and vegetables (NCBI, 2017). About 45-96% of total plant sterols are comprised of β-sitosterol (3beta-stigmast-5-en-3-ol) with stigmasterol (Stigmasta-5, 22-dien-3beta-ol) prominently found in seed oils (Shils, Shike, Ross, Caballero & Cousins, 2006). Figure 3 represents the structures of β-sitosterol and stigmasterol. Stigmasterol has been shown to have anti-osteoarthritic properties (Gabay et al., 2010). The presence of β-sitosterol, a main dietary phytosterol (NCBI, 2017), has been shown to impart anti-fungal, anti-inflammatory and anti-viral properties (Malini & Vanithakumari, 1990), including anti-carcinogenic and anti-atherogenic properties (NCBI, 2017).

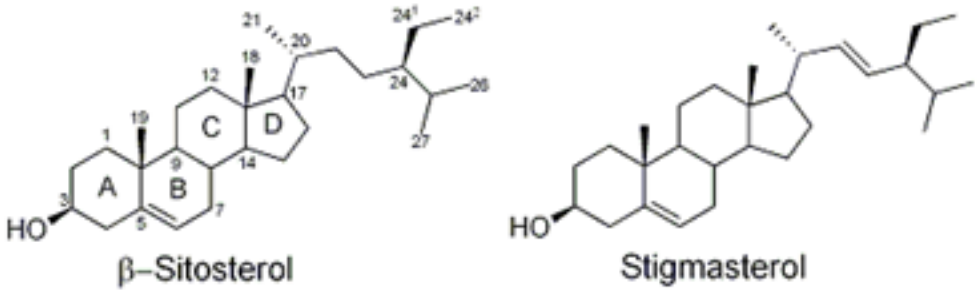


Figure 3: Structure of β-sitosterol and stigmasterol (Adopted from Winkler-Moser, 2011).

2.2. Profile and characteristics of the indigenous seed/nut oils

2.2.1. *Schinziophyton rautanenii* (Schinz) Radcl.-Sm. (Manketti) nut oil

Schinziophyton rautanenii (Schinz) Radcl.-Sm., is a large spreading dioecious tree (15-20 m in height) in the family of the Euphorbiaceae (the euphorbia family) with fruits appearing from February (Palgrave, 1983). The species was also formerly known as *Ricinodendron rautanenii* Schinz (Vermaak et al., 2011).

The tree is locally known as Omunkete (Oshiwambo), Omungete (Otjiherero), Ngongo (Kavango) and Mungongo (Caprivi) (Curtis & Mannheimer, 2005). This tree is commonly found in the north-eastern part of Namibia and an important food source to the local communities (European Commission, 1998), but also grows in Angola, South Africa, Botswana and Zambia (Atabani et al. 2014).

Figure 4 (a-d): Tree (a) and leaves (b), dried kernels (c) and cold pressed oil (d) of *S. rautanenii*.

Figure 4 (a-d) represents the tree (a) and leaves (b), dried kernels (c) and cold pressed oil from Mungongo Trading Enterprise (d) of *S. rautanenii* and Figure 5 shows the occurrence of *S. rautanenii* tree populations throughout Namibia.

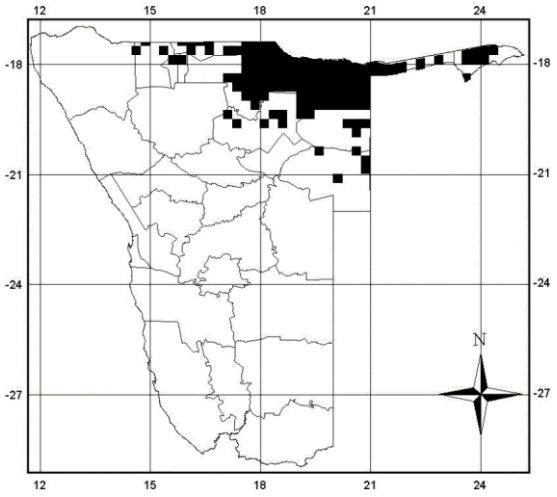


Figure 5: Geographical distribution of *S. rautanenii* in Namibia (Adopted from Graz, 2002).

From April to May, fruits fall on the ground and the ripening process starts, softening the fruit flesh (Vermaak et al., 2011). The different fruit parts have various uses but the seed kernel or nut is of most value (European Commission, 1998), as it is highly nutritious and contains high amounts of an edible oil (van Wyk & van Wyk, 2013), referred to as the Manketti nut oil.

The kernel is used for oil extraction after removal from the fruit with an axe (European Commission, 1998) or crushed between two rocks (Vermaak et al., 2011). The inner nut is extracted from dried Manketti fruit, which is pounded and cooked to be eaten with mahangu porridge, whilst the oil produced from the nut is eaten with vegetables (Namibia Tourism Board, 2014). Apart from being used in food preparations and cooking, the Manketti oil can also be used in skin formulations for cleansing and moisturising (Kivevele & Huan, 2015). The oil has been reported to be highly stable to oxidation, with a long shelf life and its use in cosmetic formulations is because the oil is easily absorbed into the skin (Zimba, Wren & Stucki, 2005).

Interestingly, Peters (1987) reported that dried seeds stored under proper conditions for about 6 years could still be edible and palatable. This provides a continuous food source for rural communities throughout the year.

In Namibia, cold pressed Manketti oil can be purchased from Mungongo Trading Enterprise in Ngweze (Appendix A) at N$ 150.00 for 500 ml (Personal communication, 2014, Mr Calicious). Divine Oils located in Ondangwa also sells cold pressed Manketti oil and mainly for export of minimum weight of 23 kg per order (Divine Oils, 2016).

A number of researchers have characterised the Manketti oil (Table 2). Chisholm & Hopkins (1966) identified the fatty acid α-eleostearic acid (23.8%) in *R. rautanenii* Schinz. oil with a refractive index of 1.487. In 1970, Engelter & Wehmeyer (1970) investigated *R. rautanenii* nut oil from South Africa, among other edible seeds of wild plants for its fatty acid composition. It was found that the seed oil contained a good source of linoleic acid and that two unidentified fatty acids were present. Chivandi, Davidson & Erlwanger (2008) investigated the properties of *R. rautanenii* nut oil from Zimbabwe after a chloroform-methanol solvent extraction (Bligh & Dyer, 1959) and found that the nuts contained 53.3% of oil content and that the oil was composed of myristic acid (0.03%), palmitic acid (10.8%), stearic acid (3.0%), myristoleoic acid (0.01%), oleic acid (15.2%), erucic acid (21.5%) and linoleic acid (49.5%). Zimba, Wren & Stucki (2005) have reported that the pressing with a simple hand press of the Manketti nut oil from Zambia from its nut, yields about 26% of oil and has a fatty acid composition of palmitic acid (9.8%), linoleic acid (39.0%), oleic acid (19.2%), oleic acid (isomer) (0.3%), stearic acid (7.7%) and linolenic acid (16.7%). Mitei et al. (2008) reported the physico-chemical properties of seed oils from Botswana from which the *S. rautanenii* (Manketti) kernel oil had oil yield (Soxhlet: 41.5%),

saponification value (185.26 mg KOH/g), iodine value (121.76), acid value (0.36 mg KOH/g oil), peroxide value (2.51 mequiv/kg), *p*-anisidine value (3.85), density (20 °C: 0.907 g/cm^3), refractive index (25 °C: 1.481) with fatty acid composition of palmitic acid (12%), linoleic acid (52%), stearic acid (11.8%) and oleic acid (24.4%). The composition of fatty acids reported by Mitei et al. (2008) resembles that of the oil from *Zea mays* (corn oil). Corn oil has a saponification value of 187-195 mg KOH/g and an iodine value of 107-128 with a fatty acid composition of stearic acid (nd-3.3%), palimitic acid (8.0-16.5%), oleic acid (20.0-42.2%) and linoleic acid (34.0-65.6%) among other minor fatty acids (Gunstone, Harwood & Dijkstra, 2007). Mitei, Ngila, Yeboah, Wessjohann & Schmidt (2009) studied the phytosterols, tocopherols and tocotrienols composition of seed oils from Botswana of which the Manketti nut oil contained α-(5.64 µg/g) and γ–(2232.99 µg/g) tocopherols and stigmasterol (36.24 µg/g oil) and sitosterol (1326 µg/g oil). In Zambia, according to Juliani, Koroch, Simon & Wamulwange (2007), 840 metric tonnes (MT) of kernel oil could be obtained from about 3000 MT of seed and reported the fatty acid composition of this oil to be palmitic acid (8%), stearic acid (9%), oleic acid (15%), linoleic acid (37%) and linolenic acid (25%). Atabani et al. (2014) have investigated the Manketti oil for methyl ester production and ultimate use in biodiesel applications and found that the oil has potential in biofuel applications. The crude oil was also characterized and had an acid value (2.08 mg KOH/g), density (15 °C: 943.0 kg/m^3; 40 °C: 925.1 kg/m^3), a specific gravity of 0.9443 at 15°C, and a refractive index of 1.487. Phytotrade (2012) reported that the Manketti nut oil is composed mainly of polyunsaturated fatty acids such us of α-eleostearic acid (23%) and linoleic acid (49%). According to Phytotrade Africa, UV light polymerizes the α-eleostearic acid producing a film on hair and skin that then protects the skin for UV damage.

Table 2: Summary of reported physico-chemical characteristics of Manketti nut oil.

Origin (Country)	Zambia	Zimbabwe	Zambia	Zambia	Botswana
Oil yield (%)	NR	53.3	26[a]	NR	41.5[b]
Iodine value	NR	NR	NR	NR	121.76
Saponification value (mg KOH/g)	NR	NR	NR	NR	185.26
Acid value (mg KOH/g)	NR	NR	NR	NR	0.36
Peroxide value (mequiv/kg)	NR	NR	NR	NR	2.51
Refractive index	1.4872	NR	NR	NR	1.481 (25 ºC)
Specific gravity	NR	NR	NR	NR	0.907[c] (20 ºC)
α-eleostearic acid (%)	23.8	NR	NR	NR	NR
Myristic acid (%)	NR	0.03	NR	NR	NR
Palmitic acid (%)	NR	10.8	9.8	8	11.95
Stearic acid (%)	NR	3.04	7.7	9	11.77
Myristoleoic acid (%)	NR	0.01	NR	NR	NR
Oleic acid (%)	NR	15.2	19.2	15	24.35
Erucic acid (%)	NR	21.5	NR	NR	NR
Linoleic acid (%)	NR	49.5	39	37	51.93

Linolenic acid (%)	NR	NR	16.7	25	NR
Alpha (α)-tocopherol	NR	NR	NR	NR	5.64 µg/g
Beta (β)-tocopherol	NR	NR	NR	NR	NR
Gamma (γ)-tocopherol	NR	NR	NR	NR	2232.99 µg/g
Delta (δ)-tocopherol	NR	NR	NR	NR	NR
Reference	Chisholm & Hopkins (1966)	Chivandi et al. (2008)	Zimba et al. (2005)	Juliani et al. (2007)	Mitei et al. (2008, 2009)

[a]cold pressed; [b]Soxhlet extracted; [c]reported as density (g/ml)

Various patents have been documented involving the application of Manketti nut oil. The use of Manketti oil in various formulations, among others, is reported by Lucka & Mullen (2010) in Patent US 2010/0267599 A1 for use as abrasive dispersion agent in developing a microdermabrasion soap bar, its compositions and preparation methods, by Razzak (2010) in Patent US 2010/0022469 A1 for the development of Anthelminthic (parasitic) formulations for use in livestock and by Bhagat (2009) in Patent US 2009/0264520 A1 for development and application of nutritional food formulations. The chemical composition in terms of the fatty acid composition of the Manketti oil makes it unique for healing applications such as the treatment of eczema, tissue regeneration, cell repair and treatment of inflammation (Zimba et al., 2005).

2.2.2. *Sclerocarya birrea* (A. Rich) Hochst. (Marula) nut oil

Sclerocarya birrea (A. Rich) Hochst. (Marula) of the family Anacardiaceae (the mango family), is a medium sized tree (about 10 m or more in height), with fruits (indehiscent) appearing February to June and becoming yellow when ripe (Palgrave, 1983). *S. birrea* is related to the mango family which is drought resistant producing plum sized fruits that are butter yellow when ripe (NBRI, 2016a). Figure 6 (a-f) represents the tree (a), leaves and fruits (b), oil and nuts (c), traditional oil (d), bottled cold pressed oil at Eudafano Women's Cooperative (EWC) for sale (e) and the label on bottle for oil sold by EWC (f) of *S. birrea*.

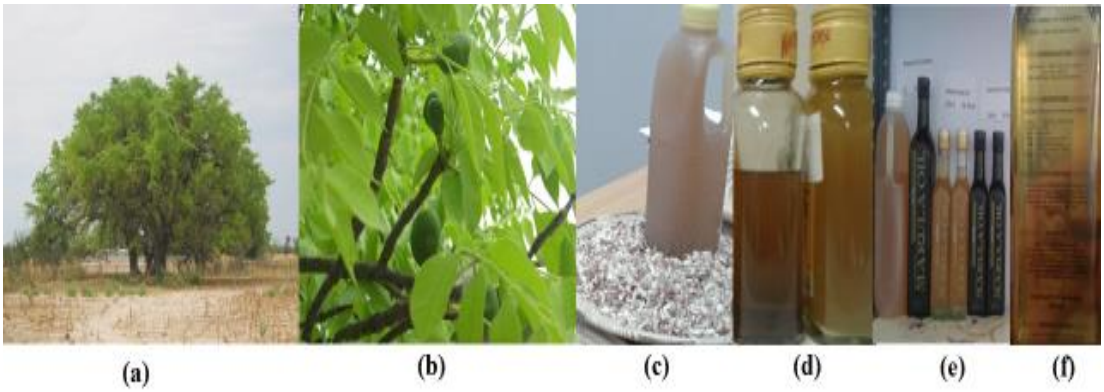


Figure 6 (a-f): Tree (a), leaves and fruits (b), oil and nuts (c), traditional oil (d), bottled cold pressed oil at Eudafano Women's Cooperative for sale (e) and label on bottle for oil sold by Eudafano Women's Cooperative (f) of *S. birrea.*

The tree is locally called Omugongo (Oshiwambo), Omukongo (Otjiherero), Uwongo (Kavango) and Mulula (Caprivi) (Curtis & Mannheimer, 2005). For the rural communities of southern Africa, the Marula tree is an important segment of their culture and food, but has over the years become a focus for commercial opportunities (Wynberg et al., 2002). The Marula tree is of great social, cultural and economic importance to the *Aawambo* communities of northern Namibia. The annual Marula fruit festival of Namibia (*Oshituthi shOmagongo* in Oshiwambo language) has been added in 2015 to the UNESCO's "Intangible Cultural Heritage of Humanity" representative list (United Nations Education, Scientific and Cultural Organization, 2016). The festival takes place for two to three days between March and April, where the communities of the *Aawambo* people gather together to enjoy a traditionally made beverage called "Omagongo" from the fruits of the Marula tree (Smit, 2015).

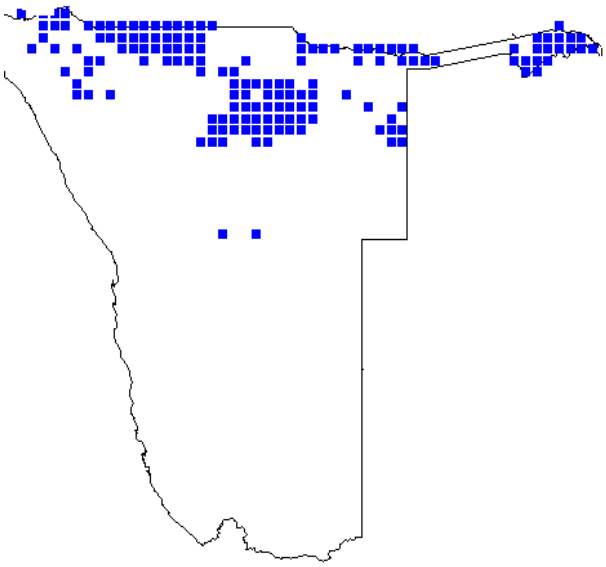


Figure 7: Geographical distribution of *S. birrea* trees across Namibia (Adopted from Curtis & Mannheimer, 2005).

In Namibia, the Marula oil is mainly produced in the north-central regions of Ohangwena, Oshana, Omusati, and Oshikoto due its available resource there (Figure 7) and the cultural heritage of Marula fruit use in those areas (Mallet & den Adel-Sheehama, 2015). In 2010, a survey done by CRIAA-SADC concluded that 85,000 to 141,000 tonnes of fruit are produced in the north-central Namibia areas of Marula production (NBRI, 2016a). Fruits are harvested from the ground in January to May and the fruit flesh is removed to make certain beverages, while the kernels are left to dry in the sun for oil extraction at a later stage. The intact seed kernels can be stored for up to one year before decortication (Travel News Namibia, 2012). The nut containing the oil is removed from the seed kernel and this process is called "decortication" and is done from June to September (Travel News Namibia, 2012).

One of the first non-governmental organization, CRIAA SADC, started investigating in 1999, the potential of producing Marula nut oil for exportation purposes to the

cosmetics industry (WIPO, 2016), and this initiative has expanded the market for rural producers dealing with Marula products (Wynberg et al. 2002). This initiative also led to the establishment of the Eudafano Women's Cooperative (EWC) located in Ondangwa (WIPO, 2016). By the year 2000, this initiative had allowed an income of US$61 500 (N$500 000) to be generated (Wynberg et al. 2002). The Eudafano Women's Cooperative produces Marula oil though the harvesting of the kernels by rural women who make up this Cooperative. The Cooperative is a member of Phyto-Trade Africa and the Body Shop Community Trade Programme. By 2008, 5000 women had joined EWC as members. According to WIPO (2016), EWC is the second largest producer of products of Marula oil as of 2010, whereby products are sold to The Body Shop, Marula Natural Products of South Africa (Marula Natural), and Distell. About 140 products developed under the Body Shop's Community Trade Program contain Marula oil, which in 2006 had generated for Namibia, US$91,450 in exports (MCA, 2008). Locally, the oil can be purchased from the EWC factory where Marula food oil is sold for N$ 75.00/500ml) and Marula oil for cosmetic for N$ 220.00/1L (EWC: Price List from March 2014).

Aurum Africa GmbH, a distributor of natural oils, reports the sourcing of Marula fruit and production of Marula oil from northern Namibia, which they sell at 50ml €14.90 – 125ml €27.90 (Aurum Africa GmbH, 2016). Shea Terra Organics (2016) also has reported their selling of Marula oil as being unadulterated, pure and harvested from the wild and sourced from Namibia (Shea Terra Organics, 2016). Rural households sell their Marula oil on the traditional markets and various locally registered enterprisers, such as Neema Cosmetics (Amukwaya, 2013), Tangi Manufacturing and Consulting Services (Lututu, 2016) also sell the Marula oil, with some such as Desert Secrets (Desert Secrets, 2016) producing value added products such as soaps, lotions

and other cosmetic formulations. The prices of the Marula oil are N$ 40.00 for 50 ml and N$ 285 for one liter (Lututu, 2016). A few restaurants also use the Marula oil for food preparation (NBRI, 2016a).

The Sustainche Farm™ Project also produces Marula oil traditionally and is used at the Restaurant Gathemann in Windhoek (The Sustainche Farm™ Project, 2016). Divine Oils located in Ondangwa also sells cold pressed Marula oil and mainly for export of minimum weight of 23 kg per order (Divine Oils, 2016). The factory of the EWC can produce eight tones of Marula oil per year with a large bag containing about 40 kg of kernel producing about 12 litres of oil (Swilling, 2013). The community buys the seed cakes from the EWC and use it as animal feed. In 2010, the EWC produced 1536 kg of oil from 3775 kg of Marula kernels for the Marula Food Oil product and was being sold from the EWC Factory for the local market (INP Market Bulletin, 2011). The INP Market Bulletin (2011) reported that the demand for Marula oil by the international markets remained stable with 6080 kg oil (N$ 920 000) being exported in 2011 and that a company in the United Kingdom was importing Marula oil for cosmetic formulations. A value of N$ 2.8 million (15 661 kg oil) was generated between 2008 and 2010 from exports. The harvesters for the EWC were paid N$ 21/kg and N$ 18/kg of kernels in 2011 and 2010, respectively and harvesters could make between N$ 400 and N$ 600 per month. Marula fruit production is affected by floods leading to poor fruiting such as was experienced in 2009 among others, whereby 14 tonnes less than what was harvested in 2007 (INP Market Bulletin, 2010). The export volume for Marula oil in 2012 was 6180 kg (N$ 1 003 200) as reported by the NBRI (2016).

In 2005, a collaboration between Phytotrade Africa and Aldivia S.A. of France registered an active botanical ingredient, called Maruline®, which is obtained through

a patented process and is 100% natural Marula oil with enhanced antioxidant properties (Phytotrade, 2005). It is estimated to bring about a commercial potential between US$120,000 to 1.7 million (MCA, 2008). The Maruline® is derived from the Marula fruit kernel, which is being harvested by the members of the EWC in Ondangwa (Phytotrade, 2005). The patented process has been filed as Patent number WO2006097806 A1, titled "Antioxidants based on Anacardiaceae species, methods for obtaining same and uses thereof", and compiles and invention concerning novel antioxidants derived from Anacardiaceae, species, particular from *S. birrea* (Marula) (WIPO, 2006). A number of researchers have characterised Marula oil from various sources of *S. birrea* (Table 3). The fatty acid composition of Marula oil from Sudan was reported by Salami in 1973 to be myristic acid (0.2%), palmitic acid (17.1%), stearic acid (10.9%), oleic acid (67%), linoleic acid (4.3%), eicosanoic acid (0.9%) and cis-11-eicosanoic acid (0.7%). Marula oil from Nigeria has been characterised by Ogbobe (1992) and reported an acid value (33.7), saponification value (162.70 mg KOH/g oil), iodine value (100.25), peroxide value (4.58 mequiv/kg), refractive index (1.460) and specific gravity (0.88) with a fatty acid composition of butyric acid (0.4%), caproic acid (1.4%), myristic acid (2.1%), palmitic acid (22.6%), stearic acid (50.8%), arachidonic acid (8.5%), behemic acid (5.1%), oleic acid (4.1%) and lignoceric acid (4.1%). Marula oil from kernels sourced from different areas in Zimbabwe was characterized by Zharare & Dhlamini (2000) and reported it characteristics to be SV (184.5 mg KOH/g oil), iodine value (67.7) and acid value (3.6%) with an fatty acid composition of palmitic acid (10.7%), stearic acid (6.9%), oleic acid (72.0%), linoleic acid (8.8%), linolenic acid (0.6%), cis-11-eicosenoic acid (0.7%) and behemic acid (0.1%). Mariod, Matthäus & Eichner (2004) reported that the Marula oil from Sudan contained oleic acid (67.2%), linoleic acid (5.9%), palmitic acid (14.1%), traces of

linolenic acid and tocopherols at an amount of 13.7 mg/100 g oil, with the γ -tocopherol being the predominant. The oil content of the seed was found to be 53.5%. The fatty acid composition for Marula oil from Niger was reported by Glew et al. (2004) to be myristic acid (0.1%), palmitic acid (15.6%), palmitoleic acid (0.2%), stearic acid (11.1%), oleic acid (63.2%), 18:1 n-11 (0.9%), linoleic (5.2%), eicosanoic acid (1.3%), cis-11-eicosenoic acid (0.5%), behenic acid (0.4%) and lignoceric acid (0.8%). The fatty acid composition of refined Marula oil was reported by Kleiman, Ashley & Brown (2008) to be myristic acid (0.1%), palmitic acid (12.7%), palmitoleic acid (0.2%), stearic acid (7.4%), oleic acid (71.3%), linoleic acid (6.7%), linolenic acid (trace amount), eicosanoic acid (0.7%), cis-11-eicosenoic acid (0.4%) and lignoceric acid (0.2%). Gandure & Ketlogetswe (2011) reported the oil content of the Marula nuts to be about 58.6 % and the Marula oil from Botswana to have an acid value (1.4 mg KOH/g) and ethyl oleate to be the dominant fatty acid. The physico-chemical, fuel and lubrication properties of *S. birrea* (Marula) seed oil were studied by Robinson, Lukman & Bello (2012) and reported the oil to have an acid value (41.4 mg KOH/g), saponification value (178.6 mg KOH/g), iodine value (100.34), peroxide value (4.58 mequiv/kg), specific gravity (15 °C: 0.88), a refractive index of 1.46 with a fatty acid composition of palmitic acid (12.8%), stearic acid (7.2%), oleic acid (73.6%), linoleic acid (6.1%) and linolenic acid (0.3%). Robinson et al. 2012 concluded in their analysis that Marula oil is a suitable candidate for use as lubricants and biofuel. Oleic acid (69.0%), palmitic acid (15.3%), linoleic acid (9.2%), palmitoleic acid (4.1%) and stearic acid (1.5%) were reported by Komane, Vermaak, Summers & Viljoen (2015) to be contained in Marula oil from South Africa.

Table 3: Summary of reported physico-chemical characteristics of Marula nut oil.

Origin (Country)	Sudan	Nigeria	Zimbabwe	Sudan	Niger	Nigeria	South Africa
Oil yield (%)	NR	NR	NR	53.5	NR	NR	NR
Iodine value	NR	100.25	67.7	NR	NR	100.34	NR
Saponification value (mg KOH/g)	NR	162.70	184.5	NR	NR	178.6	NR
Acid value (mg KOH/g)	NR	33.7	3.6%	NR	NR	41.4	NR
Peroxide value (mequiv/kg)	NR	4.58	NR	NR	NR	4.58	NR
Refractive index	NR	1.46	NR	NR	NR	1.46	NR
Specific gravity	NR	0.88	NR	NR	NR	0.88	NR
Butyric acid (%)	NR	0.4	NR	NR	NR	NR	NR
Caproic acid (%)	NR	1.4	NR	NR	NR	NR	NR
Myristic acid (%)	0.2	2.1	NR	NR	0.1	NR	NR
Palmitic acid (%)	17.1	22.6	10.7	14.1	15.6	12.8	15.3

Palmitoleic acid (%)	NR	NR	NR	NR	0.2	NR	4.1
Stearic acid (%)	10.9	50.8	6.9	NR	11.1	7.2	1.5
Arachidonic acid	NR	8.5	NR	NR	NR	NR	NR
Behemic acid	NR	5.1	0.1	NR	0.4	NR	NR
Myristoleoic acid (%)	NR	NR	NR	NR	NR	NR	NR
Oleic acid (%)	67	4.1	72.0	67.2	63.2	73.6	69.0
18:1 n-11 (%)	NR	NR	NR	NR	0.9	NR	NR
Erucic acid (%)	NR	NR	NR	NR	NR	NR	NR
Linoleic acid (%)	4.3	NR	8.8	5.9	5.2	6.1	NR
Eicosanoic acid (%)	0.9	NR	NR	NR	1.3	NR	NR
cis-11-eicosanoic acid	0.70	NR	0.7	NR	0.53	NR	NR
Lignoceric acid	NR	4.1	NR	NR	0.8	NR	NR

Linolenic acid (%)	NR	NR	0.6	traces	NR	0.3	NR
Total tocopherol (mg/100g)	NR	NR	NR	13.7	NR	NR	NR
Reference	Salami (1973)	Ogbobe (1992)	Zharare & Dhlamini (2000)	Mariod et al. (2004)	Glew et al. (2004)	Robinson et al. (2012)	Komane et al. (2015)

Komane et al. (2015) have studied the safety and efficacy of Marula oil and found it to exhibit occlusive, hydrating and moisturizing characteristics and non-irritating to the skin, concluding this oil to be safe for skin applications and ideal for cosmetic formulations. By 2002, Namibia was one of the first countries to market Marula oil to be used for the development of specialized cosmetic formulations (Travel News Namibia, 2012).

2.2.3. *Citrullus lanatus* (Thunb.) Matsum & Nakai (Kalahari melon) seed oil

The *Citrullus* genus is a member of the Cucurbitaceae family and consist of a number of melon varieties, from which, in particular fruit and seeds of the *C. lanatus* and the *C. colocynth*, are classified as great food sources in many parts of Africa (Mabaleha, Mitei & Yeboah, 2007).

Figure 8 (a-c): Fruits (a), opened fruit with seeds (b) and traditional Melon oil, (arrow, c) of *C. lanatus*.

Figure 8 (a-c) represents the fruits (a), opened fruit with seeds (b) and the traditional Melon oil, (arrow, c) of *C. lanatus*. The *C. lanatus* (Thunb.) Matsum & Nakai, commonly called, Tsamma melon or wild watermelon, is indigenous to and widespread throughout Namibia (Figure 9), with three categories of local melons

existing, namely the seed melons for oil production, cooking melons for porridge and watermelons for consumption of fresh fruit (Maggs-Kölling & Christiansen, 2003).

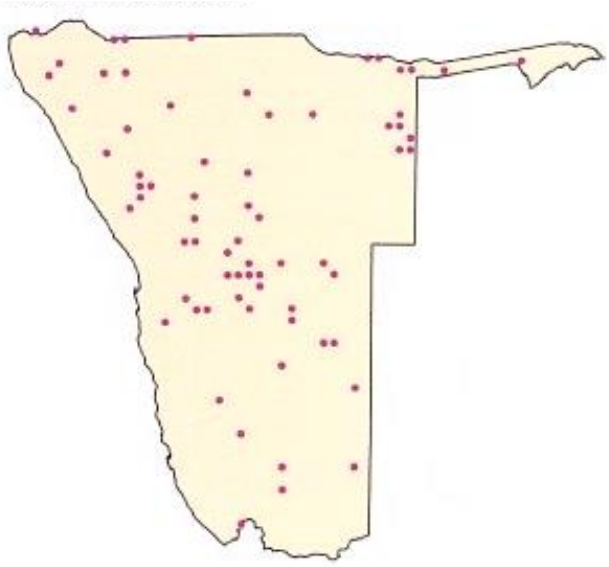


Figure 9: Distribution of *C. lanatus* across Namibia (Adopted from Mendelsohn, Jarvis, Roberts & Robertson, 2002).

In northern Namibia, the melon is also used for intercropping purposes on pearl millet fields (Maggs-Kölling & Christiansen, 2003), making this fruit a highly potential crop. The Kalahari melon seed oil is produced commercially in Kavango, Caprivi (now called Zambezi) and the north-central regions of Namibia (INP Market Bulletin, 2012). A variety of European cosmetics industries use the Kalahari melon seed oil for product development for use as moisturizers, skin regeneration and restructuring formulations (Nyam, Tan, Che Man, Lai & Long, 2009). Rural women use the Kalahari melon seed oil for healing, massages, cooking oil and as a moisturizer (Lendelvo, Munyebvu & Suich, 2012).

Tsamma melon seeds are collected by women from the north-central regions and supply them to the Eudafano Women's Co-operative in Ondangwa, which then produces a cold pressed oil (Desert Secrets, 2016). Companies such as Desert Secrets

purchase the cold pressed melon oil from the Eudafano Women's Co-operative and other entities for product formulations. Divine Oils located in Ondangwa also sells cold pressed Melon oil and mainly for export of minimum weight of 23 kg per order (Divine Oils, 2016).

The factory of the EWC can produce four tones of cold pressed melon oil per year with a large bag containing about 40 kg of seeds producing about 10 litres of oil (Swilling, 2013). The community buys the seed cakes from the EWC to be used as animal feed. Locally, the Kalahari melon seed oil can be bought at the EWC factory for N$ 100.00/L (EWC: Price List from March 2014).

The INP Market Bulletin (2011) reported a low demand in the international market for the Kalahari melon seed oil for the past years and that production was scaled down with only N$ 380 kg (N$ 40 000) of oil being exported in 2011. A value of N$ 1.5 million (1389 kg oil) was generated between 2008 and 2010 from exports. Total exports for 2011/2012 was 648 kg of oil to regional and international markets (INP Market Bulletin, 2012). In 2013, the INP Market Bulletin (2013) reported that there was still a weak international market demand for the oil, but that the Eudafano Women's Co-operative still remains to continue purchasing the seed from local farmers. Interestingly, the oil producers at household level, have started purchasing small quantities of the oil from the Eudafano Women's Co-operative (INP Market Bulletin, 2013).

Various researchers have characterised the seed oil (Table 4) from a number of different species and varieties of cucurbit species across the globe; Tsama melon from Botswana (Mabaleha, Mitei & Yeboah, 2007), *Cucumis mello* var. *agrestis*, *Cucumis melo* var. *flexuosus*, *Cucumis sativus*, *Citrullus lanatus* var. *colocynthoides*, *Cucumis*

prophetarum, and *Luffa echinata* from Sudan (Mariod et al. 2009), *Citrullus lanatus* var. *citroides* (Thrumb.) Matsum & Nakai from Ivory Coast (Gbogouri et al., 2011) and the *C. lanatus* melon (Kalahari melon) oil from northern Namibia (Nyam, Tan, Lai, Long & Che Man, 2009).

Mabaleha et al. (2007), reported on the physico-chemical properties of various melon seed oils including the Tsama melon from Botswana. The oil yield, saponification value, iodine value (Wijs), acid value, peroxide value, *p*-anisidine value, refractive index (40 °C) and relative density (30 °C) reported for the Tsama melon from Botswana seed oil, are 24.8 % (w/w), 184.4 mg KOH/g, 95.8, 1.8 mg KOH/g, 9.8 meq/kg, 2.2 mmol/kg, 1.469 and 0.889, respectively. The fatty acid composition for the Tsama melon seed oil from Botswana consisted of palmitic acid (9.9%, w/w), stearic acid (7.8%, w/w), oleic acid (15.5%, w/w) and linoleic (66.9%, w/w) (Mabaleha et al., 2007).

The characteristics of the *Citrullus lanatus* var. *colocynthoides* seed oil from Sudan were reported by Mariod et al. (2009) to be free fatty acids (2.2%), peroxide value (4.1 meq O_2/kg oil), iodine value (112.7), relative density (30 °C, 0.886), refractive index (40 °C, 1.429) and fatty acid composition as myristic acid (0.1%), palmitic acid (10.7%), stearic acid (9.0%), oleic acid (14.6%), linoleic acid (64.9%), linolenic acid (0.3%) and gondoic acid (0.4%). Gbogouri et al. (2011) have investigated the physico-chemical properties of oil from three melon species from Ivory Coast including the *Citrullus lanatus* var. *citroides* (Thumb.) Matsum & Nakai. The iodine value, acid value, peroxide value, bulk density and specific gravity reported for the *C. lanatus* seed oil from Ivory Coast were 113 g of I_2/100 g of oil, 4.30 mg of NaOH/g oil, 3.35 meq of O_2/kg of oil, 0.94 g/mL and 0.95, respectively with the fatty acid composition being; palmitic acid (11.9%), stearic acid (9.4%), oleic acid (14.4%), linoleic acid

(63.9%) and linolenic acid (0.4%). The physico-chemical properties of oil from the *C. lanatus* melon (Kalahari melon) from northern Namibia have been reported by Nyam, Tan, Lai, Long & Che Man (2009). The saponification value, iodine value, acid value, peroxide value reported are 173.2 mg KOH/g of oil, 125.0 g I_2/100g of oil, 1.1 mg KOH/g of oil and 2.3 meq of O_2/kg of oil, respectively, with the fatty acid composition being palmitic acid (12.4%), stearic acid (7.5%), oleic acid (17.1%), linoleic acid (63.1%), C18:3 (1.1%) and C20:1 (0.3%). The α-, β-, γ -, δ- tocopherols composition reported were 25.94 mg/100 g, 3.27 mg/100 g, 70.56 mg/100 g and 9.33 mg/100 g, respectively with a total of 109.10 mg/100 g of oil (Nyam et al. 2009). Nyam et al. (2009) determined the physico-chemical characteristics of Kalahari melon seed oil from Namibia after using solvent (petroleum ether) and aqueous enzymatic extraction techniques (Flavourzyme 1000 L and Neutrase 0.8 L) with the saponification value, iodine value, free fatty acid and peroxide values of the oils extracted being significantly different (p<0.05). Nyam, Tan, Karim, Lai, Long & Che Man (2010) optimised the tocopherols content recovery from Kalahari melon from Namibia and roselle seeds using supercritical fluid extraction technology. The resultant total tocopherol composition for the Kalahari melon (Namibia) seed oil under optimized conditions was 274.74 mg/100 g of oil.

Table 4: Summary of reported physico-chemical characteristics of Melon seed oil.

Origin (Country)	Botswana	Sudan	Ivory Coast	Namibia
Oil yield (%)	24.8	NR	NR	NR
Iodine value	95.8	112.7	113	125
Saponification value (mg KOH/g)	184.4	NR	NR	173.2
Acid value (mg KOH/g)	1.8	2.2%	4.30	1.1
Peroxide value (mequiv/kg)	9.8	4.1	3.35	2.3
Refractive index	1.469 (40 ºC)	1.429 (40 ºC)	NR	NR
Specific gravity	0.889[a] (30 ºC)	0.886[a] (30 ºC)	0.95	NR
Myristic acid (%)	NR	0.1	NR	NR
Palmitic acid (%)	9.9	10.7	11.9	12.4
Stearic acid (%)	7.8	9.0	9.4	7.5

Oleic acid (%)	15.5	14.6	14.4	17.1
Linoleic acid (%)	66.9	64.9	63.9	63.1
Linolenic acid (%)	NR	0.3	0.43	1.1
C20:1 (%)	NR	0.4	NR	0.3
Total tocopherol (mg/100g)	NR	NR	NR	109.10
Reference	Mabaleha et al. (2007)	Mariod et al. (2009)	Gbogouri et al. (2011)	Nyam et al. (2009)

[a]Reported as relative density

2.2.4. *Ximenia americana* (Blue sour plum) nut oil

Ximenia americana L. is a small spiny tree (about 4 m in height) from the family of Olacaceae (the sour plum family) with fruits appearing December to February, when ripe are yellow to red in colour (Palgrave, 1983). "*X. americana* L. in Namibia is known as the blue sour plum (Curtis & Mannheimer, 2005, Hoffmann, 2015) but is also known elsewhere as seaside plum, wild plum, sour plum, wild olive, monkey plum or hog plum (Řezanka & Siegler, 2007, pg 925) and is locally known as Ombeke (Oshiwambo), Omuninga (Otjiherero), Kakukuru (Kavango), Mungomba (Caprivi) (Curtis & Mannheimer, 2005).

Figure 10 (a-e): Tree (a), fruits and leaves (b), dried kernels with removed fruit flesh (c), decortication process of kernels to remove nuts (d) oil sold on traditional market (arrow, e) of *X. americana.*

Figure 10 (a-e) represents the tree (a), fruits and leaves (b), dried kernels with removed fruit flesh (c), the decortication process of kernels to remove nuts (d) and the oil sold on traditional market (arrow, e) of *X. americana*. There are eight species within the genus *Ximenia* (Mallet & den Adel-Sheehama, 2014). *X. americana* var. *americana* is the most abundant of the *Ximenia* species in the Ohangwena and Oshikoto regions and this species is used for the traditional and the cold-pressed (commercialized) oil extraction (Mallet & den Adel-Sheehama, 2014). The other species, *X. caffra* var. *natalensis* and *X. caffra* var. *caffr*a and *Ximenia americana* var. *microphylla* are also

available in Namibia, of which *X. caffra* var. *caffra* fruit is usually eaten fresh (Mallet & den Adel-Sheehama, 2014) with ripe fruits being very soft and difficult to store for long periods of time (NBRI, 2017). Figure 11 represents the distribution pattern for *X. americana* (a) *Ximenia caffra* var. *natalensis*, Large Sourplum (b), *Ximenia caffra* var. *caffra*, Large Sourplum (c) across Namibia. *X. americana* is different from *X. caffra* by having blue-green leaves, folded lengthwise and recurved (Hoffmann, 2015). The fruits of *X. americana* are yellow to orange rather than red and less tasty than the fruit of *X. caffra* changing to a dark blue colour when dried (Hoffmann, 2015).

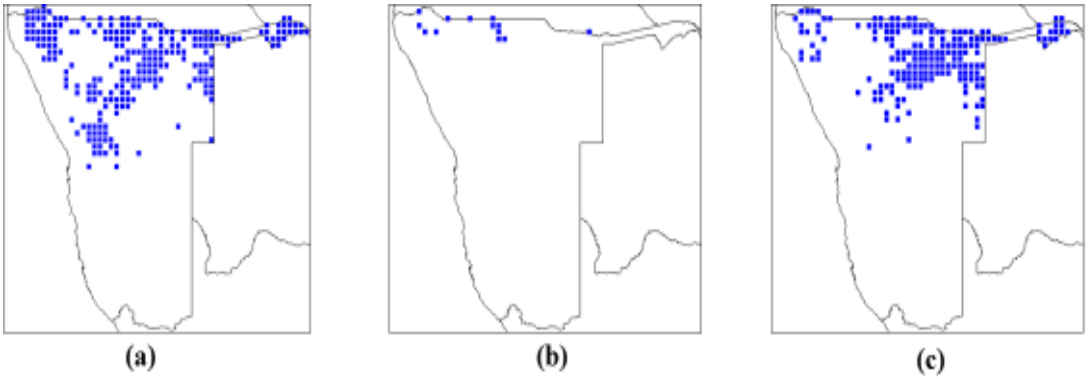


Figure 11 (a-c): Geographical distribution of *X. americana* (a) *X. caffra* var. *natalensis*, Large Sourplum (b), *X. caffra* var. *caffra*, Large Sourplum (c) across Namibia (Adopted from Curtis & Mannheimer, 2005).

Fruits are collected from the ground during December to February, which are then left to be sun-dried and then stored. In June/July, the decortication process of the kernels then starts (NBRI, 2016c). *Ximenia americana* nut oil is prepared traditionally in rural households by roasting, pounding/crushing and boiling the kernels allowing it to become brown to black in colour with a unique strong smell (Mallet & den Adel-Sheehama, 2014; NBRI, 2017). The oil is used traditionally as an emollient and as hair moisturizer (NBRI, 2016c). The oils anti-inflammatory properties, anti-ageing

properties and ability to improve blood flow has made it to become a sought-after commodity by the international market (Mallet & den Adel-Sheehama, 2014).

In 2001-2003, trials involving the purchasing of decorticated Ximenia kernels were started by CRIAA-SADC with the support of the Indigenous Plant Task Team/MAWRD, and others (NBRI, 2016c). Various technical details had to be overcome in pressing the oil from the kernel due to its sticky nature of the cold pressed version (Mallet & den Adel-Sheehama, 2014; NBRI, 2016c). Commercially, *Ximenia* kernels are harvested in the Ohangwena and Oshikoto regions where harvestors are members of the Tulongeni Twahangana Cooperative (TTC), a cooperative established in 2012 (NBRIc, 2016c). The oil is exported to France, Europe in a semi-processed (crude and decanted) version, where it is then further refined (NBRIc, 2016c). In 2012, the TTC, previously known as "Tulongeni Twahangana Project" was founded and is operating in the Ohangwena region and has about 600 members (NBRI, 2016d). Nowadays, TTC is one of the leading suppliers of *Ximenia* kernels in the southern African region (NBRI, 2016d) with Namibia being the main exporter of *X. americana* oil (Mallet & den Adel-Sheehama, 2014).

TTC is a member of Phytotrade and has about 16 certified associations registered with it (Personal communication, Johanna Amakali, MAWF, 2015). Seeds are collected during December to April from the wild and from the ground as a measure to ensure sustainability into 50 kg bags. The seeds are collected from areas such as the Oshikoto and Ohangwena regions by farmers. One farmer (18 yrs and above) can collect up to 200 kg of kernels (20 kg per day). CRIAA-SADC is the main customer for purchasing the seeds. The purchasing price in 2015 has been N$ 12 per kg. TTC experienced a bumper harvest in 2015 with more than 25 tonnes collected. Seeds are stored for a short time before being collected for further processing to avoid contaminations. The

customer has also laid down strict requirements in terms of how these seeds should be stored and packaged. There are plans in the pipeline in collaboration with the Forestry Department of the Ministry of Agriculture, Water and Forestry to try and cultivate *X. americana* to ensure the sustainable production of kernels for this oil market. The provision of this raw material depends on the weather conditions in the Ohangwena and Oshikoto regions (Mallet & den Adel-Sheehama, 2014). Currently, the TTC cooperative is planning to establish their own oil producing factory to add value to the product. The factory is planned to be in operation by 2016-2017 (Personal communication, Johanna Amakali, MAWF, 2015). Since 2008, more than 17.5 tonnes of *Ximenia* nut oil has been exported from Namibia (Mallet & & den Adel-Sheehama, 2014). The INP Market Bulletin (2011) reports that in 2011, 3150 kg *Ximenia* seed oil was exported, higher than the value reported for 2010 (3046 kg oil), with main importer, Aldivia, located in France (INP Market Bulletin, 2010). The 3150 kg of oil resulted from processing 14.4 tonnes (N$ 108 150) of *Ximenia* kernels with over 300 harvesters involved. In 2011, 16.5 tonnes of kernels generated N$ 140 250 (INP Market Bulletin, 2011). A value of N$ 0.5 million (8830 kg oil) was generated between 2008 and 2010 from exports. INP Market Bulleting (2010) reports that annually between 20 kg and 25 kg of *Ximenia* kernels are harvested per household for their own use. The export volume for *Ximenia* oil in 2012 was 383 9kg (N$ 560 561) as reported by the National Botanical Research Institute (2016b).

International companies who have developed cosmetic formulations using *Ximenia* oil (cold pressed) are; Melvita, The Body Shop, Aromatherapy Associates and Louise Galvin (Mallet & den Adel-Sheehama, 2014).

Rural households sell their traditionally prepared *Ximenia* oil on the traditional markets and various locally registered enterprises, such as Tangi Manufacturing and Consulting Services (Lututu, 2016) are also selling the *Ximenia* oil locally. The prices of the Ximenia oil are N$ 40.00 for 50 ml and N$ 270 for one litre (Lututu, 2016).

A survey of the ethnobotanical uses of *X. americana* L. among rural communities in South Angola was done by Urso, Signorini & Bruschi (2013) who then reported the uses of this oil to include health applications such ashair conditioning, improving skin tone and elasticity, prevention of stretch marks and varicose veins, soothing of joint and muscular pain and relieving of abdominal pain, mainly through massage or direct application.

Various studies have been done to characterise the oil from *Ximenia* species (Table 5); *X. caffra* (Khumalo, Majoko, Read & Ncube, 2002; Chivandi et al., 2008; Mitei et al., 2008 & 2009), and *X. americana* (Eromosele, Eromosele, Akintoye & Komolafe, 1994; Eromosele & Eromosele 2002; Eromosele & Paschal, 2003; Saeed & Bashier, 2010 and Kibuge, Kariuki & Njue, 2015). The oils of the olecaceous seeds have been found by various researchers to contain high amounts of acetylenic lipids and unsaturated fatty acids (Badami & Patil, 1980).

Three South African *Ximenia* species were studied by Ligthelm, Horn, Schwartz & von Holdt in 1954 and reported the fatty acid composition of the three species showing similarity, to contain oleic acid (32.5-40.5%), ximenynic acid (22-24.3%), octacosenoic acid (4.7-12.2%), ximenic acid (3.5-8.7%), lumequeic acid (3.0-7.0%) and tetracosenoic acid (3.0-7.0%) with the kernel containing 64-65% of oil. *X. americana* L. seed oil was found to contain about 14 fatty acids which included palmitic acid (1.0%), palmitoleic (0.2%), stearic acid (0.7%), oleic acid (48.7%),

linoleic acid (0.2%), linolenic acid (0.5%), nervonic acid (3.5%), ximenic acid (3.9%), lumequic acid (5.5%) and ximenynic acid (6.3%) and to have an iodine value (85), refractive index (40 °C: 1.4718) and oil yield (62%) (Mikolaiczak, Earle & Wolff, 1963).

Eromosele, Eromosele, Akintoye & Komolafe (1994) yielded 49.9% oil from extraction of nuts from *X. americana* from Nigeria and reported its properties to be saponification value (182.3 mg KOH/g), iodine value (149.8 g/100 g), peroxide value (29.4 mEq/kg) and an acid value (0.14 mg KOH/g). The reported iodine value makes to oil suitable to be used in paint formulations (Eromosele et al., 1994). The fatty acid composition of *X. americana* was determined by Eromosele & Eromosele (2002) to include 10 fatty acids of which it contained oleic acid (72.1%), linoleic (1.3%), linolenic (10.3%), arachidonic (0.6%), eicosatrienoic acid (3.4%), erucic acid (3.5%) and nervonic acid (1.2%), with total unsaturated fatty acids being 92.4%. The saponification value and the iodine value was reported to be 182.30 and 149.80, respectively (Eromosele & Eromosele, 2002). The density of *X. americana* was determined to be 0.9625 g/cm at 30 °C (Eromosele & Paschal, 2003). *X. caffra* was reported to contain 59% oleic acid and to be found on at the 2-position of the triglycerides (Khumalo et al., 2002).

Řezanka & Siegler (2007) identified very long chain fatty acids (VLCFA: fatty acids with more than 22 carbon atoms) up to tetracontenoic acid from *Ximenia* oil by a technique called atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy and reported its fatty acid composition of a total of 38 fatty acids, which included palmitoleic (1.9 %), palmitic acid (0.4%), linolenic acid (0.4%), heptadecenoic acid (0.1%), linoleic acid (21.3%), oleic acid (32.5%), stearic acid (2.5%), C19:1 (0.1%), C19:0 (0.1%), cis-11-eicosenoic acid (0.6%), arachidic acid

(0.2%), C21:1 (2.43%), erucic acid (2.27%), nervonic acid (C24:1: 4.7%), lumequeic acid (C30:1: 5.4%) and ximenic acid (C26:1: 6.8%). Chivandi et al. (2008) have characterised the oil of *X. caffra* (the large sour plum) from Zimbabwe and found its fatty acid composition to be myristic acid (0.02%), palmitic acid 1.5%, stearic acid (0.5%), behenic acid (0.6%), lignoceric acid (20.2%), myristoleoic acid (0.03%), oleic acid (62.8%), nervonic acid (8.6%) and α-linolenic acid (7.8%) and with an oil content of the nuts to be 47.7%.

Table 5: Summary of reported characteristics of *Ximenia americana* nut oil.

Origin (Country)	South Africa	NR	Nigeria	Sudan
Oil yield (%)	64-65	62	49.9	51.3
Iodine value	NR	85	149.8	47.59
Saponification value (mg KOH/g)	NR	NR	182.3	11.43
Acid value (mg KOH/g)	NR	NR	0.14	0.28
Peroxide value (mequiv/kg)	NR	NR	29.4	30
Refractive index	NR	1.4718 (40 ºC)	NR	1.477
Palmitic acid (%)	NR	1.0	NR	NR
Palmitoleic acid (%)	NR	0.2	NR	NR
Stearic acid (%)	NR	0.7	NR	NR
Erucic acid (%)	NR	NR	3.46	NR
Arachidonic acid (%)	NR	NR	0.60	NR
Eiosatrienoic acid (%)	NR	NR	3.39	NR

Oleic acid (%)	32.5-40.5	48.7	72.09	NR
Linoleic acid (%)	NR	0.5	1.34	NR
Linolenic acid (%)	NR	NR	10.31	NR
Nervonic acid (%)	NR	3.5	1.23	NR
Ximenynic acid (%)	22-24.3	6.3	NR	NR
Octacosenoic acid (%)	4.7-12.2	NR	NR	NR
Ximenic acid (%)	3.5-8.7	3.9	NR	NR
Lumequeic acid (%)	3.0-7.0	NR	NR	NR
Tetracosenoic acid (%)	3.0-7.0	NR	NR	NR
Methyl-14,14-dimethyl-18-hydroxy-heptatriacont-27,35-dienoate ($C_{40}H_{76}O_3$)	NR	NR	NR	major
Reference	Ligthelm et al. 1954	Mikolaiczak et al. (1963)	Eromosele et al. (1994); Eromosele & Eromosele (2002)	Saeed & Bashier (2010)

In 2004, a patent (US 2004/0115331 A1) was filed by inventors Eggink et al., titled "Ximenynic acid compositions, methods for their production and uses thereof", which involves preparing novel blends of compounds containing ximenynic acid and glycerides, which have positive effects in health (insulin resistance, body weight, skin ageing among others) and food and supplements thereof. More specifically the invention focusses on the methods of developing compositions to be used in food preparation. *Ximenia* oil is reported to contain ximenynic acid (or octadeca-trans-11-en-9-ynoic acid, 9a11t-18:2) (Eggink et al. 2004).

Kibuge et al. (2015) have studied the influence of fuel properties on the burning characteristics of *X. americana* L. seed oil from Kenya and have reported a density of 0.974 g/cm^3 at 15 °C and an acid value of 3.4 mg KOH/g among other fuel properties and concluded that this oil is a potential biofuel candidate. Saeed & Bashier (2010) characterized *X. americana* seed oil from Sudan and found it to have a saponification value (11.43), iodine value (47.59), acid value (0.28), peroxide value (30), refractive index (1.477), density (0.9376 g/ml) with the major component being methyl-14,14-dimethyl-18-hydroxy-heptatriacont-27,35-dienoate ($C_{40}H_{76}O_3$). The oil yield obtained after extraction from nuts was reported to be 51.3%.

2.2.5. *Acanthosicyos horridus* Welw. ex Hook.f. (!Nara plant) seed oil

A. horridus, a member of the Family Cucurbitaceae is a dioecious plant which occurs sporadically throughout the Namib Desert, but greatest numbers are found around the Kuiseb River Delta and some around Sossusvlei (Maggs-Kölling, Iileka, Gottlieb & Uushona, 2014).

The !Nara (Scientific Name: *Acanthosicyos horridus* Welw. Ex Hook.f.) is a leafless, thorny shrub that is endemic to the Namib Desert and is an important food source for the local Topnaar people and their livestock (Ito, 2003; Mizuni & Yamagata, 2005). Figure (a-f) represents the opened fruit with seeds (a) and plant (b), boiling of fruit flesh in drum (c), nuts (d), bottled !Nara oil (e), cosmetics from !Nara oil (f) of *A. horridus*.

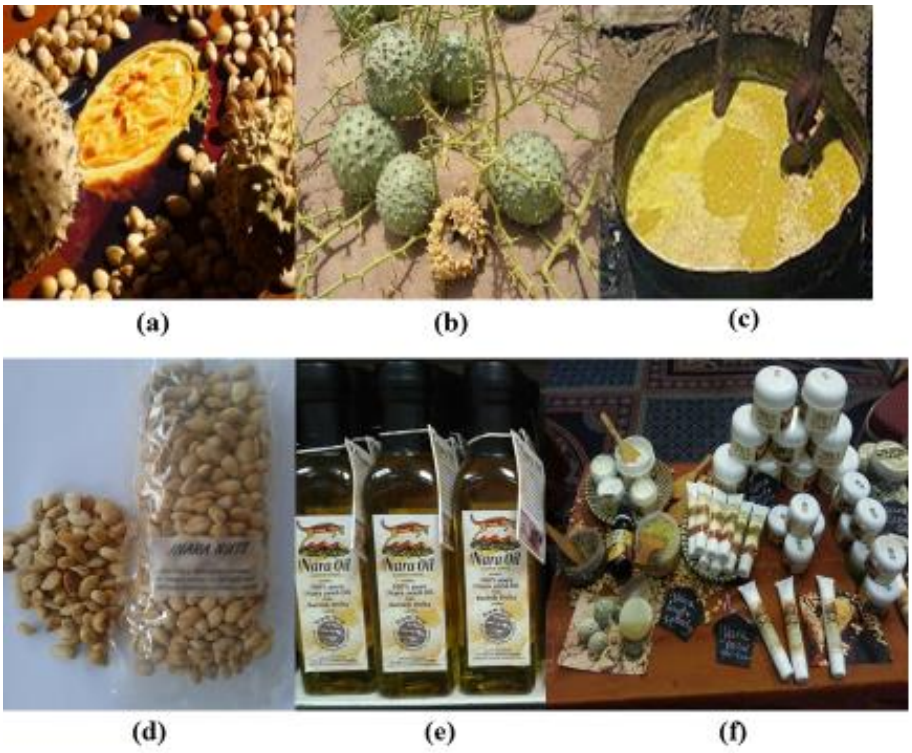


Figure 12 (a-f): Opened fruit with seeds (a) and plant (b), boiling of fruit flesh in drum (c), nuts (d), bottled !Nara oil (e), cosmetics from !Nara oil (f) of *A. horridus* (Source for (a-c): Desert Hills, 2016).

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, Dausab, Moser & Pallett, 2004). To the Topnaar communities, the !Nara plant is of a fundamental cultural value, based on a long tradition of harvesting and gathering, and provides an income in

addition to the income 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 (Mizuni & Yamagata, 2005). The ripe fruits are spiny and pale-green and can weigh between 1 to 2.5 kg (Maggs-Kölling et al., 2014).

Harvesting around 1kg of the !Nara fruit seeds takes about three hours with harvestors traveling among plants by foot and/or donkey cart, while employing the correct harvesting techniques is essential in ensuring that the plant is not damaged. Fruits are removed from bushes via traditional method by employing a long wooden stick or with modern ways, using an iron hook (Maggs-Kölling et al., 2014).

According to Henschel (2004), the flesh from fruits is removed and boiled to remove the seeds or pips. 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 & Gericke, 2000) or dried to make the so-called "!Nara-chocolate" (Maggs-Kölling, Iileka, Gottlieb & Uushona, 2014). Each !Nara bush may yield 20-500 melons with each fruit containing 50-200 seeds, requiring 10-20 melons to yield 1kg of seed, depending on the annual climate conditions and predation activities (Maggs-Kölling et al., 2014).

The market price for !Nara seed in 2015 was N$ 22.50 per kilogram which is based on an agreement reached in January 2015 between the Topnaar Traditional Authority and the buyer, Desert Hills, Swakopmund (New Era, 2015). In 2004, Henschel et al. reported the price of seeds to be about N$ 6.50/kg. The company, Desert Hills in Swakopmund, purchases the seeds in the harvesting season and produces a virgin food

oil from them (New Era, 2015, Desert Hills, 2016) and makes a number of food and cosmetic products using this oil. A spiral press is used to express the oil from the !Nara seeds producing a virgin oil (Namibia Ministry of Trade and Industry, 2013, Desert Hills, 2016).

According to Maggs-Kölling et al. (2014), the Body Shop has interest in purchasing the oil, but concerns existed that the regular supply of raw material could not be guaranteed. The oil has more than 80 % of unsaturated fatty acids of which are 58% poly unsaturated and has an acid value of 0.5 according to tests (PPECB certificate no 57212 ANLA 0808) (Namibia Ministry of Trade and Industry, 2013; Desert Hills, 2016). To date, to our knowledge, no scientific data towards the characterization of the !Nara oil has been published.

2.3. Enzymatic hydrolysis of seed oils

Hydrolysis of fats and oils also commonly known as lipolysis, is carried out to produce concentrated free fatty acids, which have significant industrial applications in producing high-value products by industries such as waste processing, oleochemical, petroleum, cosmetics, soap and detergents, pharmaceuticals, coatings, adhesives, lubricating oils, shampoos and food industries (Albasi, Bertrand & Riba, 1999; Murty, Bhat & Muniswaran, 2002; Beuve & Morison, 2010). The market size for fatty acids was estimated at over 21 million tons in 2014, which are mainly derived from hydrolysis of tallow (hard animal fats), soybean, palm kernel, coconut and crude tall oil (Global Market Insights Inc., 2016). The natural fatty acid's global market was $7.2 billion in 2011 and $6.8 billion in 2012 of which the market is expected to reach $13 billion by 2017, with a 13.6% compound annual growth rate (CAGR) from 2012 to 2017 (BCC Research, 2013).

Hydrolysis of triglycerides is generally achieved *via* the Colgate-Emery process using high pressures (5000 kPa) and high temperatures (250 °C), where undesirable side-reactions occur and side-products are formed, leading to low yield of desirable product (Pongket, Piyatheerawong, Thapphasaraphong & H-Kitikun, 2015). Alkaline hydrolysis and enzymatic hydrolysis are two other processes to be applied in the production of fatty acids (Murty, Bhat & Muniswaran, 2002).

Various difficulties are experienced with alkaline hydrolysis, whereby the resulting soaps need to be acidified to produce the products of fatty acids resulting in additional energy costs. The most energy efficient process is the enzymatic hydrolysis, taking place at 35 °C and atmospheric pressures, consisting of a reaction system with a liquid-liquid dispersion of an aqueous lipase solution and the oil (Murty, Bhat & Muniswaran, 2002).

Enzymatic hydrolysis is carried out using esterases, also termed, lipases or more systematically, triacylglycerol ester hydrolases [EC 3.1.1.3] (Schmid & Verger, 1998). The sources for lipases can be microorganisms, plants and animals and are used in a number of applications in food, paper, and detergent industry among others (Schmid & Verger, 1998).

Lipases catalyse the cleaving of ester bonds in triglycerides to free fatty acids and glycerol (Barros, Fleuri & Macedo, 2010; Mendes, Oliveira & Castro, 2012; Santos et al. 2013) at the boundary or interface of water and oil (Schmid & Verger, 1998; Murty et al., 2002). The active site of the lipase is opened when a lipid is present in the reaction mixture (Schmid & Verger, 1998). At the surface of the substrate, continuous adsorption and absorption of the lipase takes place (Al-Zuhair, Ramachandran & Hasan, 2008). In any given reaction system, the hydrolysis is affected by various

reaction parameters such as enzyme concentration, oil concentration, temperature, initial pH and agitation speed. It is therefore necessary to investigate and optimize these reaction conditions for each new lipase for hydrolysis in order to achieve maximum hydrolysis of the triglyceride.

A number of lipases have been efficiently employed to carry out hydrolysis of oil, such as *C. rugosa* lipase for sunflower oil (Pongket, Piyatheerawong, Thapphasaraphong & H-Kitikun, 2015), for palm oil (Serri, Kamarudin &Abdul Rahaman, 2008); *Thermomyces lanuginosus* for soybean oil (d'Avila Cavalcanti-Oliveira, da Silva, Ramos, Aranda & Freire, 2011); lipase from dormant castor bean seeds, *Ricinus communis* L. for canola, olive and soybean oils (Avelar et al., 2013); Lipozyme TL IM from *Thermomyces lanuginosus*, Lipozyme CALB L from *Candida antarctica*, *Yarrowia lipolytica* IMUFRJ 50682 lipase for buriti (*Mauritia vinifera*) oil (Ribeiro, Coelho & Barreto, 2012); and *Mucor miehei* lipase for blackcurrant (*Ribes nigrum*) oil (Vacek et al. 2000).

Candida rugosa lipase is unique in that it shows no positional specificity catalyzing the hydrolysis of triglycerides to mono- and diglycerides, glycerol and free fatty acids provding for effective hydrolysis of fatty acids from all three positions of the triglyceride (Enzyme Institute, 2017). To produce biodiesel by hydroesterification, the oil needs to be hydrolysed first to free fatty acids and glycerol, and then the free fatty acids are esterified with a short-chain alcohol (d'Avila Cavalcanti-Oliveira et al., 2011).

The hydrolysis of soybean oil by *Thermomyces lanuginosus* lipase for biodiesel production, has been investigated by d'Avila Cavalcanti-Oliveira et al. (2011) using a Central Composite Rotatable Design (CCRD) and have reported that the optimum

conditions were 50% (v/v) soybean oil, 2.3% (v/v) lipase in distilled water at 60 °C, achieving a conversion of 89 % after 48 hours of reaction time. Serri, Kamarudin & Abdul Rahaman (2008), carried out hydrolysis of cooking palm oil using *Candida rugosa* lipase and achieved a hydrolysis degree of 97.18% at oil load of 0.1 g/ml and enzyme load of 7.46 kLU/ml isooctane.

Enzymatic hydrolysis was employed by Ribeiro et al. (2012) to extract and concentrate β-carotene from buriti oil (crude and refined) by increasing fatty acid composition and minimizing the carotenoid loss. A Central Composite Design (CCD) was employed as the experimental design resulting in optimized parameters of 31 °C, 0.0047 g lipase/ml, 2.33 (oil:water), achieving 73.0% FFA, and 45 °C, 0.0066 g lipase/ml, 1.80 (oil:water), achieving 74.8% FFA for crude and refined oil, respectively after 4 hours and at 300 rpm (Ribeiro et al. 2012).

The hydrolysis of soybean oil, olive oil and canola oil with lipase extract from castor bean seeds (*Ricinus communis* L.), was studied by Avelar et al. (2013), who reported that the highest degree of hydrolysis was obtained with canola oil of 88.2% and was selected for the optimization of reaction conditions using a 2^3 full factorial design with three replicates at the center point. The optimized conditions for achieving complete hydrolysis of the canola oil were at a temperature of 37.5 °C, with no additives such as calcium chloride, and an oil to buffer ratio of 30 wt. %, which was achieved after 2 hours of reaction time (Avelar et al. 2013). Pongket et al. (2015) obtained a hydrolysis degree of 76.07% when hydrolysing sunflower oil with *C. rugosa* lipase at optimal conditions of initial pH of 6.7, enzyme:oil (750.28 U/g) and buffer:oil (4:1, w/w) for 19 hours of reaction time, after a Central Composite Design (CCD) was employed to determine optimum conditions. The aim of this hydrolysis was to with the addition of urea complex fractionation, to enhance the purity/concentration of the linoleic acid in

the sample and to ultimately efficiently apply this fatty acid as ingredient in the cosmetics and nutraceutical industry (Pongket et al., 2015). Eggink et al. (2004) reported in their patent (US 2004/0115331 A1) for preparing novel blends of compounds containing ximenynic acid and glycerides, a method for extracting and enriching an oil in Ximenynic acid which involves partially hydrolysing the solvent extract from crushed *X. americana* nuts with *C. rugosa* lipase to a partially hydrolyzed product; and then partitioning it into fractions rich in ximenynic acid and one which is lesser in the amount of ximenynic acid.

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials and reagents

The reagents and solvents used in the experiments were of analytical grade, unless otherwise stated. Hexane, methanol, isooctane (2,2,4-trimethylpentane), chloroform, glacial acetic acid, anhydrous sodium sulphate, hydrochloric acid, phenolphthalein, Hanus solution (Sigma-Aldrich, Johannesburg, South Africa), sodium thiosulphate, ammonium thiocyanate, ferrous chloride, ethanol, sodium hydroxide, potassium hydroxide, *p*-anisidine reagent (Sigma-Aldrich, Johannesburg, South Africa), ascorbic acid, 5α-cholestane, BSTFA, TCMS, potassium hydrogen phthalate, potassium dichromate, starch, potassium iodide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate and *Candida rugosa* lipase (≥ 700 units/mg) (Sigma-Aldrich, Johannesburg, South Africa).

3.2. Documentation of traditional processing methods for seed oil extraction

The process of the traditional oil production of the Manketti oil (one homestead), Marula and Melon oil (one homestead) was documented after receiving their informed consent (Appendix B) with respective candidates by conducting interviews and with pictures and video recordings. The questionnaire used for interviewing candidates is presented in Appendix A.

3.3. Sample collection

Kernels of *C. lanatus* (Kalahari melon), *S. rautanenii* (Manketti), *S. birrea* (A. Rich.) Hochst (Marula) and *X. americana* (Blue sour plum) were purchased from rural homesteads. The traditionally prepared oils of *C. lanatus* (Kalahari melon), *S.*

rautanenii (Manketti) and *S. birrea* (Marula) were freshly prepared in homesteads near Outapi. Informed consent was obtained from the heads of the households who prepared the traditional oils. Traditionally prepared *X. americana* (Blue sour plum) nut oil was purchased from the Omuthiya traditional market. Cold pressed oil and seeds of *A. horridus* (!Nara) were purchased from Desert Hills Company, Swakopmund. Cold pressed oils of *C. lanatus* (Kalahari melon) and *S. birrea* (A. Rich.) Hochst (Marula) were purchased from the Eudafano Women's Cooperative in Ondangwa, as the only commonly known major local producer in Namibia for the cold pressed oils chosen and which are also sold locally. Cold pressed *S. rautanenii* (Manketti) nut oil was purchased from Mungongo Trading Enterprise in Ngweze (Annex 2). Cold pressed *X. americana* (Sour plum) nut oil was obtained from the Divine Oils Company in Ondangwa.

3.4. Sample preparation

Nuts of *S. rautanenii, X. americana* and *S. birrea* were manually removed from their shells and crushed using a mortar and pestle. Seeds of *A. horridus* and *C. lanatus* were macerated using a coffee grinder. The finely ground nuts and seeds were then stored at -20 °C until further use.

3.5. Oil extraction

The macerated seeds (20 g) and nuts (20 g) were extracted with a Soxhlet apparatus. Macerated seeds or nuts were put into a cellulose thimble and extracted with 250 ml of hexane for 6 hrs in Soxhlet apparatus at 60 ºC. The solvent was removed under vacuum at 40 °C using a rotary evaporator (Heidolph, Germany). The oil yield was determined and samples were stored in dark at 4 °C for further analysis. The oils were

extracted in triplicate. Oil yield was then calculated using the equation (Yang et al., 2013):

Yield (%) = (Weight of the extracted oil (g)/Weight of seed sample (g)) x100% Eq.1

The oils were dried with anhydrous sodium sulphate and filtered at 50 °C according to the Association of Official Analytical Chemists (AOAC) Official Method: 981.11 (1998). Anhydrous Na_2SO_4 was added to the sample in proportions of 1-2g/10 g sample. The sample was then held in oven at 50 °C. Sample was vigorously stirred and then filtered and then stored at 4 °C for further analysis in glass bottles.

3.6. Determination of physico-chemical characteristics

3.6.1. Colour and state of oils

The state of oil (liquid or solid) and colour of the oils was determined by visual observation at 25 °C (Onyeike & Acheru, 2002; Ajayi, 2010).

3.6.2. Determination of the acid value

The acid value (AV) reflects the total acidity of the oil sample, while the percentage of free fatty acids (% FFA) reflects the amount of free fatty acids in the oil sample (Wrolstad et al., 2005). AV is defined as mg KOH needed to neutralize free fatty acid groups present (Wrolstad et al., 2005). The AV was determined according to the AOAC Official Method: 940.28 (1998).

The AV was then calculated from Equation 1a) and 1b) (Wrolstad et al., 2005);

FFA as % oleic acid = (ml NaOH x normality of NaOH × 28.2)/ sample weight (g)

Eq. 2a)

Acid value (mg KOH/g oil) = % FFA (as oleic acid) × 1.99

Eq. 2b)

3.6.3. Determination of the saponification value

Evidence as to the lengths of relative chains of fatty acids present in the triglyceride was obtained by the determination of the saponification value (SV) and refers to the alkali amount needed to saponify a fixed amount of oil sample (Wrolstad et al., 2005). The SV (mg KOH/g of oil) was determined according to the AOAC Official Method: 920.160 (1998).

3.6.4. Determination of the average molecular weight

The average molecular weight (AMW) is calculated from the saponification value. The average molecular weight of the seed oils was calculated according to the equation, Eq.3 (Yang, Pan, Zeng, Shupe & Hse, 2013);

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

3.6.5. Determination of the ester value

Ester value (EV) was calculated from the difference between the determined SV and the AV of the oil.

3.6.6. Determination of the iodine value (Hanus method)

To determine the level of unsaturation in an oil, the iodine value (IV) is determined and is expressed as the number of grams of I_2 that reacts with 100 g of the oil sample (Wrolstad et al., 2005). The iodine value was determined according to the AOAC Official Method: 920.158 (1998).

3.6.7. Determination of the peroxide value

To measure the level of oxidation or rancidity in an oil sample, the peroxide value (PV) was determined. The primary products of oil oxidation are the hydroperoxides formed between unsaturated fatty acids and oxygen (O'Brian, 2009). The ferric thiocyanate method (FTC) according to Jayaprakasha, Singh & Sakariah (2001) in Uluata & Özdemir (2012), was used to determine the PV of the oil samples. Data was expressed in milliequivalents O_2/kg oil.

3.6.8. Determination of the *para*-anisidine value

The amount of α and β unsaturated aldehydes in an oil sample are measured by the anisidine value and is a measure of secondary oxidation (O'Brian, 2009). The *p*-anisidine value (*p*-AV) was determined according to the AOAC Official Method: Cd 18-90 (1993).

3.6.10. Determination of specific gravity

Specific gravity (SG) was determined according to the AOAC method No. 40.1.08 (1990) in Janporn et al. (2015) using a 25 ml specific gravity bottle. Specific gravity was calculated from equation 2:

$$\text{Specific gravity} = (W_1-W_0) / (W_2-W_0), \text{ where} \quad \text{Eq. 4}$$

W_1 = weight of bottle with oil,

W_0 = weight of empty 25 ml bottle, and

W_2 = weight of bottle with water

3.6.11. Determination of the refractive index

The refractive index (RI) of oils is dependent on the fatty acid, chain length, degree of unsaturation and conjugation and molecular weight (O'Brian, 2009) of a particular oil. The refractive indices of oil samples were determined using an ABBE refractometer (K7135, MRC Ltd, Holon, Israel) at 25 °C.

3.7. Determination of the tocopherol (α, β, γ, δ) and major sterol content

The major soluble vitamin in extracted oils is a mixture of tocopherols, comprised of α-, β-, γ -, δ- tocopherols (Chen, McClements & Decker, 2011). The tocopherols content and selected major sterol content contained within selected Namibian indigenous seed oils was carried out at the GC-MS laboratory of the Central Analytical Facility, Stellenbosch University, South Africa. The oil samples were prepared and analysed according to Du & Ahn (2002). About 100 mg of oil 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 then briefly vortexed and incubated for 60 min at 50 °C. The mixture was then cooled for 10 minutes in ice. Deionized water (5 ml) and hexane (5 ml) were added. To allow the sample to separate into phases, it was vortexed and left for 15 hours. A speedvac was then used to dry 1000 µl of the supernatant. The dry samples were reconstituted with 200 µl pyridine followed with 100 µl BSTFA with 1% TMCS. The mixture was vortexed and then derivatized by incubating for 1 hour at 50 °C. The mixture was vortexed and then transferred into a GC vial containing an insert.

The tocopherols and major sterols were separated using GC with 1µl of injection volume on an Agilent 6890 N GC (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5975 MS detector, using a Zebron AB-MultiResidue (30m, 0.25mm ID, 0.25 µm film thickness) column (Part No. 7HG-G016-11) for a runtime of 40 minutes. 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 minutes and finally at 20 °C/min to 320 °C held for 12 minutes. The carrier gas was helium at a flow rate of 1.2 mL/min and the injector temperature was maintained at 200 °C and operated in a splitless mode. The mass spectral data was recorded on a MSD operated in full scan mode (35-600 *m/z*) with both the ion source and quadrupole temperatures maintained at 240 °C and 150 °C, respectively. The transfer line temperature was maintained at 200 °C. Solvent Delay was held at 5.00 min.

3.8. Triacylglycerol analysis with Nuclear Magnetic Resonance (NMR) spectroscopy

3.8.1. ^{1}H NMR analysis

^{1}H NMR spectroscopic analysis was performed using the Bruker Avance 400 MHz spectrometer (Germany) to determine acyl composition of selected Namibian indigenous seed oils using deuterated chloroform ($CDCl_3$) as solvent at 25℃. Data as "chemical shifts (δ)" were reported in ppm.

3.8.2. ^{13}C NMR analysis

^{13}C NMR spectroscopic analysis was performed using the Bruker Avance 100 MHz spectrometer (Germany) to determine acyl composition of selected Namibian indigenous seed oils using $CDCl_3$ as solvent at 25 ºC. Data as "chemical shifts (δ)" were reported in ppm.

3.9. Fatty acid composition

3.9.1. Esterification of fatty acids

The fatty acids were converted to their fatty acid methyl esters (FAME's) according to Yang et al. (2013). About 50 mg of seed oil was dissolved in 5 ml of hexane in a capped tube and then 0.5 ml of 2 M potassium hydroxide in methanol solution was added. The tube was then agitated vigorously for 5 min and then centrifuged at 3000 rpm (Eppendorf Centrifuge 5810R, Hamburg, Germany) for 15 min. The top hexane layer containing the FAME's was then removed into a capped vial and briefly stored at -20 °C until further analysis.

3.9.2. GC-MS analysis of FAMEs

The fatty acid profile as FAMEs of the seed oils was determined by gas chromatography, model 7820A coupled with mass spectrometry unit, model 5977E MSD and Agilent ChemStation software (Agilent Technologies, Palo Alto, CA). The sample injection volume was 1µl and separation was done on an Agilent capillary column HP5 MS (30 m×0.25 mm, 0.25µm). The carrier gas was helium 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°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. The composition of FAMEs was determined as percentages of the total peak areas of methyl esters contained in the sample. The identification of FAMEs was based on the comparison of mass spectra with those available from the NIST Library Mass Spectral Database and those available from literature.

3.10. Statistical methods and analysis

All experiments were carried out in triplicate, unless otherwise stated, 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. Values with different letters within rows indicate significant differences ($p<0.05$).

3.11. Enzymatic hydrolysis of selected Namibian indigenous seed oils

3.11.1. Experimental design

The enzymatic hydrolysis was carried out at 35 °C, 300 rpm, 10% (w/w) oil concentration in 200 mM potassium phosphate buffer and 20 mg/g of *C. rugosa* lipase in 250-mL shake-flasks to determine the optimum pH condition for the hydrolysis system. Lipase from *Candida rugosa* (CRL) (Sigma-Aldrich) was used in all experiments. *C. rugosa* is a non-specific enzyme, which was formerly classified as *C. cylindracea* (Schmid & Verger, 1998) and can hydrolyze tri-, di and mono-glycerides (Sharma et al., 2013). Cold pressed Manketti and Marula nut oil were used for the hydrolysis experiments due to their readily availability in large quantities in Namibia. To determine the effect of different factors such as temperature (A: °C), oil concentration (B: % w/w) and enzyme concentration (C: mg/g oil), a 2^3 full factorial design with 4 center points to evaluate experimental error, was used using Design Expert® Version 10 software (Stat-Ease, Minneapolis, MN, USA). Agitation was carried out at 300 rpm in shaking incubator (TOU-120-2, MRC Ltd, Holon, Israel) and samples were analysed after 5 hours of reaction time.

3.11.2. Determination of the percentage of hydrolysis/degree of hydrolysis

The effect of different factors on the hydrolysis system was measured with the determination of the percentage of hydrolysis obtained according to Sharma, Chaurasia & Dalai (2013). Percentage hydrolysis (%H) = (acid value time at time t - acid value at time t=0)/saponification value of the oil) × 100%. Eq.5

CHAPTER 4: RESULTS

4.1. Traditional processing methods of Namibian indigenous seed oils

The traditional processing methods of *S. birrea* (Marula) oil, *S. rautanenii* (Manketti) oil and *C. lanatus* (Melon) oil in Namibia were documented and described here. The processing methods were documented by conducting interviews with candidates in their homesteads and following the process with video recordings and pictures.

4.1.1. Traditional process of Marula oil production

The *S. birrea* (Marula) tree is an intrinsic part of the Aawambo culture and considered of high value as it is a source of food, beverage and income if products are sold that are not consumed in the household. Figure 13 represents the *S. birrea* (Marula tree) (a), the leaves and fruits (b), dried and complete kernels where the fruit flesh has been removed (c) and crushed Marula nuts after decortication of the kernels (d).

Figure 13 (a-d): *S. birrea* (Marula) tree (a) leaves and fruits (b), dried kernels (c) and crushed nuts (d).

The harvesting period of Marula fruits is from January to April of every year and each household can make up to 20 litres of traditional prepared Marula oil annually if climate conditions are appropriate in a given year to yield sufficient Marula fruits (Personal communication). Figure 14 (a-p) outlines the traditional process of Marula

oil production. The process of Marula oil production was documented in the Village of Uutangatse, Tsandi constituency, Omusati region. An axe and wooden stick is used to crack open the Marula kernel (*omahuku*) and then a needle is used to remove the nut from inside the kernel (a-d). A designated area is used for the pounding of the kernels that is made up of a flattened surface with a hole in the middle, or a so-called mortar (e). The extracted nuts are then poured into the hole (f) and pounded with a heavy and long wooden stick, the so-called pestle (g). During the pounding process, small amounts of water are added (h-k). During this process, the Marula oil (*ondjove*) is being squeezed out from the crushed Marula nuts. The seedcake (*eedi*) is skilfully removed from the hole (l, m) and the Marula oil is scooped out from the hole into a bowl (n, o). Marula oil is either then used directly for food purposes and the rest is poured into small 200-mL glass bottles for storage. Some households prefer to boil the Marula oil to improve shelf-life of this oil. The oil can be stored up to one year and longer when boiled and stored in a dark, cool place and unboiled for up to 6 months. The Marula oil is then used for food purposes mainly, as an ingredient to prepare food (addition to meat or vegetables) or as a side platter during meals. The oil is also used as a skin lotion. In a good harvest of Marula kernels for a particular year, the Marula oil is also sold on the traditional markets. The prices for this oil ranges from N$ 20.00 to N$ 30.00 for about 200 ml. Dried marula kernels are generally not sold unless there are certai agreements in place with local enterprises such as the EWC and others. The vernicular name for the Marula oil is Ondjove oil.

Figure 14 (a-p): Traditional process of Marula oil production.

4.1.2. Traditional process of Manketti oil production

Households harvest the Manketti fruits from the ground from April to May. The production of Manketti oil is more common among communities in the north-east of Namibia as the tree is most abundant there. Various households having this indigenous knowledge of Manketti oil production in the north and north-central areas, also produce the Manketti oil in small quantities. They purchase the kernels from communities in areas, where the tree is most abundant. Figure 15 represents the Manketti tree (a), leaves of the Manketti tree (b), complete and dry kernels without the fruit flesh (c) and crushed nuts used for oil extraction (d).

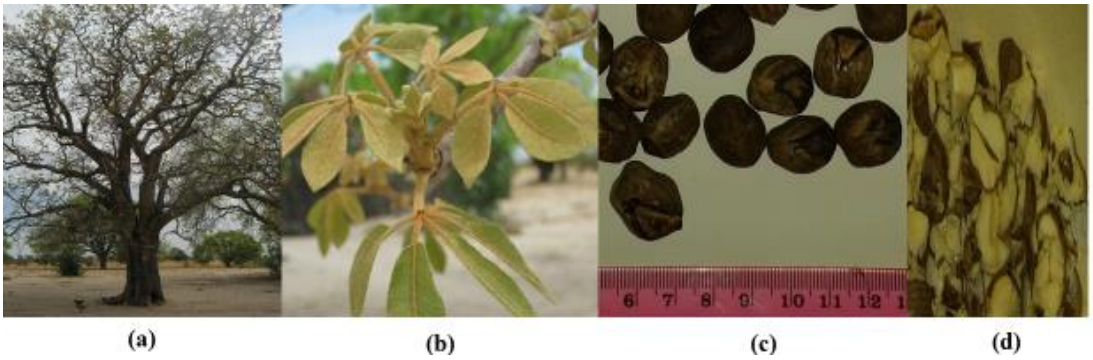


Figure 15 (a-d): *S. rautananii* (Manketti) tree (a), leaves (b), kernels (c) and cracked nuts (d).

Figure 16 (a-u) represents the traditional process of producing Manketti oil. The process of Manketti oil production was documented in the Village of Oshikulufitu, Omusati region. The seed shell is cracked open to remove the oil-bearing nut from inside (a). The nuts are slightly roasted by adding hot coals on top of the manketti nuts which aims to improve the flavour of the oil (b, c, d). The manketti nuts are then pounded using mortar (hole in cemented ground) and pestle (wooden stick) (e,f). The pounding action produces a sticky nut paste which is then removed from the mortar

and put in a bowl (g,h,i). The paste is then mixed with some boiled water (j,k,l) and the resulting solution, called “Omwai” is then decanted into a boiling pot (m). Care is to be taken not to decant the remaining undissolved seed particles (n,o). The solution is then boiled for a time period depending on the amount of starting materials (p,q,r). Continous boiling eventually allows the water to evaporate and the Manketti oil remaining (s,t). The oil is then poured in to glass bottles and stored for future use (u) or chicken is added directly to pot containing the oil for food preparations. The oil can also be added to spinach and prepared chicken at the final stages of cooking. When larger amounts of Manketti oil are prepared, greater amounts of nuts are used with a bigger boiling pot. During the boiling process, the oil is then continuously scooped off with a small calabash that has been shaped into a spoon. On a larger scale, a bigger pot is utilised and during the cooking process, oil is slowly scooped off with a calabash. The residue that remains in the boiling pot after all the oil has been scooped off is eaten as a snack after the addition of some salt. Traditional households in the Omusati region can make up to 5 litres of Manketti oil annually, but may vary depending on the source and amount of raw material (Personal communication). The oil can be stored up to 6 months. The traditional prepared Manketti oil is generally not sold in the traditional markets of the Omusati region. In the Kavango region, where the Manketti tree is most abundant, traditional houselholds can prepare between 5 to 100 litres or more of Manketti oil annually. The traditional Manketti oil is sold at N$ 30.00 for 375 ml.

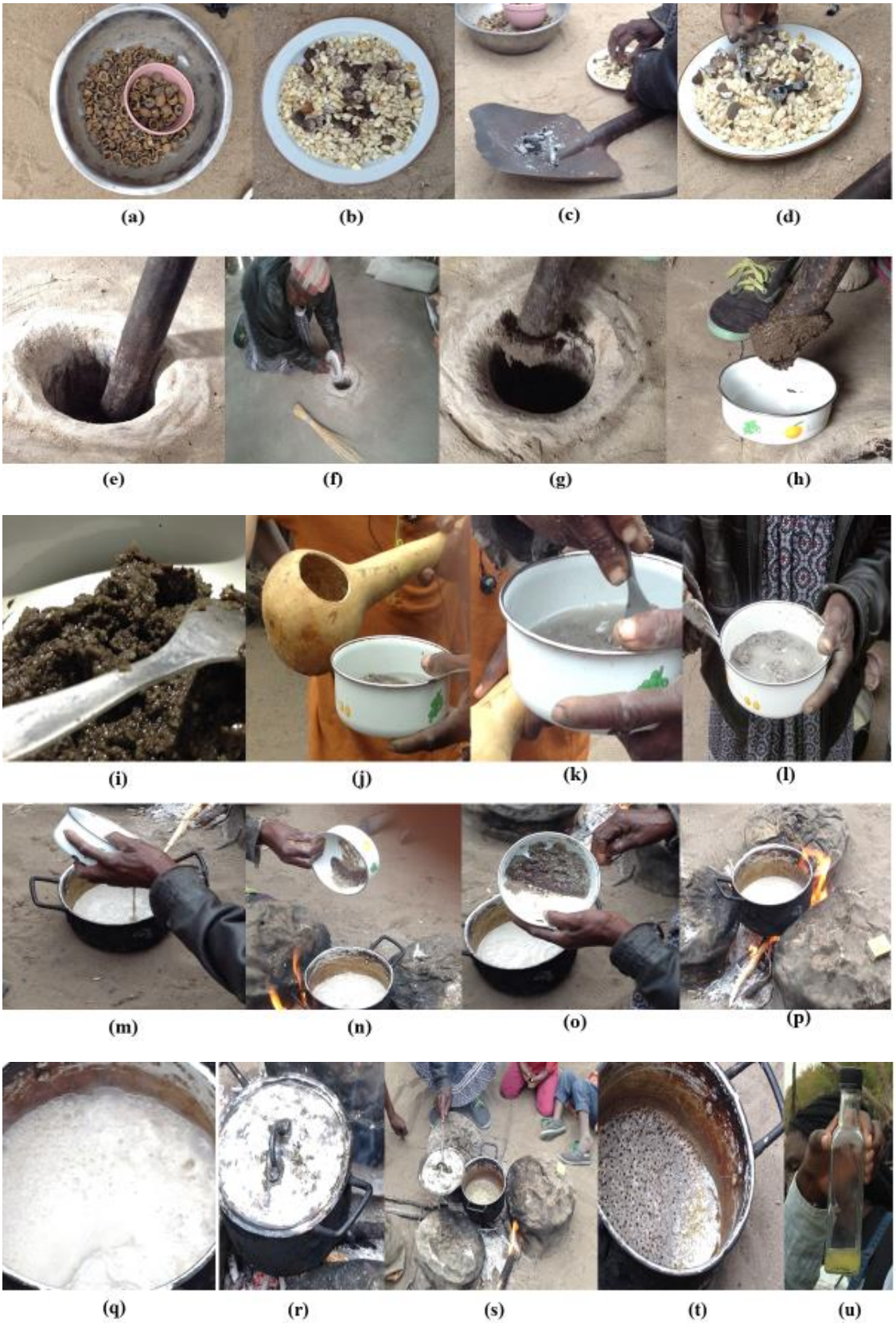


Figure 16 (a-u): Traditional process of Manketti oil production.

4.1.3. Traditional process of Melon oil production

Melons are planted in the fields among the Mahango crops as a form of intercropping and harvested during April to May. The melon is only used for oil making and the flesh is not eaten by humans. The melon seeds are removed from the fresh fruit and are then dried in the sun. The dried seeds are then stored until time during the year is made for oil extraction. Figure 17 (a-d) represents harvested fruits (a,b), opened fruit with seeds (b) and dried seeds of *C. lanatus*.

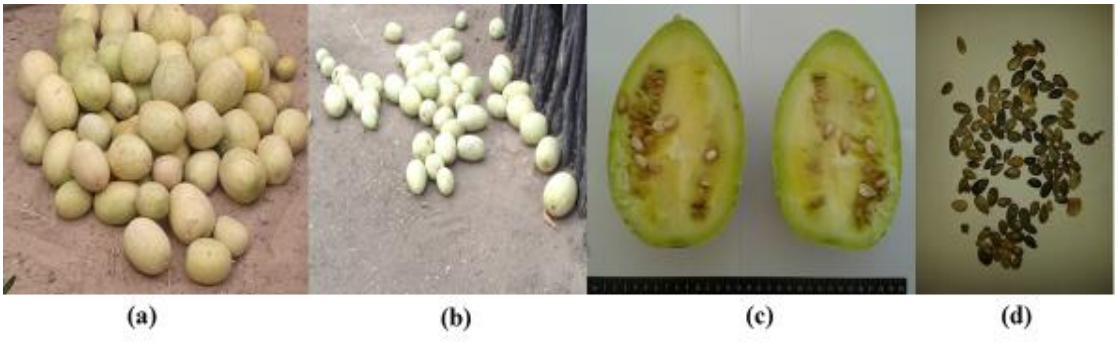


Figure 17 (a-d): Fruits (a,b), open fruit with seeds (c) and dried seeds (d) of *C. lanatus*

Traditional households can make up to 20 L annually. The process of melon oil production was documented in the Village of Uutangatse, Tsandi constituency, Omusati region. Figure 18 (a-y) outlines the traditional process of melon oil production. Sun-dried seeds are pounded to powder and mixed with water in a 20 L bucket. The fire is prepared with a three leg potjie pot (a). A basket containing grass is prepared over a bucket (b-c) to be used as a filtering device for the pounded seed powder-water mixture. A calabash serves as spoon and is used to remove portions of the mixture from the 20 L bucket (d-e).

The basket containing the grass is placed over the pot on the fire and the mixture is filtered out over the pot through the grass. Seed residues are also layered over the grass filter serving as an extra filtering device (f-o). Once the whole mixture has been filtered through the grass filter, the "basket-grass filter" is discarded (p). The mixture in the pot is then stirred using a reed stick (q). The basket is replaced back onto the pot (r) and sealed using clay between the interface of the pot and the basket (s,t). Throughout the boiling process, the mixture is occasionally stirred (t). Continuous boiling of 3 to 4 hours, allows the oil to separate out from the mixture forming a layer on top (v). Eventually the basket is removed (w) and the melon oil is carefully scoped from the top layer of the boiling mixture (x) and put into a bowl (y). The oil is later decanted into 200 ml glass bottles and stored. The oil is used for food preparation but also as skin lotion. The oil can be stored for one year and longer (Personal communication). The prices for this oil ranges from N$ 20.00 to N$ 30.00 for about 200 ml on the traditional markets. The dried melon seeds are generally not sold. The vernicular name for the Melon oil is "eentanga oil".

(a) (b) (c) (d)

(e) (f) (g) (h)

(i) (j) (k)

(l) (m) (n) (o)

Figure 18 (a-y): Traditional process of Melon oil production.

4.2. Oil extraction yields for Soxhlet extraction method

The data for the percentages of oil yields obtained from selected Namibian indigenous plant seeds after extraction with a Soxhlet apparatus using hexane for 6 hours is presented in Table 6. The highest yield of oil of 57.0 ±4.37% was obtained from *S. birrea* (Marula) kernels. The lowest oil yield was obtained from *X. americana* kernels (25.6 ±3.37%). An oil yield of 37.8 ±7.26%, 40.2 ±3.45% and 42.6 ±0.84% was obtained from *A. horridus* (!Nara plant) seeds, *C. lanatus* (Kalahari melon) seeds and *S. rautanenii* (Manketti) nuts, respectively.

Table 6: Oil yields of indigenous nut/seed oils obtained using the Soxhlet extraction method.

Plant Species	**Oil yield (%)**
Citrullus lanatus (Kalahari melon)	40.2 ±3.45
Schinziophyton rautanenii (Manketti)	42.6 ±0.84
Sclerocarya birrea (Marula)	57.0 ±4.37
Ximenia americana (Blue sour plum)	25.6 ±3.37
Acanthosicyos horridus (!Nara plant)	37.8 ±7.26

4.3. Characterization of Namibian indigenous seed oils

4.3.1. Physico-chemical characteristics of Manketti nut oil

The physico-chemical characteristics of Manketti nut oil from cold pressed, traditional and Soxhlet extraction method were determined as presented in Table 7 and compared for significant ($p<0.05$) and non-significant ($p\geq0.05$) differences using Tukey's HSD test. The tocopherol, stigmasterol and the β-sitosterol of Manketti nut oil from different extraction methods were determined using GC-MS and data is presented in Table 8. The gas chromatograms for eluted tocopherols and major sterols are presented in Appendix D: Figures 29-31. Manketti nut oil is a liquid yellow to light yellow oil at room temperature at 25 °C, depending on the extraction method used. Cold pressed and Soxhlet extracted oils are lighter in colour as compared to the traditionally extracted oil (Figure 19). Main assignments from ^{1}H and ^{13}C NMR spectral data are presented in Table 9 and Table 10, respectively, with individual spectra presented in Appendix E: Figures 43-45 (^{1}H NMR); Figures 46-48 (^{13}C NMR). The fatty acid profile presented in Table 11 was determined using GC-MS and individual gas chromatograms presented in Appendix F: Figure 72 (a-c).

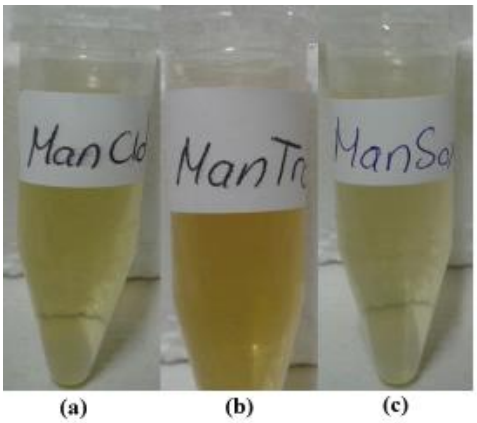


Figure 19 (a-c): Cold pressed (a), traditionally extracted (b) and Soxhlet extracted Manketti nut oil.

Table 7: Physico-chemical characteristics of Manketti nut oil from different extraction methods.

Characteristic	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[B]	Olive oil[B,E]	Soybean oil[B]
Oil appearance	Yellow	Light Yellow	Light Yellow				
State of oil at 25 ºC	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Saponification value (mg KOH/g oil)	189.06 ±1.28 [a]	187.66 ±1.52[a]	184.30 ±1.17[b]	172.16[A]	188-194	184-196	124-139
Average molecular weight (g/mol)	890.2 ±6.03[a]	896.9 ±7.30[a]	913.19 ±6.44[b]				
Acid value (mg KOH/g oil)	2.44 ±0.17[a]	2.06 ±0.03[b]	0.959 ±0.15[c]	26.22[A]	3.09[G]	6.6[F]	2.72[H]
Ester value	186.62 ±1.12[a]	185.61 ±1.55[a]	183.34 ±1.29[b]				
Peroxide value (mequiv/kg)	3.98 ±0.26[a]	2.19 ±0.27[b]	1.80 ±0.021[b]	1.02[D]	12.6[G]	≤20[I]	21.38[H]
p-anisidine value	2.51 ±0.18[a]	0.877 ±0.08[b]	0.245 ±0.07[c]				
Iodine value (g/100g)	120.24 ±0.11[a]	131.30 ±1.37[b]	126.39 ±2.43[c]	75.06[A]	118-145	75-94	124-139
Specific gravity (20 ºC)	0.922 ±0.002[a]	0.923 ±0.003[a]	0.903 ±0.006[b]	0.98[A]	0.918-0.923	0.910-0.916	0.919-0.925
Refractive index (25 ºC)	1.4827 ±0.001[a]	1.4847 ±0.001[b]	1.4767 ±0.001[c]	1.452[C] (40℃)	1.467-1.469 (40℃)	1.468-1.470 (20℃)	1.466-1.470 (40℃)

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. [A]Bhutada et al. (2016); [B]Gunstone et al. (2007); [C]Abdulkarim et al. (2007); [D]Babatunde et al. (2014); [E]not a seed oil;[F]CODEX STAN 210-1999; [G]Abitogun et al. (2008a); [H]Abitogun et al. (2008b); [I]CODEX STAN 33-1981.

Table 8: Tocopherol and major sterol content of Manketti nut oil from different extraction methods.

Tocopherol (mg/100g oil)	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[b]	Olive oil[b]	Soybean oil[b]
β-Tocopherol	nd	0.73	0.77	-	-	1.00	-
γ-Tocopherol	163.87	122.62	188.63	9.37[c]	5.00	1.00	59.00
α-Tocopherol	14.82	9.55	11.79	13.44[c]	49.00	20.00	10.00
δ-Tocopherol	3.84	4.22	4.45	4.80[c]	1.00	-	26.00
Total-Tocopherol	183.23	143.73	205.64	27.61[c]	55.00	22.00	96.00
Stigmasterol	44.28	45.34	42.33	23.10[a]	33.70	-	57.70
β-Sitosterol	586.61	667.61	682.43	45.58[a]	265.30	130.30	173.40

nd = not detected; [a]Tsaknis et al. (1998); [b]Gunstone et al. (2007); [c]Anwar et al. (2003).

The assignments of the main resonances in the ^{1}H NMR spectra (Appendix E) were assigned according to Sacchi, Addeo & Paolillo (1997), Popescu et al. (2015), Timilsena, Vongsvivut, Adhikari & Adhikari (2017) and Yeboah et al. (2017) and are presented in Table 9. The presence of protons of the main components of the Manketti nut oil resulted in a total of 11 spectral signal groupings (Table 9).

Table 9: 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—C**H**$_3$	0.83-0.88	0.83-0.88	0.84-0.82
(C**H**$_2$)$_n$	1.22-1.34	1.23-1.33	1.22-1.26
—C**H**$_2$—CH_2—COOH	1.58	1.58	1.56
—C**H**$_2$—CH=CH—	1.97-2.15	1.96-2.16	2.00-2.12
—C**H**$_2$—COOH	2.26-2.30	2.26-2.30	2.25-2.26
—CH=CH—C**H**$_2$—CH=CH—	2.75-2.82	2.72-2.75	2.73
—C**H**$_2$—OCO—R	4.09-4.13	4.09-4.14	4.08-4.12
—C**H**$_2$—O—COR	4.25-4.29	4.25-4.29	4.24-4.27
C**H**—OCO—R	5.21-5.26	5.21-5.26	5.22-5.24
–C**H**=C**H**–	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).

The assignments of the main resonances in the ^{13}C NMR spectra (Appendix E) were assigned according to Sacchi, Addeo & Paolillo (1997), Ixtaina et al. (2011), Popescu et al. (2015) and Yeboah et al. (2017) and are presented in Table 10. Four signal groupings were observed (Ixtaina et al., 2011), namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm.

Table 10: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13} NMR spectra of Manketti nut oil obtained from different extraction methods.

Assignments (Carbon)	Traditional	Cold -pressing	Soxhlet
CH_2,ω2	22.16-22.62	22.17-22.63	22.10-22.57
C3	24.76-24.80	24.70-24.73	24.70-24.73
C11	25.56	25.57	25.49
C8, C11	27.13-27.73	27.14-27.74	27.06-27.65
$(CH_2)_n$	29.01-29.70	29.02-29.71	29.05-29.65
CH_2-C=C, *trans* all[a]	32.43	32.44	32.38
C2, *sn*-1,3	33.94	33.95	33.85
C2, *sn*-2	34.11	34.12	34.02
$CH_2$0-, *sn*-1,3	62.02	62.03	61.93
CHO-, *sn*-2	68.82	68.83	68.78
Solvent ($CDCl_3$)	76.68-77.31	76.68-77.32	76.67-77.31
C12 (of α-ESA)[b]	125.86	125.87	125.80
C12	127.82	127.83	127.75-127.93
C9, *sn*-2; C10, *sn*-1,3	129.62-129.90	129.63-129.92	129.52-129.98
C9, C13	130.11-130.51	130.13-130.51	130.50
C1, *sn*-2	172.73	172.75	172.59
C1, *sn*-1,3	173.14	173.15	172.93

[a]Hutton, Garbow & Hayes (1999); [b]Yeboah et al. (2017).

Table 11: Fatty acid profile (percentage composition) of Manketti nut oil from different extraction methods.

Fatty acids	Cold pressed	Traditional	Soxhlet	*M. oleifera*[a]	Sunflower oil[b]	Olive oil[c]	Soybean oil[b]
Palmitic acid (16:0)	14.3	11.2	10.4	5.8	5.0-7.6	10.0	8.0-13.5
Stearic acid (18:0)	16.3	8.6	9.3	5.7	2.7-6.5	2.0	2.0-5.4
Arachidic acid (20:0)	0.4	0.4	0.5	3.9	0.1-0.5	-	0.1-0.6
α-Eleostearic acid (9c,11t,13t-18:3)	24.2	34.0	35.7	-	-	-	-
Oleic acid (18:1)	13.0	12.9	11.2	65.9	14.0-39.4	78	17.0-30.0
Linoleic acid (18:2)	31.2	32.2	32.1	0.6	48.3-74.0	7	48.0-59.0
Eicosenoic acid (11-20:1)	0.6	0.7	0.8	2.1	nd-0.3	-	nd-0.5

[a]Zhao & Zhang (2013); [b]Gunstone et al. (2007); [c]Gunstone (1996).

4.3.2. Physico-chemical characteristics of Marula nut oil

The physico-chemical characteristics of Marula nut oil from cold pressed, traditional and Soxhlet extraction method were determined as presented in Table 12 and compared for significant (p<0.05) and non-significant (p≥0.05) differences using Tukey's HSD test. The tocopherol, stigmasterol and the β-sitosterol of Marula nut oil from different extraction methods were determined using GC-MS and data is presented in Table 13. The gas chromatograms for eluted tocopherols are shown in Appendix D: Figures 32-34. Marula nut oil is a light yellow liquid oil at room temperature (25 °C). In Figure 20, oil samples for the cold pressed (a), traditionally (b) and Soxhlet extracted (c) Marula nut oil are shown. Cold pressed and Soxhlet extracted Marula nut oil are slightly lighter in colour as compared to the traditional extracted oil. Main assignments from ^{1}H and ^{13}C NMR spectral data are presented in Table 14 and Table 15, respectively, with individual spectra presented in Appendix E: Figures 52-54 (^{1}H NMR) and 55-57 (^{13}C NMR). The fatty acid composition as determined by GC-MS is presented in Table 16 with data represented by gas chromatograms in Appendix F: Figure 73 (a-c).

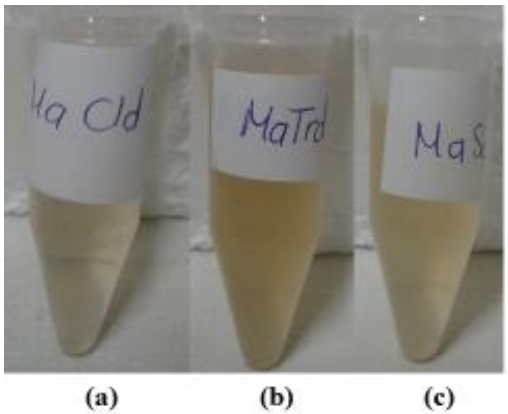


Figure 20 (a-c): Cold pressed (a), traditionally extracted (b) and Soxhlet extracted (c) Marula nut oil.

Table 12: Physico-chemical characteristics of Marula nut oil obtained from different extraction methods.

Characteristic	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[B]	Olive oil[B,E]	Soybean oil[B]
Oil appearance	Light yellow	Yellow	Light yellow				
State of oil at 25 ºC	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Saponification value (mg KOH/g oil)	186.61 ±0.85[a]	187.94 ±0.48[a]	178.67 ±1.15[b]	172.16[A]	188-194	184-196	124-139
Average molecular weight (g/mol)	901.88 ±4.08[a]	895.52 ±2.28[a]	941.96 ±4.63[b]				
Acid value (mg KOH/g oil)	1.64 ±0.10[a]	5.16 ±0.16[b]	2.69 ±0.39[c]	26.22[A]	3.09[G]	6.6[F]	2.72[H]
Ester value	184.98 ±0.75[a]	182.77 ±0.63[a]	175.98 ±1.50[b]				
Peroxide value (mequiv/kg)	0.190 ±0.01[a]	0.122 ±0.02[b]	0.412 ±0.01[c]	1.02[D]	12.6[G]	≤20[I]	21.38[H]
p-anisidine value	0.083 ±0.01[a]	0.076 ±0.02[a]	0.08 ±0.02[a]				
Iodine value (g/100g)	66.96 ±0.41[a]	70.22 ±1.10[b]	59.71 ±0.39[c]	75.06[A]	118-145	75-94	124-139
Specific gravity (20 ºC)	0.916 ±0.001[a]	0.914 ±0.003[a]	0.883 ±0.015[b]	0.98[A]	0.918-0.923	0.910-0.916	0.919-0.925
Refractive index (25 ºC)	1.4645 ±0.001[a]	1.4645 ±0.003[a]	1.4530 ±0.002[b]	1.452[C] (40℃)	1.467-1.469 (40℃)	1.468-1.470 (20℃)	1.466-1.470 (40℃)

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.
[A]Bhutada et al. (2016); [B]Gunstone et al. (2007); [C]Abdulkarim et al. (2007); [D]Babatunde et al. (2014); [E]not a seed oil;[F]CODEX STAN 210-1999; [G]Abitogun et al. (2008a); [H]Abitogun et al. (2008b); [I]CODEX STAN 33-1981.

Table 13: Tocopherol and major sterol content of Marula nut oil from different extraction methods.

Tocopherol (mg/100g oil)	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[b]	Olive oil[b]	Soybean oil[b]
β-Tocopherol	nd	nd	nd	-	-	1.00	-
γ-Tocopherol	21.51	19.85	6.22	9.37[c]	5.00	1.00	59.00
α-Tocopherol	4.14	4.67	4.14	13.44[c]	49.00	20.00	10.00
δ-Tocopherol	4.07	3.89	3.77	4.80[c]	1.00	-	26.00
Total-Tocopherol	29.72	28.41	14.13	27.61[c]	55.00	22.00	96.00
Stigmasterol	53.11	50.79	35.79	23.10[a]	33.70	-	57.70
β-Sitosterol	447.16	439.94	222.29	45.58[a]	265.30	130.30	173.40

nd = not detected; [a]Tsaknis et al. (1998); [b]Gunstone et al. (2007); [c]Anwar et al. (2003).

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 are represented in Table 14. The presence of protons of the main components of the Marula nut oil resulted in a total of 10 spectral signal groupings (Table 14).

Table 14 Chemical shifts and assignments of main resonances in ppm (δ) of the ^{1}H NMR spectra of Marula nut oil obtained from different extraction methods.

Assignment (Proton)	Traditional	Cold - pressing	Soxhlet
—CH_2—CH_2—CH_2—C**H**$_3$	0.83-0.86	0.83-0.86	0.83-0.86
(C**H**$_2$)$_n$	1.22-1.27	1.22-1.27	1.22-1.27
—C**H**$_2$—CH_2—COOH	1.57	1.58	1.57
—C**H**$_2$—CH=CH—	1.97-1.98	1.97-1.99	1.97-1.98
—C**H**$_2$—COOH	2.25-2.29	2.26-2.30	2.25-2.29
—CH=CH—C**H**$_2$—CH=CH—	2.72-2.75	2.72-2.75	2.72-2.73
—C**H**$_2$—OCO—R	4.09-4.13	4.09-4.13	4.08-4.13
—C**H**$_2$—O—COR	4.24-4.28	4.25-4.29	4.24-4.28
C**H**—OCO—R	5.22-5.26	5.22-5.25	5.22-5.25
–C**H**=C**H**–	5.30-5.33	5.29-5.34	5.29-5.30
Solvent ($CDCl_3$)	7.24	7.24	7.24

The assignments of the main resonances in the ^{13}C NMR spectra (Appendix E) were assigned according to Sacchi, Addeo & Paolillo (1997), Ixtaina et al. (2011) and Popescu et al. (2015) and are presented in Table 15. Four signal groupings were observed (Ixtaina et al., 2011), namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm.

Table 15: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13} NMR spectra of Marula nut oil obtained from different extraction methods.

Assignments (Carbon)	Traditional	Cold -pressing	Soxhlet
CH_2,ω2	22.63	22.63	22.60-22.63
C3	24.78-24.82	24.79-24.83	24.70-24.73
C8, C11	27.11-27.16	27.12-27.17	27.10-27.16
$(CH_2)_n$	29.03-29.71	29.00-29.72	29.03-29.71
C2, *sn*-1,3	33.95	33.97	33.95
C2, *sn*-2	34.12	34.14	34.11
$CH_2$0-, *sn*-1,3	62.02	62.04	62.02
CHO-, *sn*-2	68.83	68.84	68.83
Solvent ($CDCl_3$)	76.68-77.32	76.68-77.31	76.68-77.31
C9, *sn*-2; C10, *sn*-1,3	129.62-129.92	129.64-129.95	129.61-129.91
C1, *sn*-2	172.73	172.77	172.14
C1, *sn*-1,3	173.15	173.20	172.73

Table 16: Fatty acid profile (percentage composition) of Marula nut oil from different extraction methods.

Fatty acids	Cold pressed	Traditional	Soxhlet	*M. oleifera*[a]	Sunflower oil[b]	Olive oil[c]	Soybean oil[b]
Palmitic acid (16:0)	16.7	16.3	16.5	5.8	5.0-7.6	10.0	8.0-13.5
Stearic acid (18:0)	13.2	14.1	13.3	5.7	2.7-6.5	2.0	2.0-5.4
Arachidic acid (20:0)	1.6	1.9	1.6	3.9	0.1-0.5	-	0.1-0.6
Oleic acid (18:1)	67.3	66.6	67.6	65.9	14.0-39.4	78	17.0-30.0
Eicosenoic acid (11-20:1)	1.1	1.2	1.0	2.1	nd-0.3	-	nd-0.5

[a]Zhao & Zhang (2013); [b]Gunstone et al. (2007); [c]Gunstone (1996).

4.3.3. Physico-chemical characteristics of *Ximenia* nut oil

The physico-chemical characteristics of *Ximenia* nut oil from cold pressed, traditional and Soxhlet extraction method were determined as presented in Table 17 and compared for significant (p<0.05) and non-significant (p≥0.05) differences using Tukey's HSD test. The tocopherol, stigmasterol and the β-sitosterol of *Ximenia* oil from different extraction methods were determined using GC-MS and data is presented in Table 18. The gas chromatograms for eluted tocopherols is presented in Appendix D: Figures 35-37. In Figure 21, the oil samples for the cold pressed (a), traditionally extracted (b) and Soxhlet extracted (c) *Ximenia* nut oil are shown. *Ximenia* nut oil (cold pressed and Soxhlet extraction) is a light yellow oil at room temperature (25 °C) and of a sticky consisteny. The traditional extracted *Ximenia* nut oil is dark brown to black in colour. Cold pressed *Ximenia* nut oil is stickier then the traditional and cold pressed *Ximenia* nut oil. Main assignments of ^{1}H and ^{13}C NMR spectral data are presented in Table 19 and Table 20, respectively, with individual spectra presented in Appendix E: Figures 56-58 (^{1}H NMR) and 59-61 (^{13}C NMR). The fatty acid composition as determined by GC-MS is presented in Table 21 with data represented by gas chromatograms in Appendix F: Figure 74(a-c).

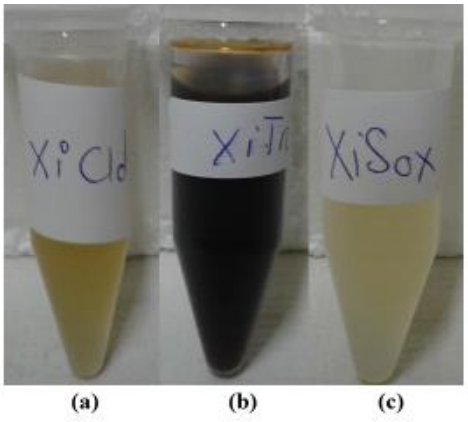


Figure 21 (a-c): Cold pressed (a), traditionally extracted (b) and Soxhlet extracted (c) *Ximenia* nut oil.

Table 17: Physico-chemical characteristics of *Ximenia* nut oil from different extraction methods.

Characteristic	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[B]	Olive oil[B,E]	Soybean oil[B]
Oil appearance	Dark brown to black	Pale yellow	Pale yellow				
State of oil at 25 ºC	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Saponification value (mg KOH/g oil)	164.53 ±0.56[a]	164.89 ±0.53[a]	156.22 ±0.80[b]	172.16[A]	188-194	184-196	124-139
Average molecular weight (g/mol)	1016.72 ±72[a]	1020.69 ±3.31[a]	1077.33±6.26[b]				
Acid value (mg KOH/g of oil)	6.07 ±0.07[a]	5.90 ±0.16[a]	1.49 ±0.44[b]	26.22[A]	3.09[G]	6.6[F]	2.72[H]
Ester value	159.46 ±0.60[a]	158.99 ±0.39[a]	154.73 ±0.37[b]				
Peroxide value (mequiv/kg)	2.12 ±0.02[a]	0.47 ±0.01[b]	0.47 ±0.06[b]	1.02[D]	12.6[G]	≤20[I]	21.38[H]
p-anisidine value	1.24 ±0.27[a]	0.085 ±0.005[b]	0.067 ±0.003[b]				
Iodine value (g/100g)	85.01 ±0.97[a]	82.01 ±1.80[a]	79.58 ±0.95[b]	75.06[A]	118-145	75-94	124-139
Specific gravity (20 ºC)	0.912 ±0.002[a]	0.913 ±0.003[a]	0.903 ±0.006[b]	0.98[A]	0.918-0.923	0.910-0.916	0.919-0.925
Refractive index (25 ºC)	1.4715 ±0.001[a]	1.4710 ±0.001[b]	1.4605 ±0.002[c]	1.452[C] (40℃)	1.467-1.469 (40℃)	1.468-1.470 (20℃)	1.466-1.470 (40℃)

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.
[A]Bhutada et al. (2016); [B]Gunstone et al. (2007); [C]Abdulkarim et al. (2007); [D]Babatunde et al. (2014); [E]not a seed oil;[F]CODEX STAN 210-1999; [G]Abitogun et al. (2008a); [H]Abitogun et al. (2008b); [I]CODEX STAN 33-1981.

Table 18: Tocopherol and major sterol content of *Ximenia* nut oil from different extraction methods.

Tocopherol (mg/100g oil)	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[b]	Olive oil[b]	Soybean oil[b]
β-Tocopherol	0.61	nd	nd	-	-	1.00	-
γ-Tocopherol	nd	nd	nd	9.37[c]	5.00	1.00	59.00
α-Tocopherol	7.07	3.69	4.05	13.44[c]	49.00	20.00	10.00
δ-Tocopherol	3.07	3.19	3.67	4.80[c]	1.00	-	26.00
Total-Tocopherol	10.75	6.88	7.72	27.61[c]	55.00	22.00	96.00
Stigmasterol	39.95	42.65	42.44	23.10[a]	33.70	-	57.70
β-Sitosterol	332.39	347.26	388.88	45.58[a]	265.30	130.30	173.40

nd = not detected; [a]Tsaknis et al. (1998); [b]Gunstone et al. (2007); [c]Anwar et al. (2003).

The assignments of the main resonances in the ^{1}H NMR spectra (Appendix E) were assigned according to Vickery, Whitfield, Ford & Kennett (1984), Sacchi et al. (1997), Popescu et al. (2015), Timilsena et al. (2017) and are represented in Table 19. The presence of protons of the main components of the *Ximenia* nut oil resulted in a total of 11 spectral signal groupings (Table 19).

Table 19: Chemical shifts and assignments of main resonances in ppm (δ) of the 1H NMR spectra of *Ximenia* nut oil obtained from different extraction methods.

Assignment (Proton)	Traditional	Cold -pressing	Soxhlet
—CH_2—CH_2—CH_2—C**H**$_3$	0.83-0.86	0.83-0.86	0.83-0.86
(C**H**$_2$)$_n$	1.22-1.27	1.23-1.27	1.22-1.27
—C**H**$_2$—CH_2—COOH	1.44-1.50	1.46-1.48	1.45-1.47
—C**H**$_2$—CH=CH—	1.58-1.64	1.58-1.65	1.57-1.64
—C**H**$_2$—COOH	1.97-2.06	1.97-2.05	1.97-2.04
—CH=CH—C**H**$_2$—CH=CH—	2.22-2.29	2.22-2.30	2.23-2.29
—C**H**$_2$—OCO—R	4.09-4.13	4.09-4.14	4.08-4.13
—C**H**$_2$—OCO—R	4.24-4.28	4.25-4.29	4.24-4.28
C**H**—OCO—R	5.07-5.24	5.08-5.25	5.22-5.27
–C**H**=C**H**–	5.26-5.43	5.27-5.43	5.30-5.42
Protons of ximenynic acid[a] and Octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid	5.97-6.04	5.97-6.04	5.98-6.01
Solvent ($CDCl_3$)	7.24	7.24	7.24

[a]Vickery et al. (1984).

The assignments of the main resonances in the ^{13}C NMR spectra (Appendix E) were assigned according to Sacchi, Addeo & Paolillo (1997), Ixtaina et al. (2011) and Popescu et al. (2015) and are presented in Table 20. Four signal groupings were observed (Ixtaina et al., 2011), namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm.

Table 20: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13} NMR spectra of *Ximenia* nut oil obtained from different extraction methods.

Assignments (Carbon)	Traditional	Cold -pressing	Soxhlet
CH_2,ω2	22.54-22.64	22.55-22.64	22.62
C3	24.74-24.81	24.75-24.82	24.73-24.80
C8, C11	27.12-27.16	27.12-27.17	27.10-27.14
$(CH_2)_n$	29.04-29.72	29.00-29.73	29.02-29.71
CH_2-C=C, *trans* all[a]	32.15-32.92	32.15-32.93	32.91
C2, *sn*-1,3	33.93-33.99	33.99	33.96
C2, *sn*-2	34.13	34.14	34.11
$CH_2$0-, *sn*-1,3	62.03	62.04	62.00
CHO-, *sn*-2	68.83	68.84	68.82
Solvent ($CDCl_3$)	76.68-77.32	76.68-77.31	76.68-77.32
C9, *sn*-2; C10, *sn*-1,3	129.61-129.95	129.62-129.96	129.59-129.91
C1, *sn*-2	172.77	172.78	172.70
C1, *sn*-1,3	173.19	173.21	173.11

[a]Hutton, Garbow & Hayes (1999).

Table 21: Fatty acid profile (percentage composition) of *Ximenia* nut oil from different extraction methods.

Fatty acid	Cold pressed	Traditional	Soxhlet	*M. oleifera*[a]	Sunflower oil[b]	Olive oil[c]	Soybean oil[b]
Palmitic acid (16:0)	3.2	2.8	3.0	5.8	5.0-7.6	10.0	8.0-13.5
Stearic acid (18:0)	17.9	15.4	18.4	5.7	2.7-6.5	2.0	2.0-5.4
Arachidic acid (20:0)	4.0	17.1	4.3	3.9	0.1-0.5	-	0.1-0.6
Oleic (18:1)	47.3	44.1	46.3	65.9	14.0-39.4	78	17.0-30.0
Eicosenoic acid (11-20:1)	4.2	10.8	4.8	2.1	nd-0.3	-	nd-0.5
Ximenynic acid (9a11t-18:2)	11.9	6.5	12.0	-	-	-	-
Octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid	11.6	3.4	11.3	-	-	-	-

[a]Zhao & Zhang (2013); [b]Gunstone et al. (2007); [c]Gunstone (1996).

4.3.4. Physico-chemical characteristics of !Nara seed oil

The physico-chemical characteristics of the !Nara seed oil from cold pressed and Soxhlet extraction methods were determined and compared for significant (p<0.05) and non-significant (p≥0.05) differences using Tukey's HSD test and are presented in Table 22. The tocopherol, stigmasterol and the β-sitosterol of the !Nara oil from the two different extraction methods were determined using GC-MS analysis and data is presented in Table 23 with gas chromatograms of eluted tocopherols presented in Appendix D: Figures: 38-39. The !Nara oil is a yellow oil at room temperature (25 °C). In Figure 22, !Nara nuts (a), the oil for the cold pressed (b), and Soxhlet extracted (c) !Nara seed oil are shown. The main assignments of the ^{1}H and ^{13}C NMR spectral data are presented in Table 24 and Table 25, respectively, with individual spectra presented in Appendix E: Figures 62-63 (^{1}H NMR) and 64-65 (^{13}C NMR). The fatty acid composition as determined by GC-MS is presented in Table 26 with data represented by gas chromatograms in Appendix F: Figure 75 (a,b).

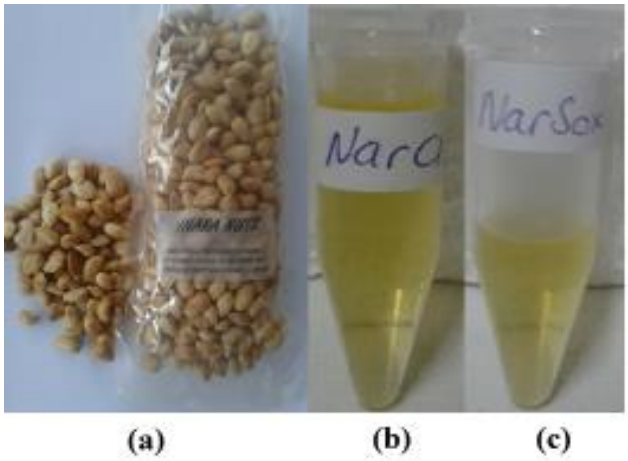


Figure 22 (a-c): !Nara nuts (a), cold pressed (b) and Soxhlet extracted (c) !Nara seed oil.

Table 22: Physico-chemical characteristics of !Nara seed oil from different extraction methods.

Characteristic	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[B]	Olive oil[B,E]	Soybean oil[B]
Oil appearance	Yellow	Yellow				
State of oil at 25 ºC	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Saponification value (mg KOH/g oil)	186.19 ±2.21[a]	180.48 ±0.85[b]	172.16[A]	188-194	184-196	124-139
Average molecular weight (g/mol)	903.98 ±10.78[a]	932.51 ±5.14[b]				
Acid value (mg KOH/g of oil)	0.643 ±0.041[a]	0.708 ±0.098[a]	26.22[A]	3.09[G]	6.6[F]	2.72[H]
Ester value	185.55 ±2.21[a]	179.77 ±0.93[b]				
Peroxide value (mequiv/kg)	2.77 ±0.22[a]	2.51±0.12[a]	1.02[D]	12.6[G]	≤20[I]	21.38[H]
p-anisidine value	0.427 ±0.14[a]	0.616 ±0.18[a]				
Iodine value (g/100g)	111.03 ±1.70[a]	108.27 ±0.94[b]	75.06[A]	118-145	75-94	124-139
Specific gravity (20 ºC)	0.921 ±0.001[a]	0.901 ±0.008[b]	0.98[A]	0.918-0.923	0.910-0.916	0.919-0.925
Refractive index (25 ºC)	1.4724 ±0.001[a]	1.4676 ±0.001[b]	1.452[C] (40℃)	1.467-1.469 (40℃)	1.468-1.470 (20℃)	1.466-1.470 (40℃)

Data shown as means with ±SD of three replicates. Means with different letters (a and b) in the same row are significantly different ($p<0.05$) as determined with the Tukey's HSD test. [A]Bhutada et al. (2016); [B]Gunstone et al. (2007); [C]Abdulkarim et al. (2007); [D]Babatunde et al. (2014); [E]not a seed oil;[F]CODEX STAN 210-1999; [G]Abitogun et al. (2008a); [H]Abitogun et al. (2008b); [I]CODEX STAN 33-1981.

Table 23: Tocopherol and major sterol content of !Nara seed oil from different extraction methods.

Tocopherol (mg/100g of oil)	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[b]	Olive oil[b]	Soybean oil[b]
β-Tocopherol	0.69	0.79	-	-	1.00	-
γ-Tocopherol	35.54	45.13	9.37[c]	5.00	1.00	59.00
α-Tocopherol	4.66	6.31	13.44[c]	49.00	20.00	10.00
δ-Tocopherol	3.62	4.14	4.80[c]	1.00	-	26.00
Total-Tocopherol	44.51	46.10	27.61[c]	55.00	22.00	96.00
Stigmasterol	20.53	24.63	23.10[a]	33.70	-	57.70
β-Sitosterol	19.84	26.80	45.58[a]	265.30	130.30	173.40

[a]Tsaknis et al. (1998); [b]Gunstone et al. (2007); [c]Anwar et al. (2003).

The assignments of the main resonances in the ^{1}H NMR spectra (Appendix E) were assigned according to Sacchi et al. (1997), Popescu et al. (2015), Timilsena et al. (2017) and are represented in Table 24. The presence of protons of the main components of the !Nara seed oil resulted in a total of 10 spectral signals groupings (Table 24).

Table 24: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{1}H NMR spectra of !Nara seed oil obtained from different extraction methods.

Assignment (Proton)	Cold -pressing	Soxhlet
—CH_2—CH_2—CH_2—C**H**$_3$	0.84-0.85	0.83-0.85
(C**H**$_2$)$_n$	1.22-1.26	1.22-1.27
—C**H**$_2$—CH_2—COOH	1.57	1.57
—C**H**$_2$—CH=CH—	1.98-2.01	1.96-2.03
—C**H**$_2$—COOH	2.25-2.27	2.25-2.29
—CH=CH—C**H**$_2$—CH=CH—	2.73	2.72-2.75
—C**H**$_2$—OCO—R	4.08-4.12	4.08-4.13
—C**H**$_2$—OCO—R	4.24-4.27	4.24-4.28
C**H**—OCO—R	5.23-5.24	5.21-5.25
–C**H**=C**H**–	5.30-5.33	5.30-5.35
Solvent ($CDCl_3$)	7.24	7.24

The assignments of the main resonances in the ^{13}C NMR spectra (Appendic E) were assigned according to Sacchi, Addeo & Paolillo (1997), Ixtaina et al. (2011) and Popescu et al. (2015) and are presented in Table 25. Four signal groupings were observed (Ixtaina et al., 2011), namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm.

Table 25: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13} NMR spectra of !Nara seed oil obtained from different extraction methods.

Assignments (Carbon)	Cold -pressing	Soxhlet
CH_2,ω2	22.49-22.60	22.50-22.61
C3	24.74-24.77	24.76-24.79
C11	25.53	25.55
C8, C11	27.10	27.12
$(CH_2)_n$	29.02-29.68	29.01-29.69
C2, *sn*-1,3	33.93	33.91
C2, *sn*-2	34.09	34.08
$CH_2$0-, *sn*-1,3	62.00	61.98
CHO-, *sn*-2	68.81	68.80
Solvent ($CDCl_3$)	76.68-77.32	76.68-77.31
C12	127.79-127.97	127.81-127.98
C9, *sn*-2; C10, *sn*-1,3	129.58-129.85	129.60-129.89
C9, C13	130.06	130.09
C1, *sn*-2	172.68	172.69
C1, *sn*-1,3	173.09	173.12

Table 26: Fatty acid profile (percentage composition) of !Nara seed oil from different extraction methods.

Fatty acid	Cold pressed	Soxhlet	*M. oleifera*[a]	Sunflower oil[b]	Olive oil[c]	Soybean oil[b]
Palmitic acid (16:0)	15.6	15.6	5.8	5.0-7.6	10.0	8.0-13.5
Stearic acid (18:0)	11.9	11.7	5.7	2.7-6.5	2.0	2.0-5.4
Oleic (18:1)	13.9	12.8	3.9	0.1-0.5	-	0.1-0.6
Eicosenoic acid (11-20:1)	4.4	4.4	65.9	14.0-39.4	78	17.0-30.0
Linoleic acid (18:2)	53.1	54.5	0.6	48.3-74.0	7	48.0-59.0
α-Eleostearic acid (9,11,13-18:3)	1.0	1.0	-	-	-	-

[a]Zhao & Zhang (2013); [b]Gunstone et al. (2007); [c]Gunstone (1996).

4.3.5 Physico-chemical characteristics of Melon seed oil

The physico-chemical characteristics of Melon seed oil obtained from cold pressed, traditionally and Soxhlet extraction method were determined as presented in Table 27 and compared for significant (p<0.05) and non-significant (p≥0.05) differences using Tukey's HSD test. The tocopherol, stigmasterol and the β-sitosterol of Melon seed oil from different extraction methods were determined using GC-MS and data is presented in Table 28 with gas chromatograms of eluted tocopherols in Appendix D: Figures 41-42. Melon seed oil is a yellow oil at room temperature (25 °C). In Figure 23, the oil for the cold pressed (a), traditionally extracted (b) and Soxhlet extracted (c) Melon seed oil is shown. ^{1}H and ^{13}C NMR spectral data main assignments of spectra peaks are presented in Table 29 and Table 30, respectively, with individual spectra presented in Appendix E: Figures 66-68 (^{1}H NMR) and 69-71 (^{13}C NMR). The fatty acid composition as determined by GC-MS is presented in Table 31 with data represented by gas chromatograms in Appendix F: Figure 76 (a-c).

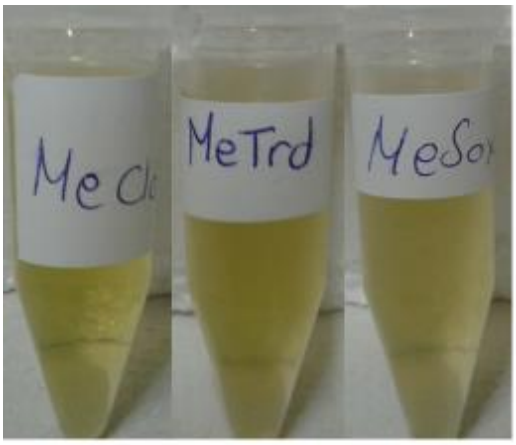


Figure 23 (a-c): Cold pressed (a), traditionally extracted (b) and Soxhlet extracted (c) Melon seed oil.

Table 27: Physico-chemical characteristics of Melon seed oil from different extraction methods.

Characteristic	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[B]	Olive oil[B,E]	Soybean oil[B]
Oil appearance	Yellow	Yellow	Yellow				
State of oil at 25 ºC	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Saponification value (mg KOH/g oil)	187.86 ±2.42[a]	189.26 ±1.40[a]	181.12 ±1.85[b]	172.16[A]	188-194	184-196	124-139
Average molecular weight (g/mol)	895.99 ±11.56[a]	889.30 ±6.58[a]	929.22 ±12.00[b]				
Acid value (mg KOH/g of oil)	1.63 ±0.059[a]	1.09 ±0.029[b]	0.947 ±0.13[b]	26.22[A]	3.09[G]	6.6[F]	2.72[H]
Ester value	186.25 ±2.47[a]	188.16 ±1.41[a]	180.17 ±1.95[b]				
Peroxide value (mequiv/kg)	2.98 ±0.20[a]	2.12 ±0.052[b]	1.69 ±0.092[c]	1.02[D]	12.6[G]	≤20[I]	21.38[H]
p-anisidine value	1.69 ±0.17[a]	1.18 ±0.11[b]	0.79 ±0.21[b]				
Iodine value (g/100g)	118.61 ±1.03[a]	117.64 ±1.96[a]	110.28 ±2.60[b]	75.06[A]	118-145	75-94	124-139
Specific gravity (20 ºC)	0.920 ±0.001[a]	0.922 ±0.001[a]	0.910 ±0.011[b]	0.98[A]	0.918-0.923	0.910-0.916	0.919-0.925
Refractive index (25 ºC)	1.4720 ±0.001[a]	1.4726 ±0.001[b]	1.4665 ±0.001[c]	1.452[C] (40℃)	1.467-1.469 (40℃)	1.468-1.470 (20℃)	1.466-1.470 (40℃)

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 Tukey's HSD test. [A]Bhutada et al. (2016); [B]Gunstone et al. (2007); [C]Abdulkarim et al. (2007); [D]Babatunde et al. (2014); [E]not a seed oil;[F]CODEX STAN 210-1999; [G]Abitogun et al. (2008a); [H]Abitogun et al. (2008b); [I]CODEX STAN 33-1981.

Table 28: Tocopherol and major sterol content of Melon seed oil from different extraction methods.

Tocopherol (mg/100g of oil)	Traditional	Cold-pressing	Soxhlet	*M. oleifera*	Sunflower oil[b]	Olive oil[b]	Soybean oil[b]
β-Tocopherol	0.78	0.74	0.73	-	-	1.00	-
γ-Tocopherol	16.33	59.99	44.81	9.37[c]	5.00	1.00	59.00
α-Tocopherol	6.22	9.23	7.07	13.44[c]	49.00	20.00	10.00
δ-Tocopherol	4.28	4.43	4.54	4.80[c]	1.00	-	26.00
Total-Tocopherol	27.61	74.39	57.15	27.61[c]	55.00	22.00	96.00
Stigmasterol	37.13	44.11	28.30	23.10[a]	33.70	-	57.70
β-Sitosterol	39.66	42.08	58.05	45.58[a]	265.30	130.30	173.40

[a]Tsaknis et al. (1998); [b]Gunstone et al. (2007); [c]Anwar et al. (2003).

The assignments of the main resonances in the ^{1}H NMR spectra (Appendix E) were assigned according to Sacchi et al. (1997), Popescu et al. (2015), Timilsena et al. (2017) and are represented in Table 29. The presence of protons of the main components of the Melon seed oil resulted in a total of 10 spectral signals groupings (Table 29).

Table 29: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{1}H NMR spectra of Melon seed oil obtained from different extraction methods.

Assignment (Proton)	Traditional	Cold -pressing	Soxhlet
—CH_2—CH_2—CH_2—C**H**$_3$	0.83-0.88	0.83-0.84	0.85-0.86
(C**H**$_2$)$_n$	1.23-1.34	1.21-1.25	1.23-1.28
—C**H**$_2$—CH_2—COOH	1.58	1.55	1.58
—C**H**$_2$—CH=CH—	1.97-2.04	1.99-2.00	1.97-2.04
—C**H**$_2$—COOH	2.26-2.30	2.26	2.26-2.30
—CH=CH—C**H**$_2$—CH=CH—	2.72-2.76	2.72	2.73-2.76
—C**H**$_2$—OCO—R	4.09-4.14	4.07-4.10	4.09-4.13
—C**H**$_2$—OCO—R	4.25-4.29	4.23-4.26	4.25-4.29
C**H**—OCO—R	5.22-5.26	5.22	5.22-5.26
–C**H**=C**H**–	5.29-5.37	5.29	5.31-5.36
Solvent ($CDCl_3$)	7.24	7.24	7.24

The assignments of the main resonances in the ^{13}C NMR spectra were assigned according to Sacchi, Addeo & Paolillo (1997), Ixtaina et al. (2011) and Popescu et al. (2015) and are presented in Table 30. Four signal groupings were observed (Ixtaina et al., 2011), namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm.

Table 30: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13} NMR spectra of Melon seed oil obtained from different extraction methods.

Assignments (Carbon)	Traditional	Cold -pressing	Soxhlet
CH_2,ω2	22.53-22.64	22.45-22.57	22.54-22.65
C3	24.79-24.82	24.70-24.73	24.80-24.83
C11	25.58	25.50	25.59
C8, C11	27.15	27.05	27.16
$(CH_2)_n$	29.04-29.72	29.05-29.64	29.01-29.67
C2, *sn*-1,3	33.97	33.86	33.98
C2, *sn*-2	34.14	34.02	34.15
$CH_2$0-, *sn*-1,3	62.05	61.93	62.05
CHO-, *sn*-2	68.84	68.78	68.84
Solvent ($CDCl_3$)	76.68-77.31	76.68-77.32	76.68-77.31
C12	127.85	127.76-127.93	127.85
C9, *sn*-2; C10, *sn*-1,3	129.65-129.94	129.52-129.98	129.66-129.93
C9, C13	130.16	130.16	130.15
C1, *sn*-2	172.78	172.57	172.77
C1, *sn*-1,3	173.19	172.99	173.20

Table 31: Fatty acid profile (percentage compositon) of Melon seed oil from different extraction methods.

Fatty acid	Cold pressed	Traditional	Soxhlet	*M. oleifera*[a]	Sunflower oil[b]	Olive oil[c]	Soybean oil[b]
Palmitic acid (16:0)	16.3	16.6	14.8	5.8	5.0-7.6	10.0	8.0-13.5
Stearic acid (18:0)	14.1	14.6	13.8	5.7	2.7-6.5	2.0	2.0-5.4
Arachidic acid (20:0)	1.3	1.4	1.0	3.9	0.1-0.5	-	0.1-0.6
Oleic acid (18:1)	11.4	10.5	17.7	65.9	14.0-39.4	78	17.0-30.0
Linoleic acid (18:2)	56.8	57.0	52.6	0.6	48.3-74.0	7	48.0-59.0

[a]Zhao & Zhang (2013); [b]Gunstone et al. (2007); [c]Gunstone (1996).

4.4. Enzymatic Hydrolysis of selected Namibian indigenous seed oils

4.4.1. Enzymatic hydrolysis of Marula nut oil: Optimization of system factors

4.4.1.1. Effect of pH on enzymatic hydrolysis of Marula nut oil

The effect of pH on the enzymatic hydrolysis of Marula nut oil is shown in Figure 22. A rapid increase in %H was observed within the first 2 hrs after which a gradual increase in the %H was observed over a time period of six hours at the different tested pH levels. Highest %H was observed at pH 6.0 and lowest %H at pH 8.0.

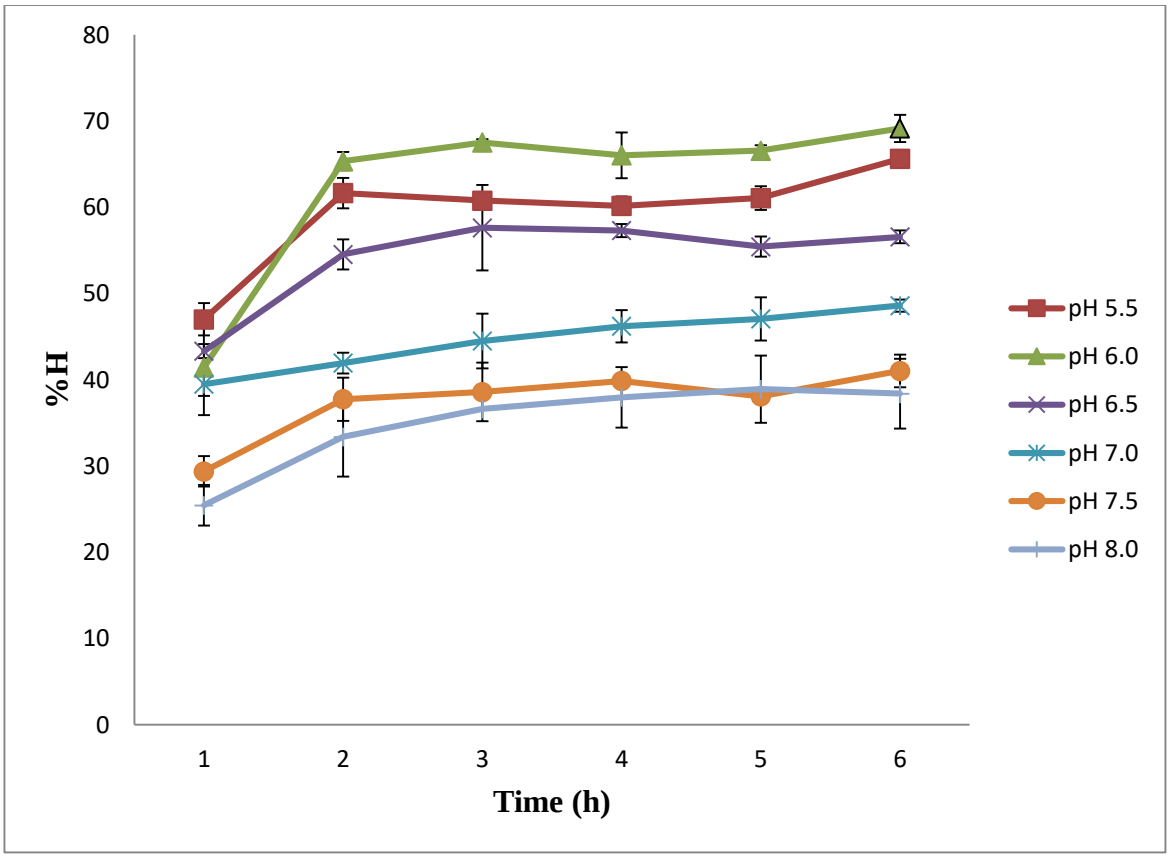


Figure 24: Effect of pH on the enzymatic hydrolysis of Marula nut oil

4.4.1.2. Optimization of system factors for the enzymatic hydrolysis of Marula nut oil

The assigned factors and coded levels (Table 32) were used in the Design Expert Version 10.0 Software (Stat-Ease, Inc., Minneapolis, USA) to generate the experimental setup for a 2^3 full factorial design as outlined in Table 33. The design was then used to analyse the optimal parameters for the enzymatic hydrolysis of Marula nut oil. The selected response for the design was the degree of hydrolysis (%H). The experimental setup (Table 33) consisted of 20 runs with four centre points.

Table 32: Experimental factors and coded levels in the 2^3 full factorial.

Factors	Coded levels		
	-1	**0**	**+1**
A: Temperature (°C)	30	37.5	45
B: Oil concentration (%)	10	20	30
C: Enzyme concentration (mg/g)	10	20	30

Table 33: Experimental setup as 2^3 full factorial and response for enzymatic hydrolysis of Marula nut oil.

Run	A: Temperature °C)	B: Oil concentration (%)	C: Enzyme concentration (mg/g)	Degree of Hydrolysis (%)
1	+1	-1	+1	64.4
2	-1	+1	-1	62.4
3	-1	-1	-1	61.4
4	0	0	0	67.2
5	-1	+1	-1	60.4
6	-1	-1	-1	62.2
7	+1	+1	-1	73.2
8	-1	-1	+1	64.6
9	-1	+1	+1	69.8
10	-1	-1	+1	61.5
11	+1	-1	-1	61.1
12	0	0	0	56.4
13	+1	-1	-1	58.5

14	+1	+1	+1	75.7
15	+1	+1	+1	72.5
16	0	0	0	61.3
17	+1	+1	+1	70.1
18	+1	+1	-1	70.0
19	0	0	0	66.7
20	+1	-1	+1	62.8

The full factorial model generated the following regression equation representing the correlation between %H and experimental factors (as coded units) studied:

$$\%H = 65.12+1.63*A+3.61*B+2.00*C+1.99*AB-0.45*AC+0.74*BC-1.09*ABC \quad \text{Eq. 5}$$

where %H is the degree of hydrolysis, A is temperature, B is oil concentration and C is enzyme concentration.

“The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients” (Design Expert® Version 10).

The Analysis of Variance (ANOVA) for the 2^3 full factorial model is presented in Table 34 reporting a coefficient of variation (*CV)*= 4.95%, coeffiecient of determination (R^2) = 0.7665 and standard deviation (SD) = 3.22.

Table 34: Analysis of variance (ANOVA) for the 2^3 full factorial model

Source	Degrees of freedom	Mean squares	*F*-values	*p*-values
Model	7	58.48	5.63	0.0046
A	1	42.32	4.07	0.0665
B	1	208.96	20.10	0.0007
C	1	63.91	6.15	0.0290
AB	1	63.29	6.09	0.0296
AC	1	3.26	0.31	0.5857
BC	1	8.67	0.83	0.3791
ABC	1	18.97	1.83	0.2016
Residual	12	10.39		
Lack of fit	1	25.02	2.76	0.1248
Pure error	11	9.06		
Total	19			

Note: *CV*= 4.95%, R^2= 0.7665 and Standard deviation = 3.22.

The perturbation plot (Figure 25) representing the effect of factors A, B and C on %H of Marula nut oil hydrolysis was generated by the Design Expert® Version 10 software (Stat-Ease, Inc., Minneapolis, USA).

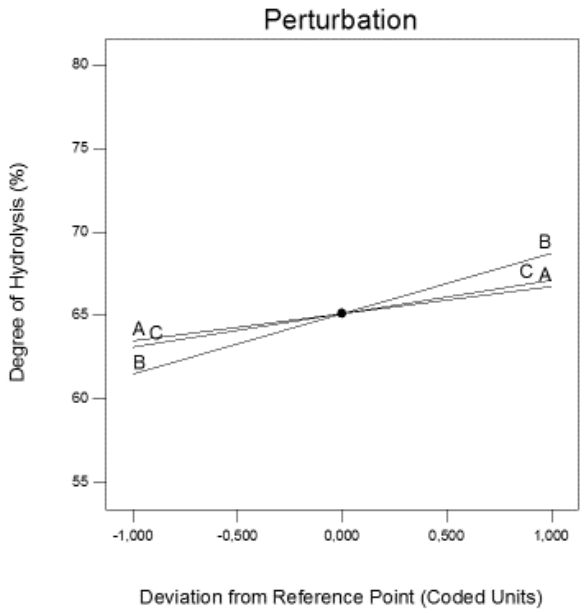


Figure 25: Effect of A: Temperature (°C), B: Oil concentration (%) and C: enzyme concentration on degree of hydrolysis (%) of Marula nut oil.

4.4.2. Enzymatic hydrolysis of Manketti nut oil: Optimization of system factors

4.4.2.1. Effect of pH on enzymatic hydrolysis of Manketti nut oil

The effect of pH on the enzymatic hydrolysis of Manketti nut oil is shown in Figure 24. A rapid increase in %H was observed within the first 3 hrs after which gradual increase in the %H was observed over a time period of six hours at the different tested pH levels. Highest %H was observed at pH 6.0 and lowest %H at pH 8.0.

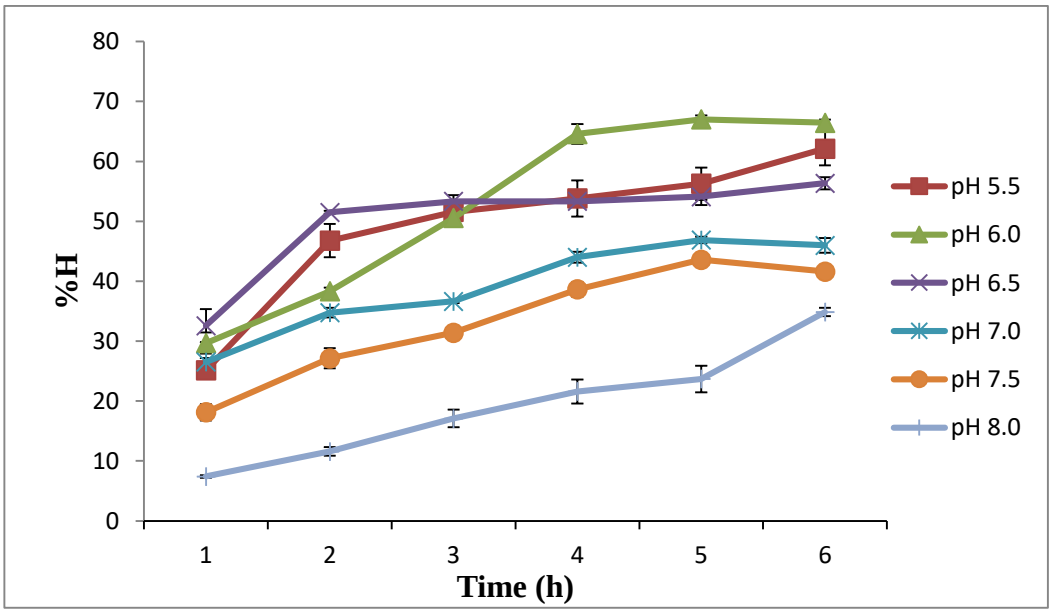


Figure 26: Effect of pH on the enzymatic hydrolysis of Manketti nut oil

4.4.2.2. Optimization of system factors for the enzymatic hydrolysis of Manketti nut oil

The assigned factors and coded levels (Table 35) were used in the Design Expert Version 10.0 Software (Stat-Ease, Inc., Minneapolis, USA) to generate the experimental setup for a 2^3 full factorial design as outlined in Table 36. The design was then used to analyse the optimal parameters for the enzymatic hydrolysis of Manketti nut oil. The selected response for the design was the degree of hydrolysis. The experimental setup (Table 36) consisted of 20 runs with four centre points.

Table 35: Experimental factors and coded levels in the 2^3 full factorial.

Factors	Coded levels		
	-1	**0**	**+1**
A: Temperature (°C)	30	37.5	45
B: Oil concentration (%)	10	20	30
C: Enzyme concentration (mg/g)	10	20	30

Table 36: Experimental setup as 2^3 full factorial and response for enzymatic hydrolysis of Manketti nut oil.

Run	A: Temperature (°C)	B: Oil concentration (%)	C: Enzyme concentration (mg/g)	Degree of Hydrolysis (%)
1	0	0	0	62.1
2	-1	+1	+1	70.1
3	-1	-1	-1	50.5
4	+1	+1	-1	66.7
5	-1	-1	-1	49.5
6	0	0	0	60.0
7	-1	-1	+1	66.7
8	-1	+1	+1	69.2
9	-1	+1	-1	77.0
10	+1	+1	+1	63.2
11	-1	+1	-1	76.1
12	+1	-1	+1	60.6
13	+1	-1	-1	58.0
14	+1	-1	+1	59.5
15	+1	+1	+1	63.2
16	-1	-1	+1	66.7
17	0	0	0	66.4
18	+1	-1	-1	58.0
19	+1	+1	-1	65.7
20	0	0	0	67.0

The full factorial model generated the following regression equation representing the correlation between %H and experimental factors (in coded factors) studied:

$$\%H = 63.81-1.93*A+5.11*B=1.11*C-2.27*AB-1.34*AC-3.58*BC+2.32*ABC \quad \text{Eq.6}$$

where %H is the degree of hydrolysis, A is temperature, B is oil concentration and C is enzyme concentration.

"The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients" (Design Expert® Version 10).

The ANOVA for the 2^3 full factorial model is presented in Table 37 reporting a *CV*= 2.74%, R^2 = 0.9607 and SD = 1.75. The R^2 =0.96 was satisfactory suggesting the suitability of the chosen model.

Table 37: Analysis of variance (ANOVA) for the 2^3 full factorial model.

Source	Degrees of freedom	Mean squares	*F*-values	*p*-values
Model	7	128.42	41.94	<0.0001
A	1	59.68	19.49	0.0008
B	1	417.18	136.25	<0.0001
C	1	19.58	6.39	0.0265
AB	1	82.36	26.90	0.0002
AC	1	28.89	9.44	0.0097
BC	1	205.21	67.02	<0.0001
ABC	1	86.03	29.09	0.0002
Residual	12	3.06		
Lack of fit	1	0.021		
Pure error	11	3.34		
Total	19			

Note: *CV*= 2.74%, R^2= 0.9607 and Standard deviation =1.75.

The perturbation plot (Figure 27) representing the effect of factors A, B and C on %H of Manketti nut oil hydrolysis was generated by the Design Expert® Version 10 software.

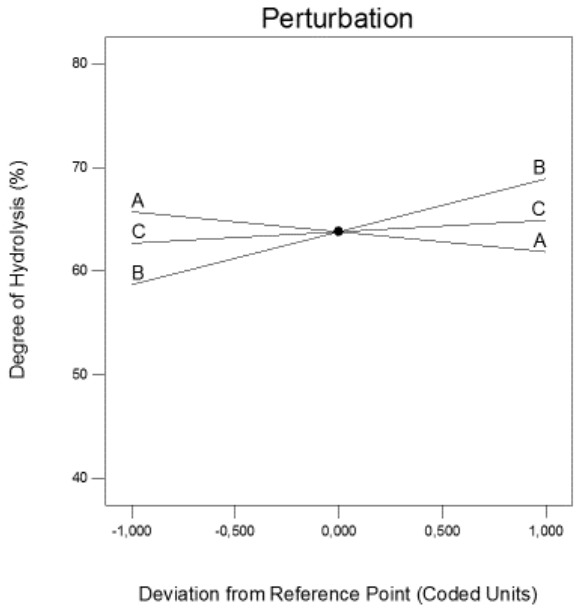


Figure 27: Effect of A: Temperature (°C), B: Oil concentration (%) and C: enzyme concentration on degree of hydrolysis (%) of Manketti nut oil.

CHAPTER 5: DISCUSSION

5.1. Traditional processing methods for Namibian indigenous seed oils

The traditional processing methods for the production of oils from *C. lanatus* (Kalahari melon) seeds, *S. rautanenii* (Manketti) nuts and *S. birrea* (Marula) nuts were documented and described. In Namibia, a rich cultural heritage and indigenous knowledge exists on the traditional oil production methods and the documentation of this indigenous knowledge is crucial, since the majority of people who have this knowledge are the elderly. This unique indigenous knowledge can be lost if efforts are not made to document this indigenous knowledge. Indigenous knowledge exists from the collection of fruits, the preparation and storage of the kernels containing the oil-bearing kernels or the seeds to the extraction of the oil, its uses in daily life for food, cosmetics and medicinal purposes and shelf-life of the oils. Traditionally produced Manketti nut oil is prepared by first roasting the nuts slightly on warm coals, crushing them and after the addition of water, the solution is boiled until the oil separates out. Traditionally produced Melon seed oil is prepared from crushed seeds, then soaked in water and then boiled for long periods until the oil separates out from the solution. The traditionally produced Marula nut oil is prepared by continuously pounding the nuts with a small addition of water until the oil separates out, which resembles a cold pressed version of the oil. For rural communities, such an indigenous resources base is critical for their food security, as oil production can be done throughout the year for the own use in the household and to be sold on the market for income generation.

5.2. Oil extraction yields for Soxhlet extraction method

Oils were extracted using a Soxhlet apparatus with hexane for 6 hours from *C. lanatus* (Kalahari melon) seeds, *S. rautanenii* (Manketti) nuts, *S. birrea* (Marula) nuts, *X. americana* (Sour plum) nuts and *A. horridus* (!Nara) seeds (Table 6). This extraction technique is a standard method and the main reference point to compare other extraction methods (Yang et al., 2013). The oil yield from *S. rautanenii* nuts (42.6 ±0.84%) is comparable to the oil yield obtained by Mitei et al. (2008) for *S. rautanenii* (Manketti) from Botswana (41.5%) using Soxhlet extraction for 6 hours with hexane/2-propanol, but is lower then the oil yield (53.3%) obtained by Chivandi et al. (2008) for *S. rautanenii* from Zimbabwe. Although it should be noted that Chivandi et al. (2008) extracted the oil according to Bligh and Dyer (1959). *S. birrea* nuts yielded an oil yield of 57.0 ±4.37%, which is comparable to the oil yield of 58.6% obtained by Gandure & Ketlogetswe (2011) from Marula nuts in Botswana. To our knowledge no reported values exist on the extraction yields of oil from *A. horridus* (!Nara) by Soxhlet extraction. Oil extracted from *C. lanatus* yielded 40.2 ±3.45% of oil, which is higher than the yield (24.8%) reported by Mabaleha et al. (2007) for melon seeds from Botswana. The oil yield from *X. americana* nuts was 25.6 ±3.37%, which is lower than the reported 62.0% by Mikolaiczak et al. (1963) and the 49.9% from Nigerian *X. americana* oil by Eromosele et al. (1994). A number of factors can affect oil yields such as quality and maturity of seed, type of extraction methods, geographical and environmental conditions among others (Adekunle, Oyekunle, Obisesan, Ojo & Ojo, 2016). Industrially, oil is extracted from seeds with low oil content (<20%) using solvents (Tasan, Gecgel & Demirci (2011). The Soxhlet extraction, an organic solvent extraction method, showed that the nuts and seeds studied here are considered to be

high yielding oilseeds. The traditional and cold pressing techniques could be industrially viable without employing the organic solvent extraction processes, which are considered unsafe with more processing steps involved (Oyinlola, Ojo & Adekoya, 2004). The mechanical pressing (Oyinlola et al., 2004) and the traditional pressing methods are safer, with less processing steps involved, although the traditional process is more labor intensive, but remains to be preserved as a valued cultural heritage and practice. Quality and stability of oils are influenced by the extraction methods employed (Bargale, 2000).

5.3. Characterization of Manketti nut oil

In Namibia, Manketti nut oil is made traditionally in households and a very limited number of small enterprises produce the cold pressed oil. The physico-chemical characteristics of the cold pressed, traditional and Soxhlet extracted Manketti nut oil were investigated (Table 7). Cold pressed and Soxhlet extracted oils are lighter in colour as compared to the traditionally extracted oil (Figure 19), which is due to the fact that the kernels have been roasted before the extraction of the oil. Roasting temperature and time of roasting affect colour development of oil due to the development of browning agents as preported by Kim et al. (2002) for rice germ oil. Similarly, roasting nuts may produce browning agents, which may result in the production of a darker oil. The oil is edible and is used in food preparation and is also used internationally by cosmetic companies in cosmetic formulations.

Significant differences ($p<0.05$) among the three extraction methods were found for the characteristics of acid value (AV), *para*-anisidine (*p*-AV), iodine value (IV) and refractive index (RI). Significant differences in saponification value (SV) and IV have been reported by Nadeem et al. (2015) between cold pressed and Soxhlet extracted

Sunflower (*Helianthus annuus* L.) seed oil. 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 SV, average molecular weight (AMW), ester value (EV) and the specific gravity (SG). Traditionally extracted Manketti nut oil was significantly higher (p<0.05) in terms of its peroxide value (PV) from the cold pressed and the Soxhlet extracted Manketti nut oil. Yang et al. (2013) have reported significant differences among different extraction methods in chemical parameters such as AV, IV, SV and AMW for seed oil from Chinese vegetable tallow and stillingia oil after extraction with supercritical carbon dioxide extraction and Soxhlet extraction method using petroleum ether, hexane, diethyl ether and ethanol. Janporn et al. (2015) 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 apparatus and maceration extraction. Ikya, Umenger & Iorbee (2013) have reported significant differences in IV, PV, AV and specific gravity of oils from Shea nut between the traditional extraction method and the solvent extraction method. Khattab & Zeitoun (2013) have reported non-significant and significant differences between solvent extraction, supercritical fluid extraction and accelerated solvent extraction methods for physico-chemical properties, fatty acid composition and tocopherols. Akbari, Vaziri &Nasab (2015) reported lower IV, AV and density value using hexane extraction compared to cold press method with higher PV, SV and RI values for pomegranate seed oil. There is a need to analyse oil samples obtained from different extraction methods as these influence the physico-chemical properties to varying extents.

The SV, AV, PV and IV of the Manketti nut oil (Table 7) are higher as compared to the *M.oleifera* seed oil (Babatunde et al., 2014; Bhutada et al., 2016), whilst the

specific gravity and the refractive index are lower than the *M.oleifera* seed oil (Abdulkarim et al., 2007; Bhutada et al., 2016). Evidence as to the lengths of relative chains of fatty acids present in the triglyceride can be obtained by the determination of the SV (Wrolstad et al., 2005). The SV for Manketti nut oil ranged between 184.30 mg KOH/g and 189.06 mg KOH/g of oil with the Soxhlet extracted Manketti nut oil being significantly lower ($p<0.05$) from the cold pressed and the traditional one. The SV obtained for Manketti oil suggests the presence of mainly the medium-chain fatty acids (C16 and C18) (Mabaleha et al., 2007), which was confirmed with the GC-MS analysis of the fatty acids. The SV for the Manketti oil is comparable to the SV value (185.26 mg KOH/g) reported by Mitei et al. (2008) for Maketti oil from Botswana. The SV obtained for the Manketti oil compares to the SV for major vegetable oils such as olive oil (184 - 196), sunflower oil (Table 7), rice bran oil (181 - 189) and canola oil (182 - 193) (Firestone, 1997 in Gunstone et al., 2007, 68).

The AV reflects the total acidity of the oil sample (Wrolstad et al., 2005) and is a measure of free fatty acids in the sample, reflecting also quaility of the respective oil (Yang et al., 2013). The AV of the oil for each extraction method was significantly different ($p<0.05$) and ranged between 0.96 to 2.44 mg KOH/g of oil, of which all are higher than the value (0.36 mg KOH/g) reported by 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), with the Soxhlet (0.96 mg KOH/g) and the traditionally extracted oil (2.44 mg KOH/g) being lower and higher, respectively. The AV of the traditional Manketti nut oil was significantly higher than compared to the cold pressed and the Soxhlet extracted oil, which could be due to the fact that the oil during the traditional extraction process is exposed to higher boiling temperatures.

The PV is an indication of the level of oxidation or degradation of the oil sample. Low levels of PV were found for which, the traditional extracted method (3.98 mequiv/kg) was significantly different (p<0.05) from the cold pressed (2.19 mequiv/kg) and the Soxhlet extracted (1.80 mequiv/kg) Manketti nut oil. PV reported by Mitei et al. (2008) was 2.51 mequiv/kg, which is higher and lower than the Soxhlet extracted and the traditionally extracted Manketti nut oil, respectively. The results obtained for AV and PV of Manketti nut oil are within the acceptable levels of the standards for edible oils described by the Codex Alimentarius Commission, which prescribes the AV to be <4.0 mg/g and the PV to be <13 (CODEX STAN 210 1999; Janporn et al., 2015). The amount of α and β unsaturated aldehydes in an oil sample is represented by the anisidine value and is a measure of secondary oxidation (O'Brian, 2009). Low levels of *para*-anisidine value (*p*-AV) were found within the Manketti nut oil. The *p*-AV was significantly different among the three different extraction methods, ranging between 0.88 for the cold pressed oil and 2.51 for the traditionally extracted oil. The traditionally extracted Manketti nut oil had a greater number of secondary oxidation products present, but was lower than the value reported by Mitei et al. (2008) of 3.85 for Manketti oil from Botswana. The low levels of PV and *p*-AV are an indication of good oil quality of the Manketti nut oil obtained from the three different extraction techniques.

The level of unsaturation can be represented by the iodine value (Wrolstad et al., 2005). The IV of the oil for the three different extraction methods was significantly different (p<0.05) and ranged between 120.24 and 131.30. The high IV recorded is due to the high content of unsaturated fatty acids (Lianhe, Xing, Li & Zhengxingm, 2012) present in the Manketti nut oil, as confirmed by the GC-MS analysis of the fatty acid composition (Table 11). The IV reported by Mitei et al. (2008) was 121.76. The

IV observed for Manketti nut oil are within the ranges of major vegetable oils such as sunflower oil (118 - 145) and soybean oil (124-139) (Firestone, 1997 in Gunstone et al., 2007, pg 68).

The SG at 20 °C of the traditional (0.922) and cold pressed (0.923) oil was significantly different 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 traditionally extracted and cold pressed Manketti nut oil. Atabani et al. (2014) reported a density of 943.0 kg/m^3 at 15°C and 925.1 kg/m^3 at 40 °C, which are higher than the observed values for the Namibian Manketti nut oil. The specific gravity of traditionally extracted and cold pressed extracted Manketti nut oil are in the range of that of sunflower and soybean oil (Gunstone et al. 2007) (Table 12). Significant differences were observed in the refractive index (RI) at 25 °C among the three different extraction methods. The RI reported by Mitei et al. (2008) at 25 °C was 1.481, which is lower than the traditionally extracted (1.4827) and cold pressed oil (1.4847) and higher than the Soxhlet extracted (1.4767) Manketti nut oil. Atabani et al. (2014) reported a RI of 1.487. The refractive index of the Manketti nut oil is higher when compared to that of sunflower, olive and soybean oil (Table 12) (Gunstone et al., 2007). Increases in the degree of saturation, in particular polyunsaturation as revealed by higher iodine values increases the refractive index of the seed oil (Mitei et al., 2008). This was observed with the data reported here for Manketti nut oil obtained using the three different extraction methods as observed from the iodine values reported and the GC-MS data.

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 major soluble vitamin in

extracted oils is a mixture of tocopherols, comprised of alpha (α)-, beta (β)-, gamma (γ)-, delta (δ)- tocopherols and their type and concentration of each types varies among different sources of oilseeds (Chen, McClements & Decker, 2011). The greatest sources of tocopherols are seed oils, with the delta (δ)-tocopherols being the most active and the (α)-tocopherols, the least active antioxidant. But the (α)-tocopherol is most active with regard to its Vitamin E activity and function (O'Brian, 2009).

The Manketti nut oil had total tocopherol content in the range of 137.12 - 205.64 mg/100g of oil, with the dominant tocopherol being γ-tocopherol (Table 8). Mitei et al. (2009) did not detect β- and δ-tocopherol in the Manketti oil from Botswana and reported the tocopherol content to be composed of α-(0.56 mg/100g) and γ-(223.3 mg/100g) tocopherols. The Manketti nut oil is comparable to the hemp seed oil (150 mg/100g oil) and the redcurrent seed oil (145 mg/100g oil) in terms of the total tocopherol content and higher than that compared to common vegetable oils such as soybean (96 mg/100g oil), sunflower (55 mg/100g oil), coconut (1 mg/100 g oil), olive (22 mg/100g oil) oil (Gunstone et al., 2007) and argan (62.9 to 66.0 mg/100g) oil (Khallouki, 2003; Khallouki et al., 2003; El Abbassi, Khalid, Zbakh & Ahmad, 2013).

The α, γ, β and δ-tocopherol has not been detected in Macadamia nut oil and contains the β-sitosterol (143.7 mg/100g) among other phytosterols (Azadmard-Damirchi, Sh. Emami, Hesari, Peighambardoust & Nemati, 2011). The stigmasterol and the β-sitosterol content of Manketti nut oil ranged from 42.33 - 45.34 mg/100g of oil and 586.61 - 682.43 mg/100g of oil, respectively (Table 8). Mitei et al. (2009) reported a stigmasterol content of 36.24 µg/g of oil and a β-sitosterol content of 1326.74 µg/g of oil in Manketti oil from Botswana, as determined by GC-MS after Solid-Phase Extraction (SPE) fractionation. Gwatidzo, Botha, McCrindle & Combrinck (2014) reported a stigmasterol content of 98.3 mg/100g, 118 mg/100g and a β-sitosterol

content of 1733 mg/100g and 1544.9 mg/100g for Soxhlet extracted and screw press, respectively, for Manketti oil from Okavango region, Namibia. The stigmasterol content of the Manketti nut oil is higher than the values reported for sunflower (33.70 mg/100g oil), cotton (5.00 mg/100g oil) and coconut (12.50 mg/100g oil) oil and lower than soybean (57.70 mg/100g oil) and corn (67.70 mg/100g oil) oil (Gunstone et al., 2007, pg 67). The β-sitosterol content of Manketti nut oil is comparable to that of the corn oil (645.70 mg/100g oil), higher than coconut (48.60 mg/100g oil), olive (130.30 mg/100g oil), soybean (173.40 mg/100g oil) and sunflower (265.30 mg/100g oil) oil (Gunstone et al., 2007, pg 67). Figure 43 represents the structures of β-sitosterol and stigmasterol (Winkler-Moser, 2011). Mitei et al. (2009) have demonstrated that Manketti oil is a rich source of γ-tocopherol and β-sitosterol. The presence of β-sitosterol imparts anti-fungal, anti-inflammatory and anti -viral properties (Malini & Vanithakumari, 1990).

The NMR spectroscopy is used routinely in lipid chemistry (Gunstone, 1994) and has been classified as a fingerprinting technique (Popescu et al., 2015). ^{1}H and ^{13}C NMR have been shown to be useful techniques (Sacchi et al., 1997; Guillén et al., 2003; Almoselhy, et al., 2014). The presence of protons of the main components in the ^{1}H NMR spectra of the Manketti nut oil resulted in a total of 11 spectral signal groupings, with similar profiles being observed among the different extraction methods (Table 9). Four signal groupings were observed (Ixtaina et al., 2011) from the ^{13}C NMR, namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm, with similar profiles among the three extraction methods observed (Table 10). Spectral signals observed in the region of 5.63 - 6.37 ppm in the H NMR spectra of Manketti nut oil indicated the presence of α-eleostearic acid in the

Manketti oil sample (Seremeta, Viana, Garcia & Carneiro, 2015, Yeboah et al., 2017), which was confirmed with the GC-MS analysis of the fatty acid composition (Table 11). The protons, H-9, H14, H-10, H-12, H-11 and H-13 (Figure 28) of the α-eleostearic acid contribute to the signals observed in the region 5.63 - 6.37 ppm (Yeboah et al., 2017).

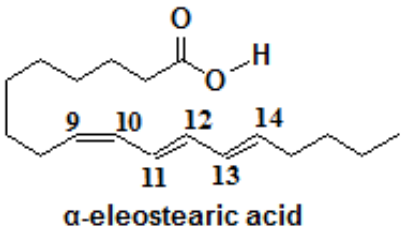


Figure 28: Structure of α-eleostearic acid (Adopted from Yeboah et al., 2017).

The fatty acids found in Manketti nut oil from Namibia as determined by GC-MS analysis were palmitic acid (10.4 - 14.3%), stearic acid (9.3 - 16.3%), arachidic acid (0.4 - 0.5%), α-eleostearic acid (24.2 - 35.7%), oleic acid (11.2 - 13.0%), linoleic acid (31.2 - 32.1%) and eicosenoic acid (0.6 - 0.8%). The palmitic acid, stearic acid and oleic acid content was highest in the cold pressed Manketti nut oil with the α-eleostearic acid content being lower when compared to the traditionally extracted and the Soxhlet extracted Manketti nut oil. The linoleic acid content for Manketti nut oil were lower than those reported by Mitei et al (2008) and Chivandi et al. (2008) for Manketti nut oil from Botswana and Zimbabwe, respectively, and higher than those reported for edible major oils such as coconut oil (1.0 - 2.5%), palm kernel oil (1.0 - 3.5%) and rapeseed oil (11.0 - 23.0%) and similiar to argan oil (31 - 37%). The level of palmitic acid (C16:0) is comparable to the levels found in corn (8.0 - 16.5%) and groundnut oil (8.0 - 14.0%).

The levels of stearic acid found in Manketti nut oil are considerable higher than those reported for the common vegetable oils (Food and Agriculture Organization (FAO) in Gunstone et al., 2007, pg 66, 67, 71), therefore the use of this unique oil has many applications, as stearic acid has a wide range of applications such as in the production of foods, cosmetics and pharmaceuticals (Zhai, 2014). Stearic acid has also been found to inhibit human cervical cancer (HOG-1) cell growth (Beesley, Soutter & White, 1993) and to lower levels of LDL cholesterol (Mensink, 2005). Compared to Manketti nut oil, *Moringa* seed oil contains higher amounts of oleic acid but with relatively lower concentrations of palmitic, stearic and linoleic acid and with no α-eleostearic acid present (Table 7) . Argan oil produced in Morocco from the kernels of the Argan tree (Argania spinosa L.) and which has become a worldwide popular commodity in the cosmetics industry with promising anti-inflammatory properties and an ability to treat scars, eczema, acne and psoriasis (Desanctis, 2016). Argan oil contains 42-47% of oleic acid and 31-37% of linoleic acid (Gunstone et al., 2007) with 12-14% palmitic acid and 4-7% stearic acid (Rueda et al., 2014). Comparatively, Manketti nut oil has similar linoleic acid concetrations with lower oleic acid and higher content of stearic and palmitic acid, including the unique presence of the fatty acid, α-eleostearic acid.

Chisholm & Hopkins (1966), Ucciani (1995), Phytotrade (2012) and Yeboah et al. (2017) have reported the presence of this rare triunsaturated fatty acid, α-eleostearic acid (9c11t13t-18:3) in Manketti oil. Yeboah et al. (2017) reported the presence of α-eleostearic acid at 25% in Manketti nut oil from Botswana using ^{13}C NMR. Others such as Chivandi et al. (2008), Zimba et al. (2005) and Mitei et al. (2008) did not report the presence of α-eleostearic acid in Manketti nut oil. According to Phytotrade (2012), UV light polymerizes the α-eleostearic acid producing a film on hair and skin protecting the skin from UV damage, making Manketti oil from Namibia an excellent

candidate for skin care formulations towards protecting skin from UV damage. Alpha-eleostearic acid, a linolenic acid isomer with a conjugated triene system, possesses potent antioxidant and anti-tumor activity (Tsuzuki, Tokuyama, Igarashi & Miyazawa, 2004; Zhang et al., 2012). Alpha-eleostearic acid (9c11t13t-18:3) has been shown to exhibit potential cytotoxicity and apoptosis induction effect on human breast cancer cells (Zhang et al., 2012) and to have potential for the prevention and treatment of invasive breast cancer in cells overexpressing HER2 (Zhuo et al., 2014). The paint, ink and coatings industry extensively use the eleostearic acid (Chang et al., 1996).

Total unsaturated fatty acids (TUSFA) for the Manketti nut oil of cold pressed, traditionally extracted and Soxhlet extracted method was found to be 69.0%, 79.9% and 79.9%, respectively. The total saturated fatty acid (TSFA) content of Manketti nut oil for traditional, cold pressing and Soxhlet extracted were 20.1%, 31.0% and 20.1%, respectively. The TSFA of cold pressed Manketti nut oil allows the oil to be highly resistant to oxidation (Choi et al., 2014), with the traditionally and Soxhlet extracted oils being less resistant to oxidation, due to the lower content of saturated fatty acids. The TUSFA content is higher than that of coconut oil (6.0 - 10.4%) and palm oil (48.5 - 53.7%) (Firestone, D. (2009) in Choi et al., 2014). Mitei et al. (2009) reported total unsaturated fatty acids and total saturated fatty acids of 76.28% and 23.72%, respectively in Manketti oil from Botswana, which is comparable to the values found for the Manketti nut oil. The composition of Manketti nut oil in terms of the high content of linoleic acid, the α-eleostearic acid, the phytosterol content of stigmasterol and β-sitosterol and its high tocopherol content, make this oil an ideal candidate for pharmaceutical and nutritional industrial applications.

5.4. Characterization of Marula nut oil

In Namibia, Marula nut oil is made traditionally in households and the cold pressed version is produced by a very limited number of small enterprises, with the biggest one being the EWC in Ondangwa. The physico-chemical characteristics of the cold pressed, traditionally extracted and Soxhlet extracted Marula nut oil were investigated (Table 12). The oil is light yellow in colour and is edible and can be used in food preparations and cosmetic applications. It is very popular by the international community for use in cosmetic formulations.

Significant differences ($p<0.05$) were found for the characteristics of acid value (AV), peroxide value (PV) and iodine value (IV). Significant differences in acid value between solvent extraction and pressing of *Salvia hispanica* L. seed oil was reported by Ixtaina et al. (2011). The Soxhlet extracted Marula nut oil was significantly different ($p<0.05$) for the characteristics of saponification value (SV), average molecular weight (AMW), ester value (EV), specific gravity (SG) and refractive index (RI) when compared to the traditionally extracted and the cold pressed Marula nut oil (Table 12). No significant differences ($p\geq0.05$) were found for the *p*-AV among the three extraction techniques. Significant and non-significant differences in physico-chemical characteristics in using different solvents for extraction have been reported by Yang et al. (2013). The SV, SG and RI of the Marula nut oil were higher compared to the *M.oleifera* seed oil (Table 12), whilst the AV, PV and IV were lower (Abdulkarim et al., 2007; Babatunde et al., 2014; Bhutada et al., 2016). The saponification value (SV) for the traditionally extracted (186.61 mg KOH/g oil), cold pressed (187.94 mg KOH/g oil) were significantly different ($p<0.05$) from the Soxhlet extracted (175.98 mg KOH/g oil) Marula nut oil. The SV obtained for Marula nut oil

suggests the presence of mainly the medium-chain fatty acids (C16 and C18) (Mabaleha et al., 2007), which was confirmed with the GC-MS analysis of the fatty acids. Ogbobe (1992) and Robinson et al. (2012) reported SV of 162.70 mg KOH/g oil and 178.6 mg KOH/g oil, respectively, for Marula nut oil from Nigeria, whilst Zharare & Dhlamini (2000) reported SV 184.5 mg KOH/g oil for Marula oil from Zimbabwe. The SV for Marula nut oil from Namibia is comparable to the values reported by Robinson et al. (2012) and Zharare & Dhlamini (2000). The SV values for the Marula nut oils compares to that of major vegetable oils such as castor oil (176-187) with the traditional and cold pressed Marula nut oil comparing to canola oil (182 - 193), olive oil (184 - 196) and rice bran oil (181 - 189) (Firestone, 1997 in Gunstone et al., 2007, pg 68).

The acid value (AV) for the traditionally extracted (1.64 mg KOH/g oil), cold pressed (5.16 mg KOH/g oil) and the Soxhlet extracted (2.69 mg KOH/g oil) were significantly different ($p<0.05$). Ogbobe (1992), Zharare & Dhlamini (2000), Gandure & Ketlogetswe (2011) and Robinson et al. (2012) reported an acid value of 33.70, 3.6%, 1.4 mg KOH/g and 41.4 mg KOH/g, respectively. The AV of 5.16 mg KOH/g oil is slightly higher than the acceptable standard of CODEX of 4.0 mg/g for cold pressed and virgin oils (CODEX STAN 210-1999), although the CODEX standard for virgin plam oil is at 10.0 mg KOH/g of oil. Phytotrade Africa (2016) has reported the AV for "Ubuntu" Marula oil to be <5.0 mg KOH/g.

Significant differences ($p<0.05$) in peroxide values (PV) values were found for the traditionally extracted (0.19 mequiv/kg), cold pressed (0.12 mequiv/kg) and the Soxhlet extracted (0.41 mequiv/kg) Marula nut oil, but the *p*-AV values for the Marula nut oil showed no significant differences ($p>0.05$) among the three different extraction methods, of traditionally extracted (0.083), cold pressed (0.076) and Soxhlet extracted

(0.08) techniques. Ogbobe (1992) and Robinson et al. (2012) reported a peroxide value 4.58 mequiv/kg for the Nigerian Marula nut oil, which is considerably higher than the values obtained for the Namibian Marula nut oil. The PV for the Marula nut oil is within the acceptable levels of the standards for edible oils described by the Codex Alimentarius Commission, which prescribes the PV to be <13 (Janporn et al., 2015).

The iodine values (IV) for traditionally extracted (66.96) and cold pressed (70.22) and Soxhlet extracted Marula nut oil (59.71) were significantly different ($p \leq 0.05$). Ogbobe (1992) and Robinson et al. (2012) reported higher IV of 100.25 and 100.34, respectively for Marula oil from Nigeria as compared to the Marula nut oil from Namibia. The IV reported by Zharare & Dhlamini (2000) for Marula nut oil from Zimbabwe was 67.7, which is comparative to the IV obtained for the Marula nut oil from Namibia.

The specific gravity (SG) and the refractive index (RI) for the traditionally extracted (0.916; 1.4645) and the cold pressed (0.914; 1.4645) were significantly different ($p<0.05$) from the Soxhlet extracted (0.883; 1.4530) Marula nut oil. Ogbobe (1992) and Robinson et al. (2012) reported a RI of 1.46 and a specific gravity of 0.88 at 15 °C for Marula nut oil from Nigeria, with the SG being considerably lower and the RI being comparable to the Marula nut oil from Namibia.

The tocopherol content of Marula nut oil as determined by GC-MS revealed the ranges of the total tocopherol content to be 14.13-29.72 mg/100g of oil, with individual tocopherol concentrations of γ-, α- and δ-tocopherol being 6.22 - 21.51, 4.14 - 4.67, 4.07 - 3.77 mg/100g of oil, respectively (Table 13). The β-tocopherol was not detected. The γ-tocopherol was the dominant tocopherol as compared to the data reported by Mariod et al. (2004) for Marula nut oil from Sudan. The Soxhlet extracted Marula nut

oil had considerably lower total tocopherol content as compared to the traditional and cold pressed Marula oil. Mariod et al. (2004) reported a tocopherol content of 13.7 mg/100g of oil, which was extracted with Soxhlet apparatus, which is comparable to the value observed for the Soxhlet extracted Marula nut oil.

The totol tocopherol content of olive oil (22 mg/100g oil), rice (21 mg/100g oil), oats (30 mg/100g oil) and cranberry (30 mg/100g oil) oil falls within the range of the Marula nut oil. The totol tocopherol content of the Marula nut oil is lower than reported values of common vegetable oils such as soybean (96 mg/100g oil) and sunflower (55 mg/100g oil) oil (Gunstone et al. 2007, pg 67, 68). The stigmasterol and the β-sitosterol content of Marula oil ranged between 35.79-50.79 mg/100g of oil and 222.29-447.16 mg/100g of oil, respectively (Table 13). The level of the sterol component of Soxhlet extracted Marula nut oil was considerably lower than the traditional and cold pressed Marula oil.

The stigmasterol content of the Marula nut oil is higher than the values reported for sunflower (33.70 mg/100g oil), cotton (5.00 mg/100g oil) and coconut (12.50 mg/100g oil) oil and lower than soybean (57.70 mg/100g oil) and corn (67.70 mg/100g oil) oil (Gunstone et al., 2007, pg 67). The β-sitosterol content of Marula nut oil is higher than that of coconut (48.60 mg/100g oil), olive (130.30 mg/100g oil) and soybean (173.40 mg/100g oil) oil and lower than that of corn oil (645.7 mg/100g oil) (Gunstone et al., 2007, pg 67). Reported values of β-sitosterol content of cotton (401.8 mg/100g oil), rapeseed (419.8 mg/100g oil) and sunflower (265.3 mg/100g oil) oil fall within the range of the β-sitosterol content of the Marula nut oil (Gunstone et al., 2007, pg 67). The presence of β-sitosterol imparts anti-fungal, anti-inflammatory and anti-viral properties (Malini & Vanithakumari, 1990).

The NMR spectroscopy is used routinely in lipid chemistry (Gunstone, 1994). The signals and groupings observed from both the ^{1}H (Table 14) and ^{13}C NMR (Table 15) spectra are typical of those for vegetable oils. In determining the acyl group composition of oils, ^{1}H and ^{13}C NMR have been shown to be useful techniques (Sacchi et al., 1997; Guillén et al., 2003; Almoselhy, et al., 2014). The chemical shifts and their main assignments for ^{1}H and ^{13}C NMR have been summarized in Table 14 and Table 15, respectively. The presence of protons of the main components from the ^{1}H NMR spectra of the Marula nut oil resulted in a total of 10 spectral signal groupings, with similar profiles being observed among the three extraction methods (Table 14). Four signal groupings were observed (Ixtaina et al., 2011) from the ^{13}C NMR, namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm, with similar profiles observed among the three extraction methods (Table 15).

The fatty acid analysis of Marula oil from Namibia as determined by GC-MS revealed the presence of oleic acid (66.6 - 67.6%), eicosenoic acid (1.0 - 1.2%), palmitic acid (16.3 - 16.7%), stearic acid (13.2 - 14.1%) and arachidic acid (1.6 - 1.9%) (Table 16). Marula nut oil is unique in its high oleic acid concentration, higher than that found in the popular Argan oil which contains around 42-47% of oleic acid (Gunstone et al., 2007), and is comparative to that of the *Moringa* seed oil 66% (Zhao & Zhang, 2013) and the Macadamia nut (55-65%) oil (Gunstone et al., 2007). Marula nut oil contains higher amounts of stearic acid and palmitic acid, comparative to Argan oil (Gunstone et al., 2007) and *Moringa* seed oil (Zhao & Zhang, 2013). Marula nut oil fatty acid composition was dominated by the presence of oleic acid, which has also been reported

by Salami (1973), Zharare & Dhlamini (2000), Mariod et al. (2004), Glew et al. (2004), Kleiman et al. (2008) and Komane et al. (2015). The level of oleic acid determined is higher than those reported for major seed oils such as cottonseed (14.7 - 21.7%) rapeseed (8.0 - 60.0%), soybean (17.0 - 30.0%) and sunflower oil (14.0-39.4%) and of some minor oils such as Argan oil (42 - 47%) and Baobab oil (21 - 39%) (FAO in Gunstone, et al., 2007, pg 66, 67, 71). The level of oleic acid determined is comparable to some minor seed oils such as Macadamia oil (55 - 65%), Teaseed oil (58 - 87%) and Pecan nut oil (49 - 69%) (FAO in Gunstone et al., 2007, pg 72). Oleic acid has shown to have beneficial effects in disease prevention and health (Sales-Campos, Souza, Peghini, da Silva & Cardoso, 2013), in particular heart disease (Menendez, Papadimitropoulou, Vellon & Lupu, 2006, Nadeem & Imran, 2016). The presence of oleic acid at high concentration imparts a certain stability and nutritious element to the Marula oil, making it suitable for applications as a frying oil, cooking oil and for food products with enhanced nutrition (Nyam et al., 2009).

The levels of stearic acid found in Marula nut oil are higher than those reported for the common commercial vegetable oils (FAO in Gunstone et al., 2007, pg 66, 67, 71), therefore the use of this oil has many applications, as stearic acid has a wide range of applications such as in the production of foods, cosmetics and pharmaceuticals (Zhai, 2014). Stearic acid has also been found to inhibit human cervical cancer (HOG-1) cell growth (Beesley, Soutter & White, 1993) and to lower levels of LDL cholesterol (Mensink, 2005). Stearic acid compared to other saturated fatty acids has unique positive effects on plasma lipids and lipoproteins (Sampath & Ntambi, 2005; Mensink, 2005). The TUSFA for the Marula oil ranged between 67.8% and 68.6%, whilst the TSFA content of Marula oil ranged between 31.4% and 32.2%. The TSFA of Marula oil allows the oil to be highly resistant to oxidation (Choi et al., 2014). The TUSFA

content is higher than that of coconut oil (6.0 - 10.4%) and palm oil (48.5 - 53.7%) (Firestone, D. (2006) in Choi et al., 2014).

5.5. Characterization of *Ximenia* nut oil

In Namibia, traditionally prepared *Ximenia* nut oil has a dark colour (brown to black) and a strong smell. The level of darkness varies among oils purchased on the traditional markets, which could be due to the variations in roasting of nut paste and boiling temperatures and boiling durations among households (Urso et al., 2013). Cold pressed *Ximenia* nut oil, which is light yellow in colour is not readily available in Namibia, and the one produced commercially by a handful of enterprises is mainly exported. *Ximenia* nut oil is non-edible (Orwa et al. 2009) and the traditionally prepared oil is commonly used as hair oil, while the cold pressed oil is used in a number of cosmetic formulation by the international cosmetics enterprise. The physico-chemical characteristics of the cold pressed, traditional and Soxhlet extracted *Ximenia* nut oil were investigated (Table 17).

Significant differences ($p<0.05$) among the three different extraction techniques were only found for the characteristic of the RI. The Soxhlet extracted *Ximenia* nut oil was significantly different ($p<0.05$) from the traditionally extracted and the cold pressed *Ximenia* nut oil for the characteristics of SV, AMW, AV, EV, IV and SG. The traditionally extracted *Ximenia* nut oil was significantly higher ($p<0.05$) from the cold pressed and the Soxhlet extracted *Ximenia* nut oil for the characteristics of PV and *p*-AV (Table 17). The significant differences in the Soxhlet extracted *Ximenia* nut oil could be further investigated by employing different types of solvents to study their affects on the parameters and to find reasons behind this result. Significant and non-significant differences in physico-chemical characteristics in using different solvents

for extraction have been reported by Yang et al. (2013). The SV, PV and SG of the *Ximenia* nut oil were lower when compared to *M.oleifera* seed oil, whilst the AV, IV, the PV of the traditionally extracted *Ximenia* nut oil and the RI were higher (Table 12) (Abdulkarim et al., 2007; Babatunde et al., 2014; Bhutada et al., 2016). The saponification value (SV) for traditionally extracted (164.53 mg KOH/g of oil) and the cold pressed (164.89 mg KOH/g of oil) were significantly different (p<0.05) from the Soxhlet extracted (156.22 mg KOH/g of oil) *Ximenia* nut oil. Eromosele et al. (1994) reported a higher SV value for *Ximenia* nut oil from Nigeria of 182.3 mg KOH/g oil, while Saeed & Bashier (2010) reported a lower SV value of 11.43 for *Ximenia* nut oil from Sudan. The acid value (AV) for the Soxhlet extracted (1.49 mg KOH/g of oil) was significantly different (p<0.05) from the traditionally extracted (6.07 mg KOH/g of oil) and the cold pressed (5.90 mg KOH/g of oil) *Ximenia* nut oil. Eromosele et al. (1994), Saeed & Bashier (2010) and Kibuge et al. (2015) reported an AV of 0.14 mg KOH/g, 0.28 mg KOH/g and 3.4 mg KOH/g for *Ximenia* nut oil from Nigeria, Sudan and Kenya, respectively.

The peroxide values (PV) and the *para*-anisidine values (*p*-AV) values for the cold pressed (0.47 mequiv/kg; 0.085) and the Soxhlet extracted (0.47 mequiv/kg; 0.067) *Ximenia* nut oil were significantly different (p<0.05) from the traditionally extracted (2.12 mequiv/kg; 1.24) *Ximenia* nut oil, with values being considerably higher. The traditional extraction process for *Ximenia* nut oil includes roasting the crushed nut paste before boiling the mixture. The roasting and subsequent boiling process could have caused the development of additional peroxides in the resultant oil, but is still within acceptable standards. Eromosele et al. (1994) and Saeed & Bashier (2010) reported a PV of 29.4 mequiv/kg and 30.0, respectively.

The iodine values (IV) values for the traditionally extracted (85.01) and the cold pressed (82.01) were significantly different (p<0.05) from the Soxhlet extracted (79.58) Ximenia nut oils, with the Soxhlet extraction resulting in lower iodine values. Mikolaiczak et al. (1963), Eromosele et al. (1994) and Saeed & Bashier (2010) reported and IV of 85, 149.8 and 47.59, respectively, of which the *Ximenia* nut oil is comparable to the values reported by Mikolaiczak et al. (1963).

The specific gravity (SG) for the traditionally extracted (0.912) and the cold pressed (0.913) Ximenia oil were significantly different (p<0.05) from the Soxhlet extracted (0.903) *Ximenia* nut oil, with the Soxhlet extraction having lower values. Eromosele & Paschal (2003), Saeed & Bashier (2010) and Kibuge et al. (2015) reported a density of 0.9625g/ml, 0.9376 g/ml and 0.974 g/ml, respectively. Significant differences (p<0.05) were observed between the three methods of extraction for the refractive index (RI) of traditionally extracted (1.4715), cold pressed (1.4710) and the Soxhlet extraction (1.4605). Mikolaiczak et al. (1963) and Saeed & Bashier (2010) reported a RI of 1.477 and 1.4718, respectively.

The tocopherol content of *Ximenia* oil was determined using GC-MS and revealed a total tocopherol content range of 6.88 – 10.75 mg/100g of oil (Table 18). The γ-tocopherol was not detected in *Ximenia* oil among the three extraction methods investigated and only the β-tocopherol was detected in the traditionally extracted oil at low level (0.61 mg/100g oil). The cold pressed *Ximenia* oil had the highest β-tocopherol content, whilst the traditional *Ximenia* nut oil had the highest α-tocopherol content. Mitei et al. (2009) did not detect any tocopherol in a related species, *X. caffra* (moretologa-kgomo) from Botswana after analysis with High Performance Liquid Chromatography-Fluorescence Detection (HPLC-FLD). Tocopherol was also not detected by Eromosele (1994) in *X. americana* seed oil from Nigeria.

The stigmasterol and the β-sitosterol content of *Ximenia* oil range was 39.95 – 42.65 and 332.39 - 388.88 mg/100g of oil, respectively (Table 18). The presence of β-sitosterol imparts anti-fungal, -inflammatory and -viral properties (Malini & Vanithakumari, 1990). Mariod et al. (2009) reported the stigmasterol and the β-sitosterol content of *X. caffra* (moretologa-kgomo) seed oil to be 2.41 and 61.27 µg/g of oil.

The NMR spectroscopy is used routinely in lipid chemistry (Gunstone, 1994). ^{1}H and ^{13}C NMR have been shown to be useful techniques (Sacchi et al., 1997; Guillén et al., 2003; Almoselhy, et al., 2014). The chemical shifts and their main assignments for ^{1}H and ^{13}C NMR have been summarized in Table 19 and Table 20, respectively. The presence of protons of the main components from the ^{1}H NMR spectra of the *Ximenia* nut oil resulted in a total of 11 spectral signal groupings, with similar profiles being observed among the three extraction methods (Table 19). Protons of ximenynic acid (Vickery et al., 1984) and Octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid are observed at 5.97-6.04 ppm. Four signal groupings were observed (Ixtaina et al., 2011) from the ^{13}C NMR, namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm, with similar profiles observed among the three extraction methods (Table 20).

The fatty acids found in *Ximenia* oil from Namibia as determined by GC-MS revealed the presence of oleic acid (44.1 - 47.3%), eicosenoic acid (4.2 - 10.8%), ximenynic acid (6. – 12.0%), octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid (3.4 - 11.6%), palmitic acid (2.8 - 3.2%), stearic acid (15.4 -18.4%) and arachidic acid (4.0 - 17.1%). The dominat fatty acid was oleic acid (Table 21). Ligthelm et al. (1954), Mikolaiczak et al. (1963), Eromosele & Eromosele (2002), Řezanka & Siegler (2007), Chivandi et

al. (2008) also reported the oleic acid to be the dominant fatty acid at levels ranging between 30 – 72%.

The oleic acid content of the Namibian *Ximenia* oil is comparable to major oils such as groundnut oil (35.0-69.0%), palm oil (36.0-44.0%) and palm olein (39.8-46.0%) and some minor oils such as Argan oil (42-47%), Mango seed oil (34-56%), Neem seed oil (46-57%) and Shea nut oil (33-68%) (FAO in Gunstone et al., 2007, pg 66, 67, 72). The traditionally extracted *Ximenia* nut oil had a significantly higher arachidic acid and 11-eicosenoic acid content when compared to the cold pressed and Soxhlet extracted *Ximenia* nut oil, while its ximenynic acid and octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid content was lowest.

Ligthelm et al. (1954), Mikolaiczak et al. (1963) and Eggink et al. (2004) have reported the presence of Ximenynic acid (octadeca-trans-11-en-9-ynoic acid; 9a11t-18:2) at 6.0-25.0% in *Ximenia* nut oil. Ximenynic acid is also found in the seed oils of the Santalaceae family in particular, the *Santalurn* and *Exocarpos* genera (Vickery et al., 1984) and the Olacaceae family (Gunstone, Harwood & Padley, 1994). Ximenynic acid, a long –chain acetylenic, conjugated ene-ynoic fatty acid (Christie, 2014), in seed oil such as sandalwood seed oil has been reported to improve anti-inflammatory activity in rats (Li, Singh, Liu & Sunderland, 2013) and to be able to enhance the blood flow in the skin. (Phytotrade Africa, 2012). Eggink et al. (2004) reported the filing of a patent (US 2004/0115331 A1) for the development of ximenynic acid compositions and glycerides which have positive effects in health such as in insulin resistance, body weight, skin ageing among others. The octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid (conjugated ene-ynoic acetyelenic fatty acid) has been reported by Christie (2016) to be found in a seed oil of the genus *Ximenia* (author, unpublished in Christie, 2016). Traditionally extracted *Ximenia* oil had the highest level of arachidic acid and cis-11-

eicosenoic acid, whilst it had the lowest level of ximenynic acid and octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid as compared to the other extraction methods studied. Acetylenic acids have been found to have pesticidial activity (Fatoupe, Adoum & Takeda, 2000). The levels of stearic acid (C18:0) found in *Ximenia* nut oil are considerable higher than those reported for the common vegetable oils (Food and Agriculture Organization (FAO) in Gunstone et al., 2007, pg 66, 67, 71), therefore the use of this unique oil has many applications, as stearic acid has a wide range of applications such as in the production of foods, cosmetics and pharmaceuticals (Zhai, 2014). Stearic acid has also been found to inhibit human cervical cancer (HOG-1) cell growth (Beesley, Soutter & White, 1993) and to lower levels of LDL cholesterol (Mensink, 2005). The TUSFA for the *Ximenia* nut oil range was 65.0 – 75.0% and the TSFA content for the range was 25.0 - 35.2%. The TSFA of *Ximenia* oil allows the oil to be highly resistant to oxidation (Choi et al., 2014). The TUSFA content is higher than that of coconut oil (6.0-10.4%) and palm oil (48.5-53.7%) (Firestone, D. (2006) in Choi et al., 2014).

5.6. Characterization of !Nara seed oil

In Namibia, the !Nara seed oil is not prepared traditionally in households. It is only available as a cold pressed version that is commercially sold by the Desert Hills Company in Swakopmund. The oil is edible and yellow in colour with a nutty flavour. It is used in food preparations and skin care products, which are produced by the Desert Hills Company. The physico-chemical characteristics of the cold pressed and the Soxhlet extracted !Nara oils were investigated (Table 22). It should be noted that to the best of our knowledge, no data that is presented here for !Nara oil has been previously published.

Significant differences (p<0.05) among the two extraction methods for the !Nara seed oil were found for the characteristics of SV, AMW, EV, IV, SG and RI and non-significant differences (p≥0.05) for the characteristics of AV, PV and *p*-AV (Table 22). The RI, SV, PV and IV of the !Nara seed oil were higher compared to the *M.oleifera* seed oil, whilst the AV and SG were lower (Table 22) (Abdulkarim et al., 2007; Babatunde et al., 2014; Bhutada et al., 2016). Significant and non-significant differences in physico-chemical characteristics in using different solvents for extraction have been reported by Yang et al. (2013). Ixtaina et al. (2011) reported significant differences between solvent extraction and pressing of *Salvia hispanica* L. seed oil for AV and RI.

Significant differences (p<0.05) were found in the saponification values (SV) of the cold pressed (186.19 mg KOH/g of oil) and the Soxhlet extracted (180.48 mg KOH/g of oil) !Nara seed oil and the iodine values (IV), 111.03 and 108.27, respectively, with Soxhlet extracted values being lower. The SV obtained for !Nara seed oil suggests the presence of mainly the medium-chain fatty acids (C16 and C18) (Mabaleha et al., 2007), which was confirmed with the GC-MS analysis of fatty acids. The SV for !Nara oil compares to major edible oils such as canola (182 - 193) and rice bran oil (181-189). The IV values for !Nara seed oil compares to major edible oils such as rice bran oil (99-108), canola oil (110 - 126) and corn oil (107 - 128) (Firestone, 1997 in Gunstone et al., 2007, 68).

No significant differences (p≥0.05) in the acid values (AV) of the cold pressed (0.64 mg KOH/g of oil) and the Soxhlet extracted (0.71 mg KOH/g of oil) !Nara seed oil were found. The peroxide values (PV) and *para*-anisidine values (*p*-AV) were found not to be significantly different (p≥0.05) among cold pressed (2.77 mequiv/kg; 0.43) and Soxhlet extracted (2.51 mequiv/kg; 0.62) !Nara oil. The results obtained for AV

and PV of !Nara seed oil are within the acceptable levels of the standards for edible oils of cold pressed origin, described by the Codex Alimentarius Commission, which prescribes the AV to be <4.0 mg/g and the PV to be <13 (Janporn et al., 2015).

Significant differences (p<0.05) in specific gravity (SG) and refractive index (RI) were found between the cold pressed (0.921; 1.4724) and Soxhlet extracted (0.881; 1.4676) !Nara oil, with the values for the Soxhlet extracted oil being lower.

The !Nara seed oil had a high total tocopherol content range of 44.41 – 46.10 mg/100g of oil, of which the individual tocopherol concentrations ranges of β-, γ-, α- and δ-tocopherol were 0.69 - 0.79 mg/100g, 35.54 - 45.13 mg/100g, 4.66 - 6.31 mg/100g, 3.62 - 4.14 mg/100g of oil, respectively (Table 23). The total tocopherol content of the !Nara seed oil compares to that of Barley oil (45 mg/100g oil) and is higher then the total tocopherol content found in olive oil (22 mg/100 g oil), almond oil (28 mg/100g oil), peanut oil (19 mg/100g oil) and lower when compared to soybean oil (96 mg/100g oil) and sunflower oil (55 mg/100 g oil) (Gunstone et al., 2007, pg 67, 68).

The stigmasterol and the β-sitosterol content of !Nara seed oil ranged between 20.53 - 24.63 mg/100g and 19.84 – 26.80 mg/100g of oil, respectively (Table 23). The presence of β-sitosterol imparts anti-fungal, -inflammatory and -viral properties (Malini & Vanithakumari, 1990). The stigmasterol content of !Nara seed oil is comparative to the content found in peanut oil (21.9 mg/100g oil), higher when compared with coconut oil (12.5 mg/100g oil) and cotton seed oil (5.0 mg/100g oil) and lower than the stigmasterol content of soybean (57.7 mg/100g oil) and sunflower oil (33.7 mg/100g oil) (Gunstone et al., 2007, pg 67).

The NMR spectroscopy is used routinely in lipid chemistry (Gunstone, 1994). The signals and groupings observed from both the ^{1}H (Table 24) and ^{13}C NMR (Table 25) spectra are typical of those for vegetable oils. In determining the acyl group composition of oils, ^{1}H and ^{13}C NMR have been shown to be useful techniques (Sacchi et al., 1997; Guillén et al., 2003; Almoselhy, et al., 2014). The chemical shifts and their main assignments for ^{1}H and ^{13}C NMR have been summarized in Table 24 and Table 25, respectively. The presence of protons of the main components from the ^{1}H NMR of the !Nara oil resulted in a total of 10 spectral signal groupings, with similar profiles being observed among the three extraction methods (Table 24). Four signal groupings were observed (Ixtaina et al., 2011) from the ^{13}C NMR, namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around 172-173 ppm, with similar profiles being observed among the three extraction methods (Table 25).

The fatty acids found in !Nara seed oil from Namibia as determined by GC-MS (Table 26) were oleic acid (12.8-13.9%), cis-11-eicosenoic acid (4.4 - 4.4%), α-eleostearic acid (1%), palmitic acid (15.6 - 15.6%), stearic acid (11.7 - 11.9%) and linoleic acid (53.1 - 54.5%) being the dominant fatty acid present. The linoleic acid content of !Nara seed oil is comparable to oils such hemp oil (53 - 60%), raspberry oil (55%) and rose hip oil (54%), corn oil (34.0 - 65.6%), cottonseed (46.7 - 58.2%) and soybean oil (48.0 - 59.0%) and higher than that of Argan oil (31 - 37%) and Nigella seed oil (45%). More than half of the total fatty acid content is comprised of the linoleic fatty acid, an ω-6 polyunsaturated fatty acid. Linoleic acid, other than palmitic, oleic and stearic acids, is not synthesized in the body, and a lack of intake will cause a deficiency (Vermaak et al., 2011) and is necessary for the skin's normal growth (Choi et al., 2014), such as cell membrane synthesis and tissue regeneration (Górnaś & Rudzińska, 2016).

Polyunsaturated fatty acids have been found to be able to improve immune responses (Ntambi, Choi, Park, Peters & Pariza, 2002), increase insulin sensitivity (Suresh & Das, 2003), decrease plasma total cholesterol (Kris-Etherton & Yu, 1997) associated with cardiovascular disease (Nehdi, Sbihi, Tan, Al-Resayes, 2013) and decrease lipid levels in blood (Harris et al., 1997).

The fatty acid composition makes this oil suitable for applications such as for seasoning, mayonnaise and ice-creams (Gunstone, 2008). Since !Nara seed oil is free of linolenic acid, it can easily be applied for frying purposes in the food industry (Gunstone, 2008; Nehdi et al., 2013). !Nara seed oil from Namibia is a good source of linoleic acid and its intake can have beneficial health and nutritious benefits, with diverse food applications.

The levels of stearic acid found in !Nara seed oil are considerable higher than those reported for the common vegetable oils (Food and Agriculture Organization (FAO) in Gunstone et al., 2007, pg 66, 67, 71), therefore the use of this unique oil has many applications, as stearic acid has a wide range of applications such as in the production of foods, cosmetics and pharmaceuticals (Zhai, 2014). Stearic acid has also been found to inhibit human cervical cancer (HOG-1) cell growth (Beesley, Soutter & White, 1993) and to lower levels of LDL cholesterol (Mensink, 2005). The total unsaturated fatty acids (TUSFA) content for the !Nara seed oil was at 72%, and the TSFA content was observed to be 27.3 – 27.5%. The total saturated fatty acids content (TSFA) of !Nara seed oil allows the oil to be reasonably resistant to oxidation (Choi et al., 2014). The TUSFA content is higher than that of coconut oil (6.0 - 10.4%) and palm oil (48.5 - 53.7%) (Firestone, D. (2006) in Choi et al., 2014).

5.7. Characterization of Melon seed oil

In Namibia, Melon seed oil is made traditionally in households and the cold pressed version is produced by a very limited number of small enterprises, with the biggest one being the EWC in Ondangwa. The physico-chemical characteristics of the cold pressed, traditional and Soxhlet extracted Melon seed oil were investigated (Table 27). The oil is yellow, edible and is used in food preparations and skin care formulations.

Significant differences ($p<0.05$) among the three different extraction techniques for the Melon seed oil were found for the characteristics of PV and RI, while the Soxhlet extracted Melon seed oil was significantly different ($p<0.05$) for the characteristics of SV, AMW, EV, IV and SG from the traditionally extracted and the cold pressed Melon seed oil. Significant differences in the refractive index between solvent extraction and pressing for *Salvia hispanica* L. seed oils has been reported by Ixtaina et al. (2011). Significant and non-significant differences in physico-chemical characteristics in using different solvents for extraction have been reported by Yang et al. (2013). The traditionally extracted Melon seed oil was significantly different ($p<0.05$) from the cold pressed and the Soxhlet extracted Melon seed oil for the characteristics of AV and *p*-AV with higher values (Table 27). The SV, PV, IV and RI of the !Nara seed oil were higher compared to the *M.oleifera* seed oil, whilst the AV and the SG were lower (Table 27) (Abdulkarim et al., 2007; Babatunde et al., 2014; Bhutada et al., 2016). The saponification value (SV) for the traditionally extracted (187.86 mg KOH/g of oil) and the cold pressed (189.26 mg KOH/g of oil) Melon seed oil were significantly different ($p\leq0.05$) from the Soxhlet extracted (181.12 mg KOH/g of oil) Melon seed oil. The SV obtained for Melon seed oil suggests the presence of mainly the medium-chain fatty acids (C16 and C18), which was confirmed with the GC-MS analysis of fatty

acids and which was also reported by Mabaleha et al. (2007). Mabaleha et al. (2007) and Nyam et al. (2009) reported a SV of 184.4 and 173.2 mg KOH/g oil, respectively for Melon oil. The SV for Melon seed oil compare to those of major edible oils such as corn oil (187 - 195), groundnut oil (187 - 196) and sesame oil (187 - 195) (Firestone, 1997 in Gunstone et al., 2007, 68).

The acid value (AV) for the cold pressed (1.09 mg KOH/g of oil) and the Soxhlet extracted (0.95 mg KOH/g of oil) Melon seed oil were found to be significantly different ($p<0.05$) from the traditionally prepared (1.63mg KOH/g of oil) Melon seed oil, which had a higher AV value. Mabaleha et al. (2007), Gbogouri et al. (2011) and Nyam et al. (2009) reported an AV of 1.8 mg KOH/g, 4.30 mg KOH/g and 1.1 mg KOH/g oil for Melon seed oil from Botswana, Ivory Coast and Namibia, respectively.

Significant differences ($p<0.05$) in peroxide values (PV) were found between the three extraction methods of traditional (2.98 mequiv/kg), cold pressed (2.12 mequiv/kg) and Soxhlet extracted (1.69 mequiv/kg) Melon seed oil. Mabaleha et al. (2007), Gbogouri et al. (2011), Nyam et al. (2009) and Mariod et al. (2009) reported a PV of 9.8 mequiv/kg, 3.35 mequiv/kg, 2.3 mequiv/kg and 4.1 mequiv/kg, respectively. The results obtained for AV and PV of Melon seed oil are within the acceptable levels of the standards for edible oils of cold pressed origin, described by the Codex Alimentarius Commission, which prescribes the AV to be <4.0 mg/g and the PV to be <13 (Janporn et al., 2015). The *para*-anisidine values (*p*-AV) for the cold pressed (1.18) and the Soxhlet extracted (0.79) Melon seed oil were found to be significantly different ($p<0.05$) from the traditionally prepared (1.69) Melon seed oil, which had a higher *p*-AV value. Mabaleha et al. (2007) have reported a *p*-AV of 2.2 mmol/kg for Melon seed oil from Botswana.

The iodine value (IV) for the traditionally prepared (118.61) and the cold pressed (117.64) Melon seed oil were significantly different (p<0.05) from the Soxhlet extracted (110.28) Melon seed oil. Mabaleha et al. (2007), Gbogouri et al. (2011), Nyam et al. (2009) and Mariod et al. (2009) have reported an IV of 95.8 g of I_2/100g, 113.0 g of I_2/100g, 125.0 g of I_2/100g and 112.7 g of I_2/100g of oil for Melon seed oil from Botswana, Ivory Coast, Namibia and Sudan, respectively. The IV for Melon seed oil compares to major edible oils such as canola oil (110 - 126), corn oil (107 - 128) and sesame oil (104 - 120) (Firestone, 1997 in Gunstone et al., 2007, 68).

The specific gravity (SG) for the traditional (0.920) and the cold pressed (0.922) Melon seed oil were significantly different (p<0.05) from the Soxhlet extracted (0.910) Melon seed oil, with the Soxhlet extraction having a lower value. Mabaleha et al. (2007) and Mariod et al. (2009) have reported a relative density of 0.889 and 0.886, respectively. Gbogouri et al. (2011) reported a specific gravity of 0.950. Significant differences (p<0.05) in refractive index (RI) values were found between the three extraction methods of traditionally (1.4720), cold pressed 1.4726) and Soxhlet extracted (1.4665) Melon seed oil. Mabaleha et al. (2007) and Mariod et al. (2009) have reported a RI of 1.469 and 1.429, respectively.

The tocopherol content of Melon seed oil of three different extraction techniques was analysed using GC-MS (Table 28). The traditional extracted Melon seed oil was found to have a lower total tocopherol content of 27.61 mg/100g of oil, as compared to the cold pressed (74.39 mg/100g oil) and the Soxhlet extracted (57.15 mg/100g oil) Melon seed oil. The lower value for the total tocopherol content was obtained due to the much lower observed value for the γ-tocopherol of the traditionally extracted (16.33 mg/100g oil) Melon seed oil as compared to the cold pressed (59.99 mg/100g oil) and the Soxhlet extracted Melon seed oil (44.81 mg/100g oil). The β-, α- and δ- tocopherol

content range for the Melon seed oil was 0.73 – 0.78, 6.22 – 9.23 and 4.28 – 4.54 mg/100g of oil, respectively. Nyam et al. (2009) have reported the α-, β-, γ -, δ-tocopherols composition to be 25.94 mg/100g, 3.27 mg/100g, 70.56 mg/100g and 9.33 mg/100g, respectively with a total of 109.10 mg/100g of oil. The total tocopherol content of the cold pressed and Soxhlet extracted Melon seed oil is higher than the content found in rapeseed oil (53 mg/100g oil), groundnut oil (37 mg/100g oil), olive oil (22 mg/100g oil) and sunflower oil (55 mg/100g oil) (Gunstone et al., 2007, pg 67). The stigmasterol and the β-Sitosterol content of Melon seed oil ranged was 28.30-44.11 mg/100g and 39.66 – 58.05 mg/100g of oil, respectively (Table 28). Nyam et al. (2009) have reported the stigmasterol and sitosterol for Melon seed oil to be 25.87 mg/100g oil and 485.41 mg/100g oil, respectively. The presence of β-sitosterol imparts anti-fungal, -inflammatory and -viral properties (Malini & Vanithakumari, 1990).

The NMR spectroscopy is used routinely in lipid chemistry (Gunstone, 1994). The signals and groupings observed from both the ^{1}H (Table 29) and ^{13}C NMR (Table 30) spectra are typical of those for vegetable oils. In determining the acyl group composition of oils, ^{1}H and ^{13}C NMR have been shown to be useful techniques (Sacchi et al., 1997; Guillén et al., 2003; Almoselhy, et al., 2014). The chemical shifts and their main assignments for ^{1}H and ^{13}C NMR have been summarized in Table 29 and Table 30, respectively. The presence of protons of the main components from the ^{1}H NMR of the Melon seed oil resulted in a total of 10 spectral signal groupings, with similar profiles being observed among the three extraction methods (Table 29). Four signal groupings were observed (Ixtaina et al., 2011) from the ^{13}C NMR, namely, the aliphatic carbons at around 13-34 ppm, the carbons of the glycerol backbone at around 61-77 ppm, the unsaturated carbons from around 129 ppm and the carbonyls at around

172-173 ppm, with similar profiles being observed among the three extraction methods (Table 30).

The fatty acids analysis of Melon oil from Namibia as determined by GC-MS revealed the presence oleic acid (10.5 - 17.7%), linoleic acid (52.6 – 57.0%), palmitic acid (14.8 - 16.6%), stearic acid (13.8 - 14.6%) and arachidic (1.0 - 1.4%) (Table 31). Linoleic acid was determined to be the dominant fatty acid in the Namibian Melon oil. Mabaleha et al. (2009), Mariod et al. (2009) and Nyam et al. (2009) also reported the dominant fatty acid to be linoleic acid in ranges of 63 - 67%. The oleic acid content of melon oil is higher than that of coconut oil (5.0 - 10.0%) and comparable to watermelon (13 - 19%) and hemp seed oil (8 - 15%). The linoleic acid content of Melon oil is comparable to oils such hemp oil (53-60%), raspberry oil (55%) and rose hip oil (54%), corn oil (34.0 - 65.6%), cottonseed (46.7 - 58.2 and soybean oil (48.0 - 59.0%) and higher than that of Argan oil (31 - 37%) and Nigella seed oil (45%). The levels of stearic acid found in Melon seed oil are considerable higher than those reported for the common vegetable oils (Food and Agriculture Organization (FAO) in Gunstone et al., 2007, pg 66, 67, 71), therefore the use of this unique oil has many applications, as stearic acid has a wide range of applications such as in the production of foods, cosmetics and pharmaceuticals (Zhai, 2014). Stearic acid has also been found to inhibit human cervical cancer (HOG-1) cell growth (Beesley, Soutter & White, 1993) and to lower levels of LDL cholesterol (Mensink, 2005).

Linoleic acid, other than palmitic, oleic and stearic acids, is not synthesized in the body, and a lack of intake will cause a deficiency (Vermaak et al., 2011), and is necessary for the skin's normal growth (Choi et al., 2014), such as cell membrane synthesis and tissue regeneration (Górnaś &Rudzińska, 2016). Melon oil from

Namibia is a good source of linoleic acid and its intake can have beneficial health and nutritious benefits. The tocopherol, fatty acid and phytosterol coentent of the Melon seed oil allows it to be of a potential natural resource for the the cosmetics, food and pharmaceutical industry. The total unsaturated fatty acid (TUSFA) content range for Melon oil was 67.5 - 70.3% and the total saturated fatty acid (TSFA) content ranged between 31.8 - 32.5%. The TSFA of Melon seed oil allows the oil to be highly resistant to oxidation (Choi et al., 2014). The TUSFA content is higher than that of coconut oil (6.0 - 10.4%) and palm oil (48.5 - 53.7%) (Firestone, D. (2006) in Choi et al., 2014).

5.8. Enzymatic hydrolysis of Namibian Marula and Manketti nut oil

Various studies to investigate the effects of reaction parameters on enzymatic hydrolysis have involved studying one variant at a time while keeping the others constant, but with the use of full factorial designs, parameters can be varied simultaneously (Kumar, Reddy, Managuli & Pai, 2015) and interactions among independent parameters can be studied (Bozkir & Saka, 2005). In this study, the effect of pH was studied while keeping other parameters such as temperature, enzyme and oil concentration constant. For the full factorial design, three parameters investigated (Table 32 and 33) such as temperature (A), oil concentration (B) and enzyme concentration (C), at three factorial levels with coding of -1 (low), 0 (medium) and +1 (high) (Kumar et al. 2015). The Design Expert® Version 10 software computed a design consisting of 20 runs for studying the interaction of temperature (A), oil concentration (B) and enzyme concentration (C) on the degree of hydrolysis (Table 19 and 22). The response factor for the study was the degree of hydrolysis (%H). Data obtained was analysed with Design Expert® Version 10 software within the computed

design and further analysed with ANOVA for significance for suggested model design and parameter interaction.

The effect of initial pH on the %H of Marula and Manketti nut oil by *Candida rugosa* lipase (CRL) was studied and revealed that the highest %H was observed at pH 6.0 and the lowest %H at pH 8.0. Other hydrolysis studies reported optimal initial pH 7.0 for castor hydrolysed by *Aspergillus* oryzae lipase (Kulkarni & Pandit, 2005) and pH 6.7 for sunflower oil hydrolysed by *C. rugosa* (Pongket et al., 2015).

The %H data obtained from the CRL hydrolysis of Marula nut oil fluctuated from 56.37 to 75.70 % (Table 33). From Table 34, as generated by the ANOVA analysis of the Design Expert® Version 10 software, it can be seen that the Model F-value of 5.63 implies that the model used for the hydrolysis of Marula oil was significant. There was only a 0.46 % chance that an F-value this large could occur due to noise. Reaction parameters (A, B and C) were classed significant if values of "Prob > F" less than 0.050 were observed. For the Marula oil hydrolysis, the parameters B, C, AB were observed to be significant for the hydrolysis reaction (Table 34).

Linfield, Barauskus, Sivieri, Serota & Stevenson (1984) reported that the reaction rate of the hydrolysis of tallow, coconut oil and olive oil by CRL was unaffected by temperature in the range of 26-46 °C. The "Lack of Fit F-value" of 2.76 implies the Lack of Fit was not significant relative to the pure error, suggesting a good fit of the model. There was a 12.48% chance that a "Lack of Fit F-value" this large could occur due to noise. The “Pred R-Squared” of 0.6072 was in reasonable agreement with the “Adj R-Squared” of 0.6303. The Analysis of Variance (ANOVA) for the 2^3 full factorial model is presented in Table 34 reporting a coefficient of variation (*CV)*= 4.95%, coeffiecient of determination (R^2) = 0.7665 and standard deviation (SD) = 3.22. The perturbation plot (Figure 25) generated

from the effect of parameters A, B and C on the %H of Marula oil with CRL by the Design Expert® Version 10 software shows that when the parameters of temperature, oil and enzyme concentration are increased, the %H also increases, with temperature and enzyme concentration having a slightly lower effect than oil concentration. The optimal conditions for achieving a %H of 73.5% for Marula oil were an initial pH of 6.0, a temperature of 45 °C, an oil concentration of 30 % and an enzyme concentration of 30 mg/g of oil. To reduce the cost of input factors, similiarly the optimal condition for achieving %H of 62.45%, the optimal condition would then be a temperature of 36.84 °C, an oil concentration of 11.3% and an enzyme concentration of 23.58 mg/g of oil.

The %H data obtained from the CRL hydrolysis of Manketti nut oil fluctuated from 49.50 to 77.00% (Table 36). From Table 37, as generated by the ANOVA analysis of the Design Expert® Version 10 software, it can be seen that the F-value of 41.94 implies that the model used for the hydrolysis experiment for the Manketti oil was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. Reaction parameters (A, B and C) were classed significant if values of "Prob > F" less than 0.050 were observed. For the Manketti oil hydrolysis, the parameters A, B, C, AB, AC, BC, ABC were observed to be significant for the hydrolysis reaction (Table 37). The "Lack of Fit F-value" of 0.01 implied the Lack of Fit was not significant relative to the pure error, suggesting the good fit of the studied model. There was a 93.80% chance that a "Lack of Fit F-value" this large could occur due to noise. The “Pred R-Squared” of 0.9495 was in reasonable agreement with the “Adj R-Squared” of 0,9378. The ANOVA for the 2^3 full factorial model is presented in Table 37 reporting a *CV*= 2.74%, R^2 = 0.9607 and SD = 1.75. The R^2 =0.96 was satisfactory suggesting the suitability of the chosen model. The perturbation plot (Figure 27) generated from the effect of parameters A, B and C on the %H of Manketti nut oil with CRL by the Design Expert® Version 10 software shows that

there is a slight effect of enzyme concentration (C) on %H. Temperature (A) and oil concentration (B) have an effect on %H, as B increases, %H also increases and as A increases, the %H decreases. The optimal conditions for achieving a %H of 76.53% for Manketti oil were an initial pH of 6.0, a temperature of 30.05 °C, an oil concentration of 30% and an enzyme concentration of 10 mg/g of oil.

The hydrolysis of sunflower oil as reported by Pongket et al. (2015) achieved a rate of hydrolysis of 67.12 % with CRL, which was the most efficient when compared to other lipases (*Aspergillus niger*, *Pseudomonas fluorescence*, *Mucor javanicus*) employed. Hydrolysis of oils with non-specific lipases from *Candida* sp. tend to result in higher hydrolysis degrees as compared to the use of 1,3-specific lipases (Carvalho et al., 2002; Okada & Morrissey, 2007; Carvalho et al., 2009, Pongket et al., 2015). The hydrolysis of triglycerides by the use of lipases has become a favourable industrial approach due to its potential towards saving of energy, reduced downstream processing, reduction in environmental hazards and its appropriateness when dealing with compounds that are thermo-sensitive (Ramachandran, Al-Zuhair, Liu, Fong, & Gak,2006; Liu et al., 2008; Huang et al.,2010; Feiten et al., 2014).

CHAPTER 6: CONCLUSION

Currently, Namibia produces nut and seed oils such as Manketti oil, Marula oil, *Ximenia* oil, Melon oil and !Nara oil from seeds and nuts of indigenous trees, both traditionally and as cold pressed oils. The traditional method of producing Manketti nut oil, Marula nut oil and Melon seed oil was described and documented. In this study, the chemical and physical properties of these oils among three different extraction methods (traditional extraction, cold pressed and Soxhlet extraction) were determined towards establishing their biochemical profile and quality characteristics. Significant differences and non-significant differences in the physico-chemical characteristics of the seed oils studied were observed, but the values obtained were within the standards described for crude oils and reflected the good quality of these oils among the three extraction methods. Soxhlet extraction showed that the nuts and seeds used are high yielding oilseeds and this is of great economic value. The traditional process, although labor intensive, carries a long existing cultural heritage, in need to be preserved and promoted. Cold pressing is commercially being promoted as compared to the solvent extraction methods as being healthier and less process intensive.

Among the indigenous oils studied, Manketti nut oil contains the highest amount of total tocopherol, with Ximenia nut oil and Marula nut oil being among the lowest. The dominant tocopherol in Manketti, Marula nut oil, !Nara and Melon is the γ-tocopherol. The γ-tocopherol is a promising cancer-preventing agent due to its strong anti-inflammatory activity (Ju et al., 2010). Manketti nut oil, has promising health applications due to its high tocopherol content of which it is far higher than that found in argan, coconut, olive and macadamia nut oil. Commonly, most vegetable oils are comprised of only three acids at concentrations exceeding 10%, namely palmitic acid,

oleic acid, and linoleic acid (Gunstone et al., 2007). GC-MS analysis of the fatty acid composition of the oils also revealed the presence of unique fatty acids such as arachidic acid, α-eleostearic acid, eicosenoic acid, ximenynic acid and octadeca-9-yn, 11-*trans*, 13-*cis/trans*-dienoic acid. The presence of these fatty acids and the high content of polyunsaturated fatty acids makes the indigenous seed oil studied a health orientated commodity. *Ximenia* nut oil is a unique oil due to the presence of ximenynic acid and the octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid (conjugated ene-ynoic acetyelenic fatty acid), a very rare reported fatty acid but found in the Namibian *Ximenia* nut oil. Manketti nut, Nara seed and Melon seed oil are good sources of the essential fatty acid linoleic acid, making up almost 60% of the total fatty acid content, with Manketti nut oil making up around 30%. Manketti nut oil contains the unique fatty acid α-eleostearic acid and its relatively high concentrations of linoleic acid, makes it an oil with potential for further value added products development towards health and nutrition. The high oleic acid content of Marula nut oil makes it suitable as frying oil and in cooking applications. The high content of stearic acid makes the oils suitable for applications in the production of foods, cosmetics and pharmaceuticals. The oils with relatively high content of stearic acid content compared to conventional oils are recommended for industrial uses such as cosmetic and biodiesel applications.

Climate conditions such as drought or floods, severly affect the harvesting potential of raw materials, in which Namibia cannot guarantee or commit to a uniform provision of the raw material annually. But initiatives could be put in place, such a domestication, to allow for a consistent supply chain and sustainability of the raw material. Results obtained from the different extraction methods suggests that the use of the indigenous knowledge of the oil extraction method should be maintained and promoted, especially among the young generation in order to preserve this unique

indigenous knowledge. The good quality characteristics, tocopherol and phytosterol contents, fatty acid profile of the Namibian indigenous seed oils makes them a potential natural resource for the cosmetics, food and pharmaceutical industry. Knowledge of the biochemical profile of indigenous natural products assists in the development of value-added products. The consumption of the edible indigenous Namibian oils should be promoted for assisting in the reduction of malnutrition and health promotion, which in turn would provide income-generating initiatives for rural communities.

Fatty acids produced from triglycerides make up economically important compounds useful for the oleochemical industry (Avelar et al., 2013). In an attempt to to study the hydrolysis of Marula and Manketti oil for fatty acid production with *Candida rugosa* lipase, a 2^3 full factorial design was applied to study the interactions of experimental factors such as pH, temperature, oil concentration and CRL concentration. The degree of hydrolysis among runs fluctuated from 49.5% to 77.0% and 56.4% to 75.7% for Manketti oil and Marula oil, respectively. The effect of initial pH, temperature, oil and enzyme concentration and their interaction had significant effect on the degree of hydrolysis of Manketti oil. For the Marula oil hydrolysis, oil concentration, enzyme conentration and the interaction between temperature and oil concentration were observed to be significant. The optimal conditions for achieving a %H of 76.5% for Manketti oil were an initial pH of 6.0, a temperature of 30.05 °C, an oil concentration of 30% and an enzyme concentration of 10 mg/g of oil. The optimal condition for achieving %H of 62.5% for Marula oil hydrolysis, the optimal conditions were a temperature of 36.84 °C, an oil concentration of 11.3% and an enzyme concentration of 23.58 mg/g of oil. To the best of our knowledge, this is the first finding on the investigation of hydrolysis by CRL of Marula and Manketti oil using a 2^3 full factorial

design and forms a basis for further research in using unique indigenous nut or seed oils for potential industrial applications.

CHAPTER 7: RECOMMENDATIONS

- Due to the fact that the traditional oil extraction process is labour intensive and time consuming, the use of modern extraction processes (cold pressing) could be promoted among local communities. This could encourage the local communities to sell the oils as a value-added product and not only the raw material (seed/nuts), which would allow them to get higher incomes. On the other hand, the good quality of the traditionally produced oil could also be attempted to be promoted commercially by putting in place certain market incentives and structures.
- Further studies in the further processing of the crude oils depending on the application (food, cosmetics or biodiesel industry) are recommended which would further the value-addition initiative of the crude oils. This would also include studying the presence of other compunds present in the oils not studied here such as the tocotrienols, proteins, enzymes, other phytosterols, and water exclusion among others). This would also include studying the reasons for the colour development and the total pigment content of the traditionally extracted *Ximenia* nut oil and finding ways of improving the process to obtain an oil similar in colour to the commercially produced *Ximenia* nut oil.
- To understand the effect of the organic solvent extraction (Soxhlet extraction) on the physico-chemical charcteristics observed here, further studies need to be undertaken by using a variety of solvents for extraction at varying extraction times and to be able to understand any possible correlation. A new method that is becoming popular is the supercritical carbon dioxide extraction due to its

high extraction efficiency and it is recommended that further studies on this be done.

- Government is encouraged to offer farmers incentives to grow the plants to increase the oil production. Namibia could be known as major producer of indigenous oils thus generating much needed income. This activity would also contribute to reforestation, while at the same time shifting and improving the economic bases for the communities in the rural areas.
- The characterization data can eventually contribute to the classification of the oils by origin, which is critical in counterfeiting and adulteration detection in order to protect a country's natural resource from fraud, especially for instances when these oils are detected in advanced markets. In particular, NMR data and chemometrics can be further utilized in quality assessment of oils in terms of their botanical origin (Popescu et al., 2015).
- ^{1}H NMR fingerprinting can be used to evaluate oil stability/oxidative stability. The NMR analysis here could be further expanded to include measurements at 8-9.90 ppm, the range which indicates presence of hydroperoxides and aldehydes in oils (Almoselhy et al., 2014). This could be developed into a routine technique for quality analysis of oils.
- The presence of α and δ tocopherol in Namibian Manketti nut oil could be used as a marker for identification of its origin. Further studies are recommended towards assessing the identification of markers for seed oils from Namibia.
- Metabolomics studies in future studies are recommended to be incorporated for classification purposes, with the goal to shield the natural resources from fraud due to possible counterfeits in the market.

- It is recommended that further research to be conducted on the safety and the efficacy of the indigenous seed oils of study, as has been done for Marula nut oil by Komane et al. (2015).
- The high content of linoleic acid in !Nara and Kalahari melon seed oil allows it to be used in formulations for restoring the stratum corneum permeability barrier (SCPB) of skin (Nehdi et al., 2013). This could be further confirmed by subjecting the oils to UV-B wide range absorbance tests.
- Continued research in profiling the indigenous seed oils such as *Adansonia digitata* (Baobab tree), *Ximenia caffra* (Large sourplum), *Kigelia africana* (Sausage tree) and *Vigna subterranea* (Bambara nut) is to be recommended to enhance the opportunities for seed oil production markets in Namibia.
- It is also recommended to continue research towards the effect-directed analysis of the indigenous seed oils via the applications of techniques such as bioassays.
- Hydrolysis of seed oils could be further analysed using GC-MS and NMR spectroscopy to identify the concentration of individual reaction products such as fatty acids and glycerides in order to further streamline the hydrolysis reaction towards aplications such as esterification and transesterification for the production of intermediates for the oleochemical industry.

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APPENDICES

APPENDIX A: Questionnaire

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

FACULTY OF SCIENCE

SURVEY ON OILS FROM NAMIBIAN INDIGENOUS PLANT SEEDS

QUESTIONAIRE

1. Kindly identify yourself (Name, Community, Town/Village, Region, and Contact Address)

_____
_____
_____
_____

2. Are you aware of any plants in your area that are used for oil production?
If Yes: Please name them.

_____
_____
_____

4. When is the harvesting period of seeds and/or the oil producing period?

_____
_____
_____
_____
_____

Do you sell the oil and/or use the oil at home?

_____
_____
_____

For how much do you sell the oil? (provide volume)

_____
_____
_____

Do you sell the seeds for oil production and to whom?

_____
_____
_____

What do you use the oils for?

_____
_
_____
_

How do you make the oil? Cold pressing/boiling off?

_____
_
_____
_

How much oil do you make annually? (approx measurenments)

_____
_

For how long can you store the oil/s before it spoils?

_____
_

Interview conducted by:

Name: _____

Signature: _____

Date: _____

APPENDIX B: Informed consent

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pioneerspark, Windhoek, Namibia

PARTICIPANT INFORMED CONSENT FOR INTERVIEW AND SAMPLE COLLECTION

Researcher: Natascha Cheikhyoussef, PhD student, Department of Chemistry and Biochemistry, University of Namibia, Email: natapogori@gmail.com

Supervisors: Dr. Martha Kandawa-Schulz, Department of Chemistry and Biochemistry, University of Namibia, Tel. 061 2063635, Email: kschulz@unam.na

Dr. Ronnie Böck, Department of Biological Sciences, University of Namibia, Tel. 0612063423, Email: rbock@unam.na

Dear Participant

We would appreciate your assistance with this research project on the Profiling studies of Namibian Indigenous Seed Oils.

The objectives of this study are:

1) compile information on the types of oils, the types of extraction methods applied and uses of the oils by Namibian communities, through literature searches of published reports and/or conducting interviews with selected target participants; 2) assess the effects of extraction methods of oils from seeds on the oils physico-chemical characteristics; 3) compile data such as fatty acid composition, tocopherol and major sterol composition of the seed oils using techniques such as GC-MS, ^{1}H and ^{13}C NMR spectroscopy; 4) establish the effects of enzymatic hydrolysis by *Candida rugosa* lipase under different operating conditions on Manketti and Marula nut oil.

Therefore, we would like to kindly request for informed consent from you as a provider of information on the traditional processing method of the seed oil and the sample provided thereafter, which is to be used in this research project and where we declare that the information and results will be used for educational and academic purposes only and not for any commercialization aspect. The results will be provided to all parties upon request to supervisors of this project.

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pioneerspark, Windhoek, Namibia

Consent

I have read and I understand the provided information and have had the opportunity to ask questions and get all clarification needed. I also understand that I will get a copy of this consent form and agreed to take part in this study.

Participant's Name and Address:

Mrs Loide Kapolo
Ututangatse Village
Kandi - Constituency - Omusati Region

Participant's Signature: Lkapolo Date: 17/08/2015

Researcher's Signature: Ndaitaba. Date: 17/08/2015

Supervisor's Signature: RBöcke Date: 17/08/2015

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pioneerspark, Windhoek, Namibia

PARTICIPANT INFORMED CONSENT FOR INTERVIEW AND SAMPLE COLLECTION

Researcher: Natascha Cheikhyoussef, PhD student, Department of Chemistry and Biochemistry, University of Namibia, Email: natapogori@gmail.com

Supervisors: Dr. Martha Kandawa-Schulz, Department of Chemistry and Biochemistry, University of Namibia, Tel. 061 2063635, Email: kschulz@unam.na

Dr. Ronnie Böck, Department of Biological Sciences, University of Namibia, Tel. 0612063423, Email: rbock@unam.na

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1) compile information on the types of oils, the types of extraction methods applied and uses of the oils by Namibian communities, through literature searches of published reports and/or conducting interviews with selected target participants; 2) assess the effects of extraction methods of oils from seeds on the oils physico-chemical characteristics; 3) compile data such as fatty acid composition, tocopherol and major sterol composition of the seed oils using techniques such as GC-MS, ^{1}H and ^{13}C NMR spectroscopy; 4) establish the effects of enzymatic hydrolysis by *Candida rugosa* lipase under different operating conditions on Manketti and Marula nut oil.

Therefore, we would like to kindly request for informed consent from you as a provider of information on the traditional processing method of the seed oil and the sample provided thereafter, which is to be used in this research project and where we declare that the information and results will be used for educational and academic purposes only and not for any commercialization aspect. The results will be provided to all parties upon request to supervisors of this project.

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pioneerspark, Windhoek, Namibia

Consent

I have read and I understand the provided information and have had the opportunity to ask questions and get all clarification needed. I also understand that I will get a copy of this consent form and agreed to take part in this study.

Participant's Name and Address:

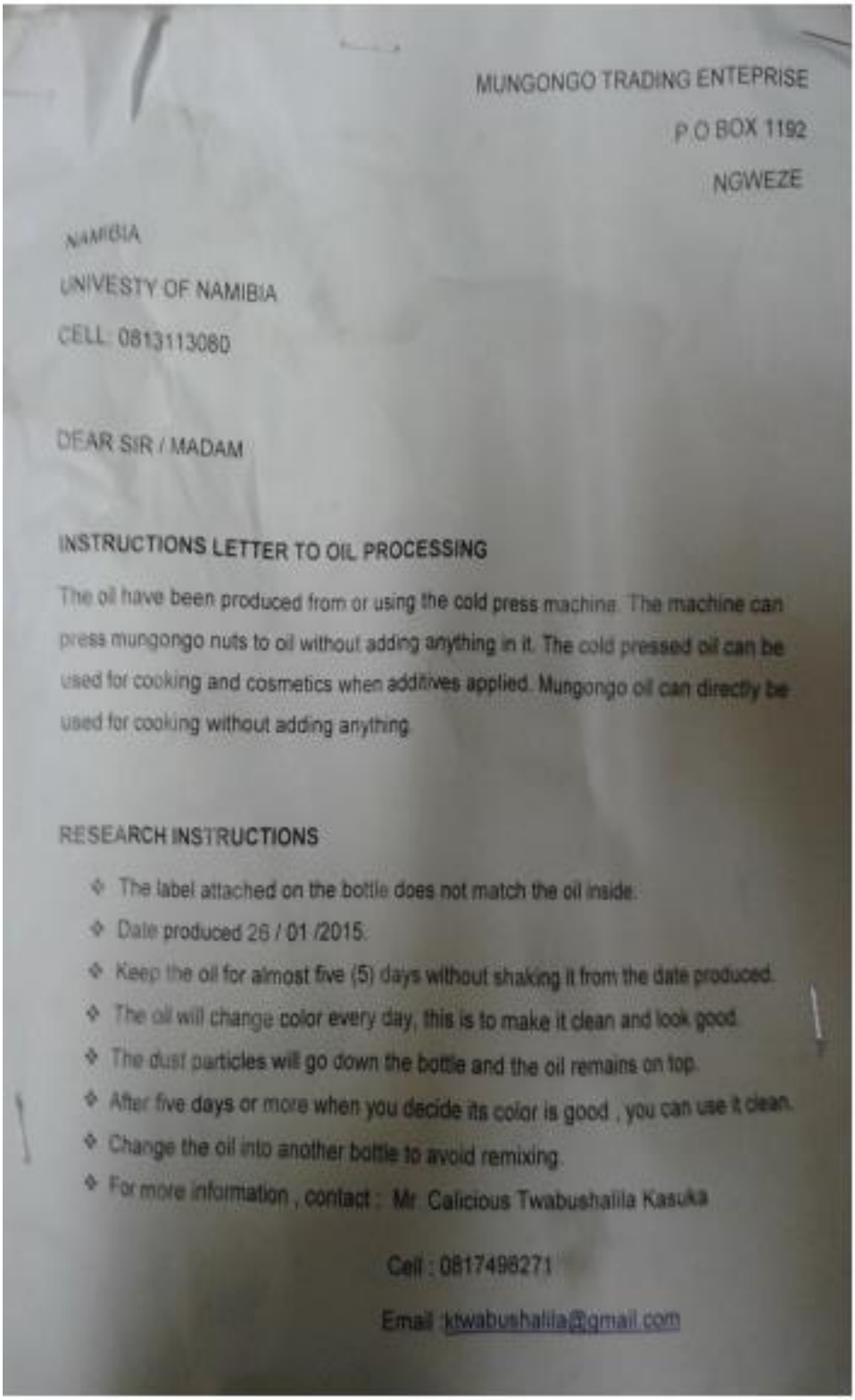
ONAITEMBU VILLAGE
UUTAPI CONSITUENCY - OMUSATI REGION
Mr Shakamba Junias

Participant's Signature: Shakamba Junias Date: 18/08/2015

Researcher's Signature: [signature] Date: 18 August 2015

Supervisor's Signature: [signature] Date: 18/8/2015

APPENDIX C: Letter from Mungongo Trading Enterprise



MUNGONGO TRADING ENTEPRISE

P.O BOX 1192

NGWEZE

NAMIBIA

UNIVESTY OF NAMIBIA

CELL: 0813113080

DEAR SIR / MADAM

INSTRUCTIONS LETTER TO OIL PROCESSING

The oil have been produced from or using the cold press machine. The machine can press mungongo nuts to oil without adding anything in it. The cold pressed oil can be used for cooking and cosmetics when additives applied. Mungongo oil can directly be used for cooking without adding anything.

RESEARCH INSTRUCTIONS

- The label attached on the bottle does not match the oil inside.
- Date produced 26 / 01 /2015.
- Keep the oil for almost five (5) days without shaking it from the date produced.
- The oil will change color every day, this is to make it clean and look good.
- The dust particles will go down the bottle and the oil remains on top.
- After five days or more when you decide its color is good , you can use it clean.
- Change the oil into another bottle to avoid remixing.
- For more information , contact : Mr. Calicious Twabushalila Kasuka

Cell : 0817498271

Email :ktwabushalila@gmail.com

APPENDIX D: Gas chromatograms for tocopherol and major sterol analysis

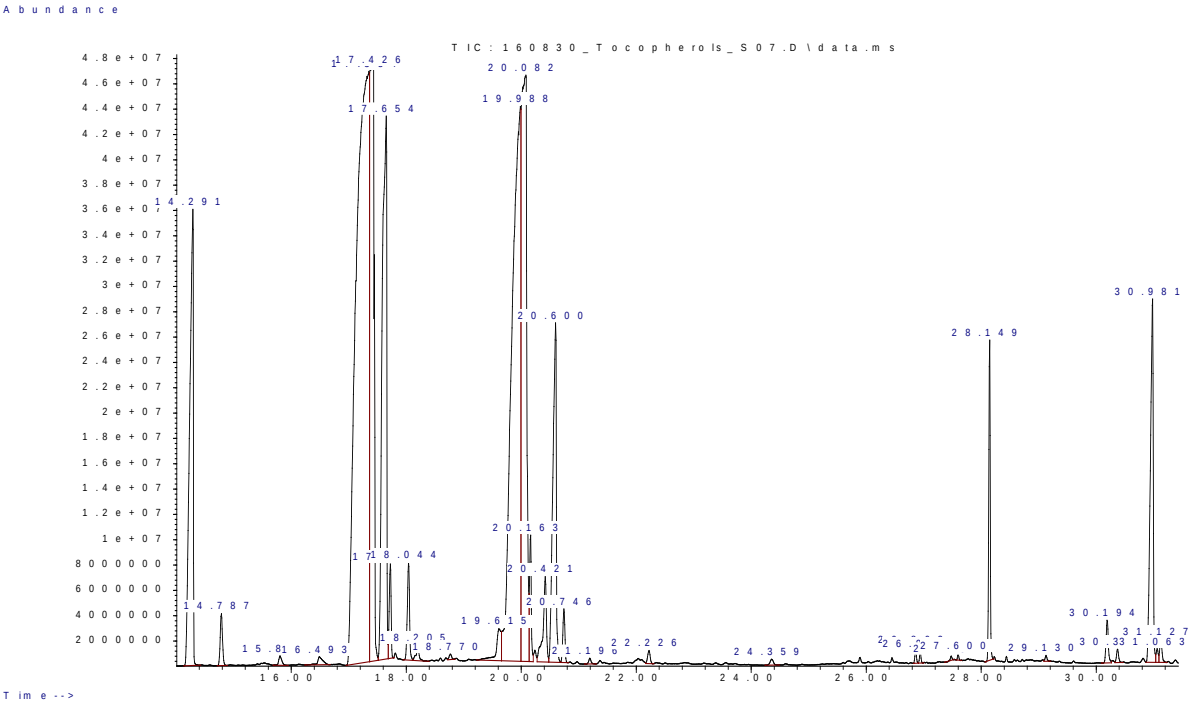


Figure 29: Gas chromatogram of Soxhlet extracted Manketti nut oil.

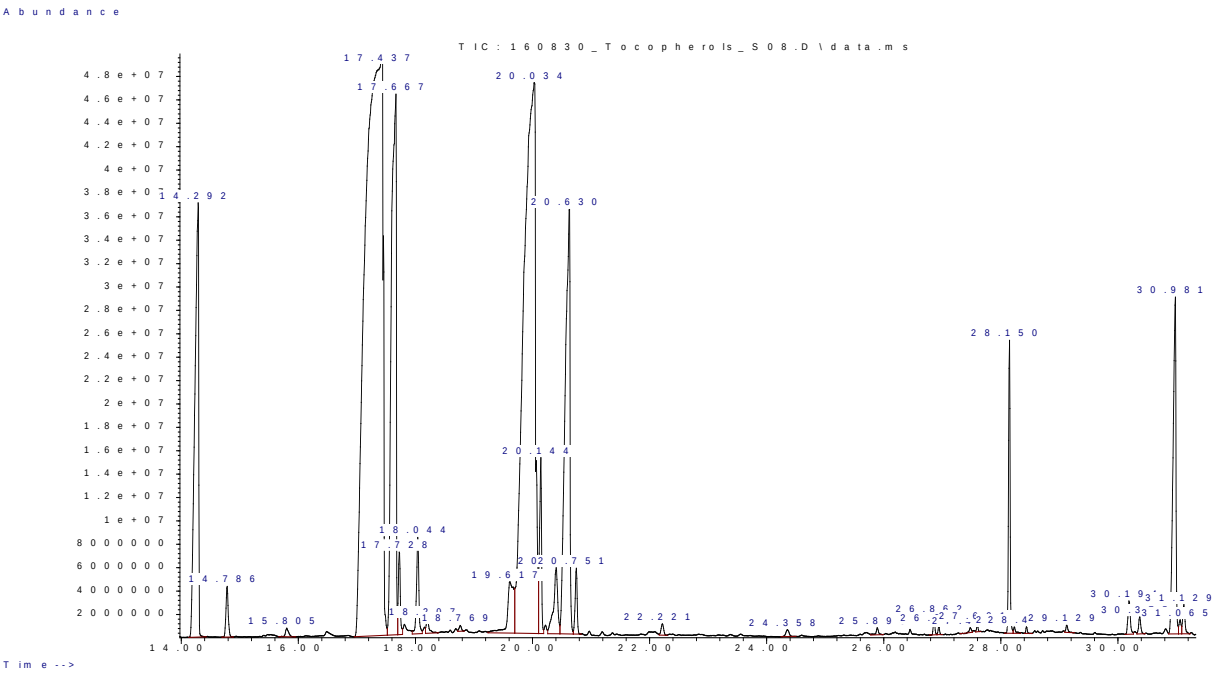


Figure 30: Gas chromatogram of traditionally extracted Manketti nut oil.

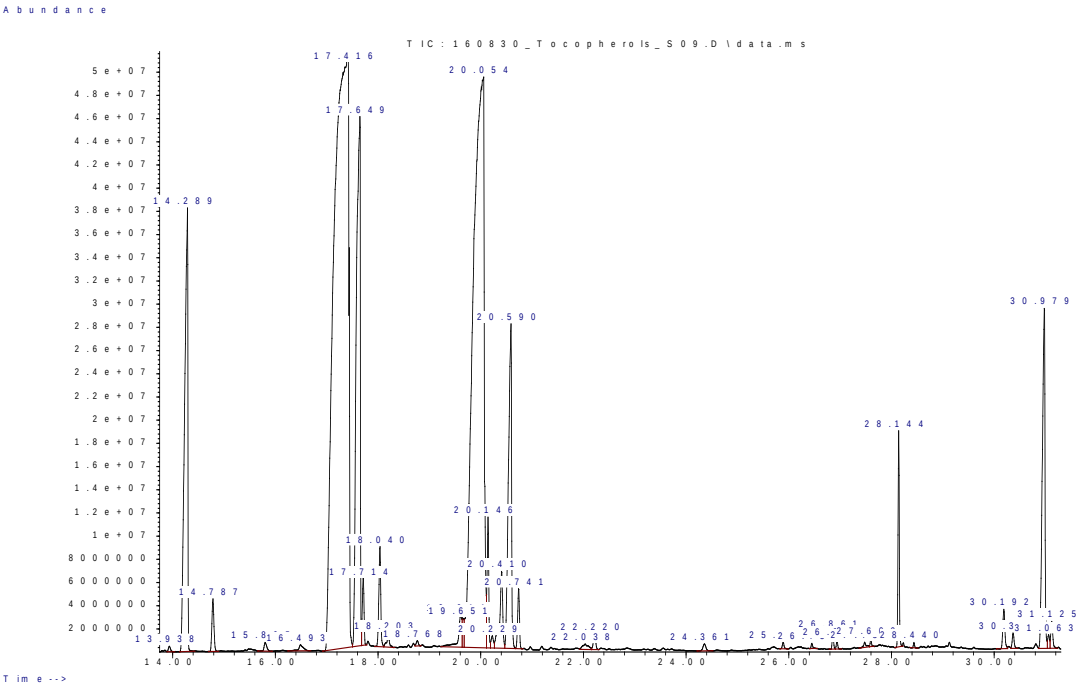


Figure 31: Gas chromatogram of cold pressed Manketti nut oil.

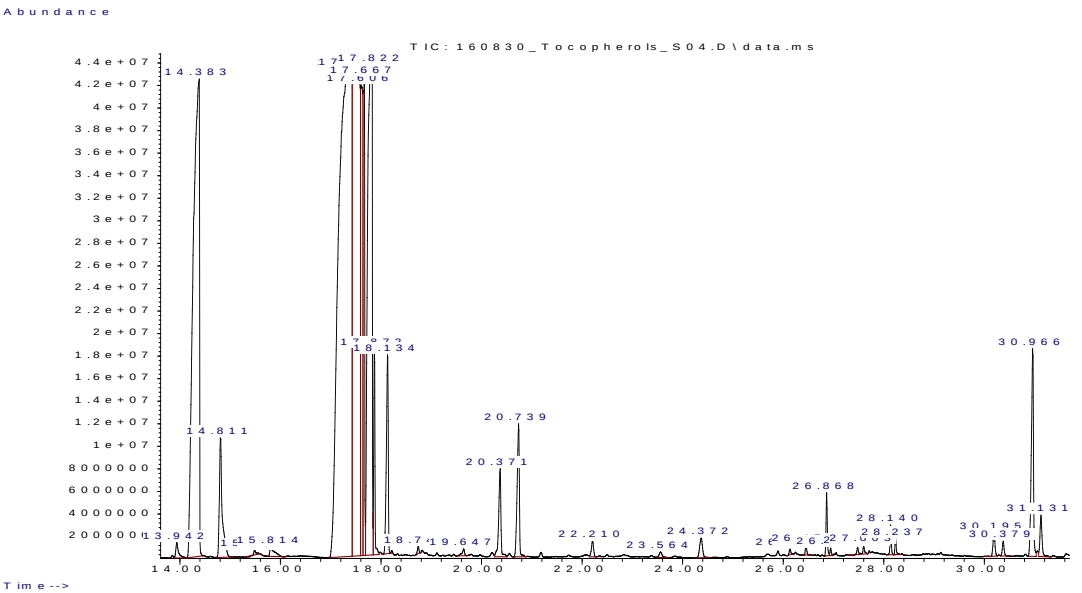


Figure 32: Gas chromatogram of Soxhlet extracted Marula nut oil.

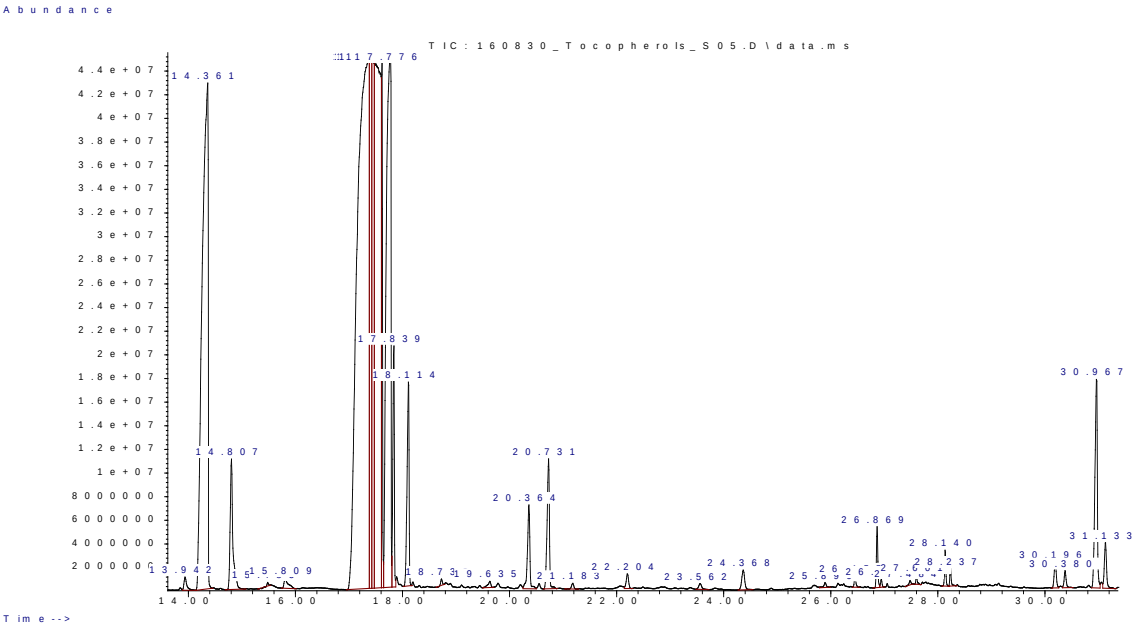


Figure 33: Gas chromatogram of traditionally extracted Marula nut oil.

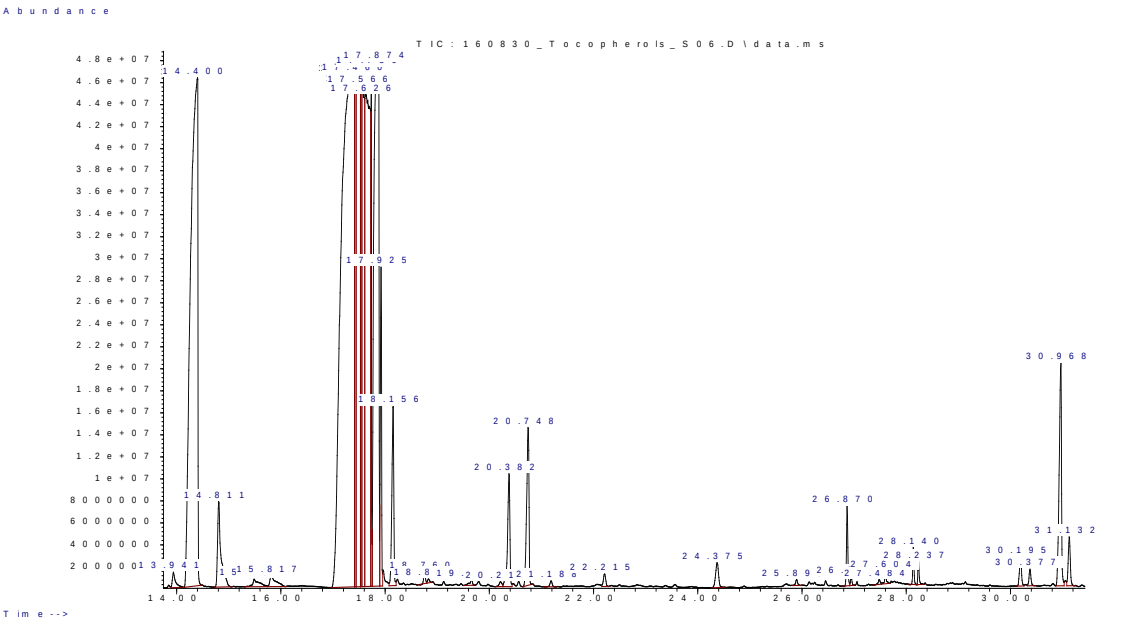


Figure 34: Gas chromatogram of cold pressed Marula nut oil.

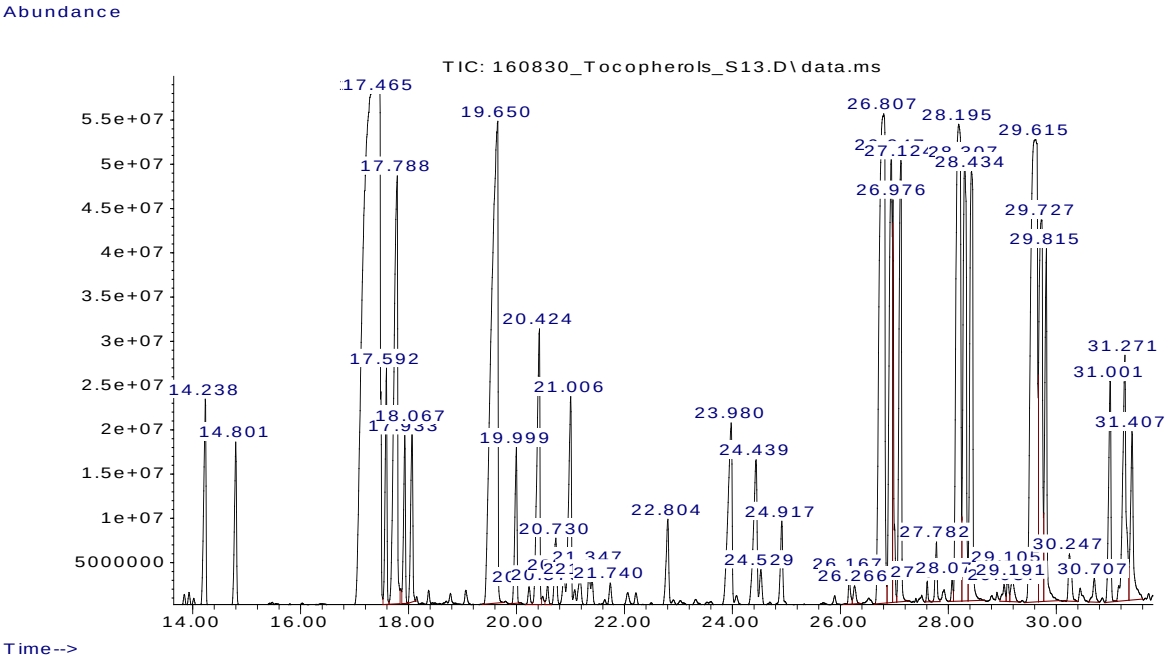


Figure 35: Gas chromatogram of Soxhlet extracted *Ximenia* nut oil.

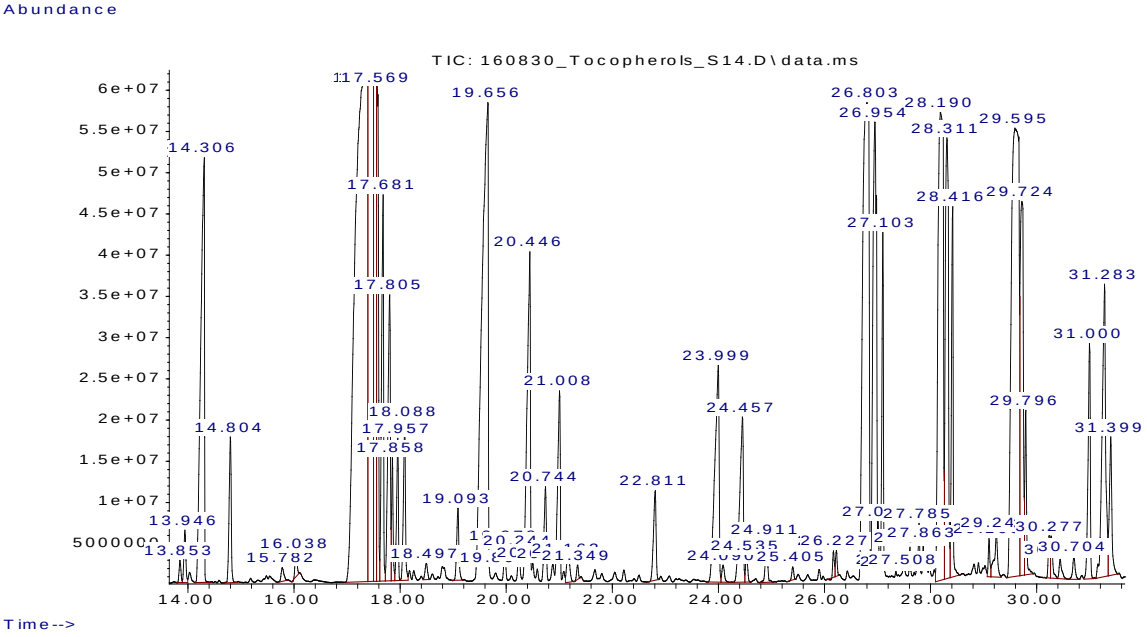


Figure 36: Gas chromatogram of traditionally extracted *Ximenia* nut oil.

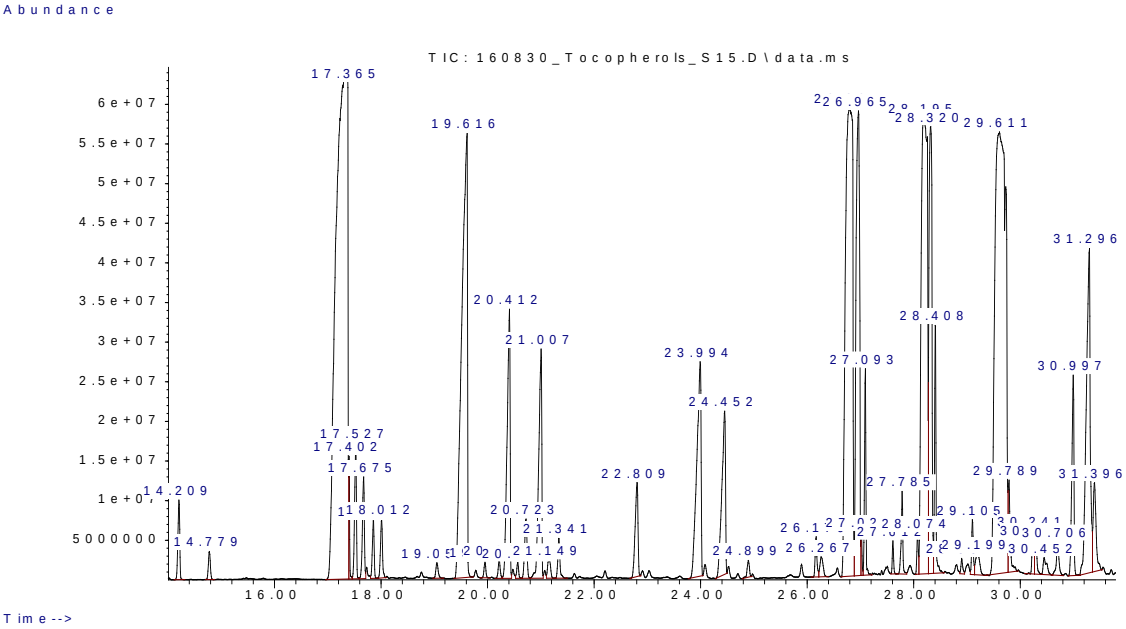


Figure 37: Gas chromatogram of cold pressed *Ximenia* nut oil.

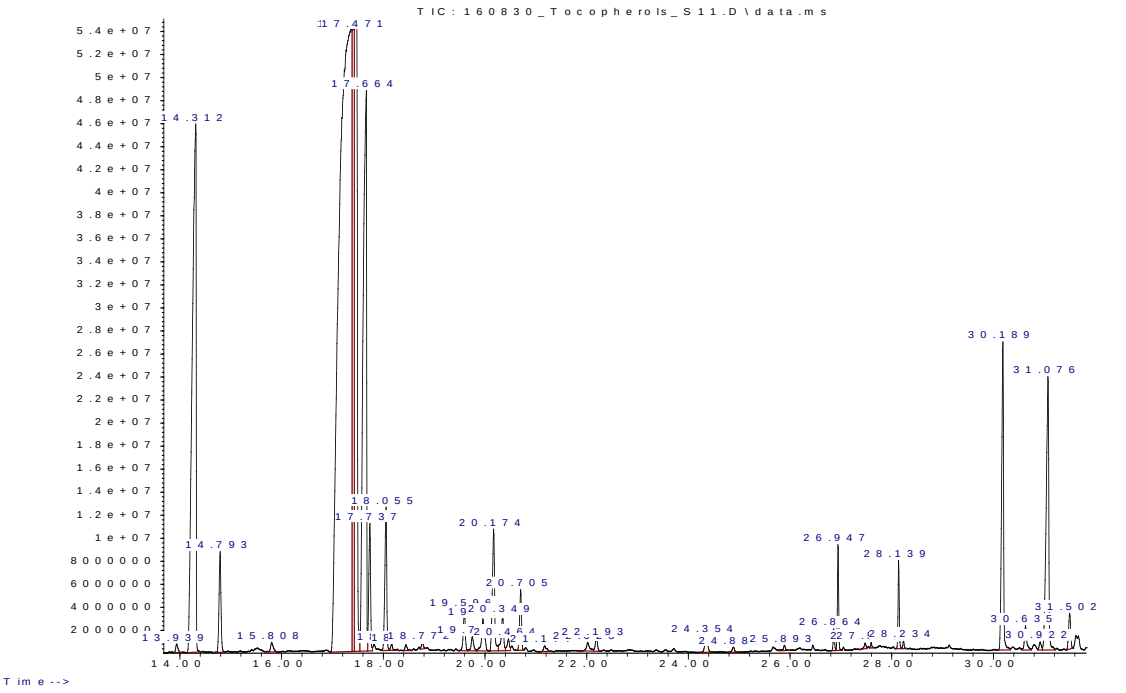


Figure 38: Gas chromatogram of Soxhlet extracted !Nara seed oil.

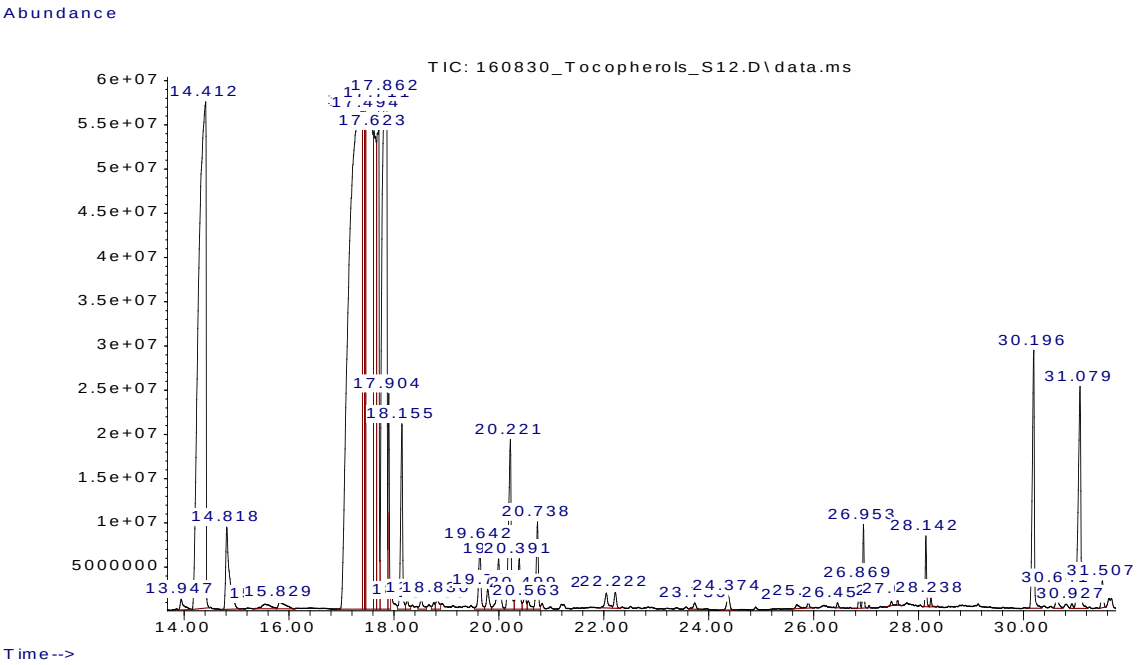


Figure 39: Gas chromatogram of cold pressed !Nara seed oil.

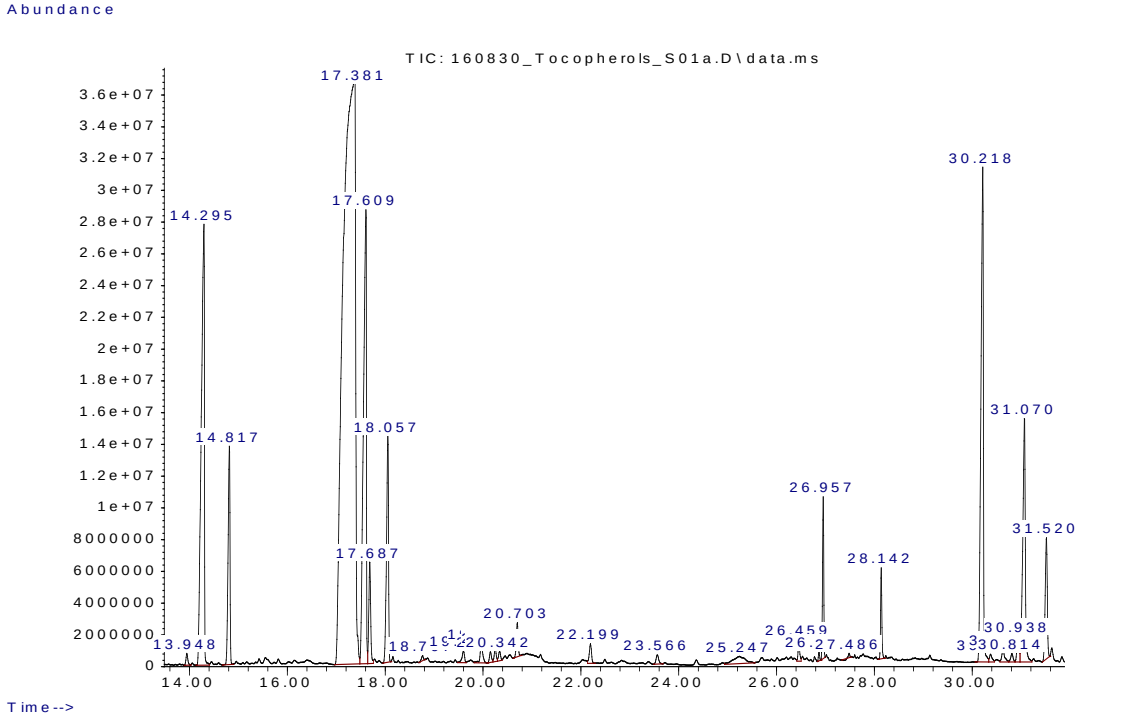


Figure 40: Gas chromatogram of Soxhlet extracted Melon seed oil.

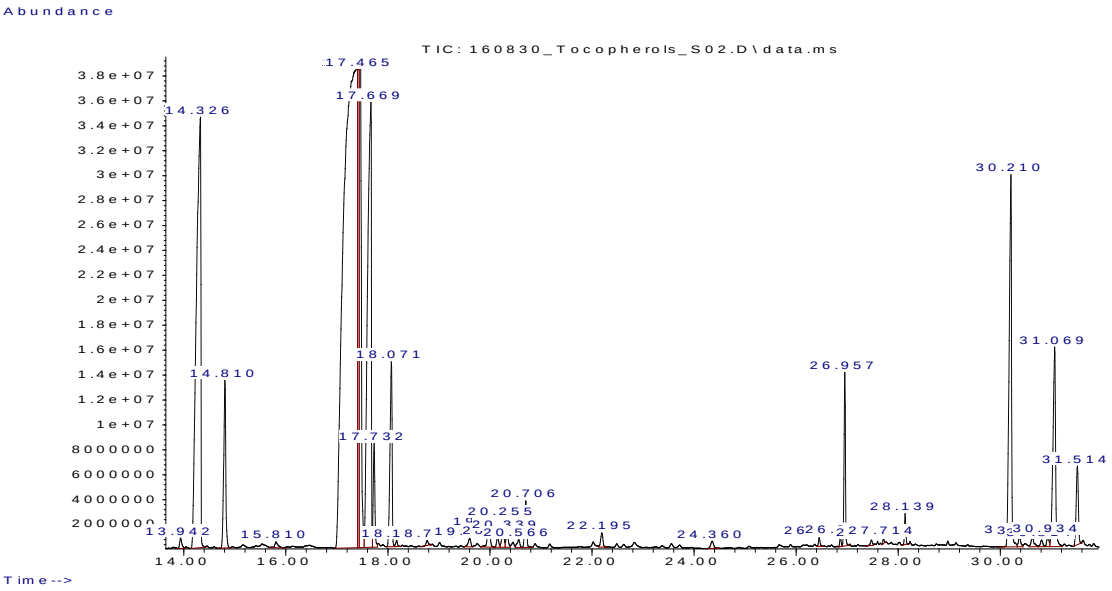


Figure 41: Gas chromatogram of traditionally extracted Melon seed oil.

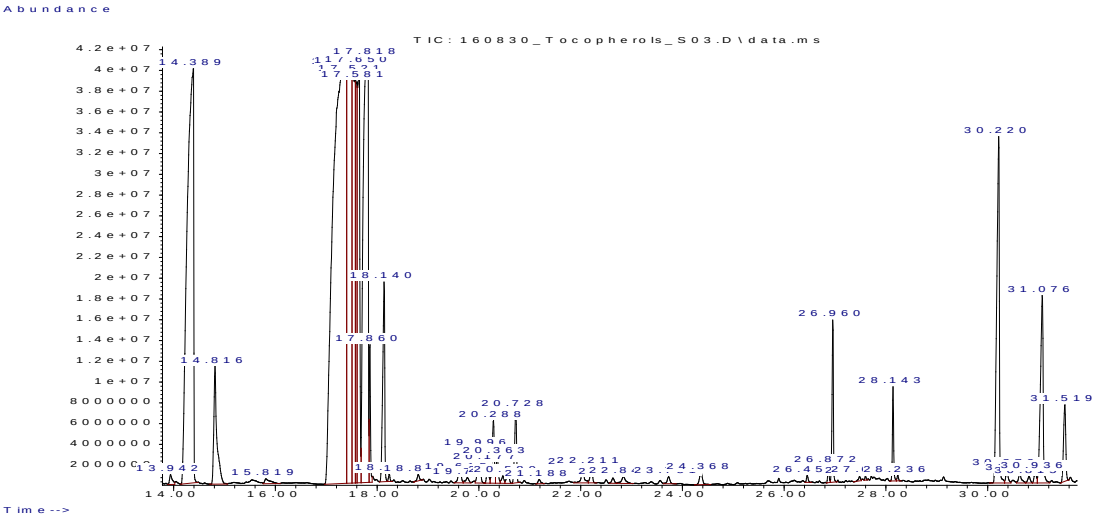


Figure 42: Gas chromatogram of cold pressed Melon seed oil.

APPENDIX E: NMR Spectra

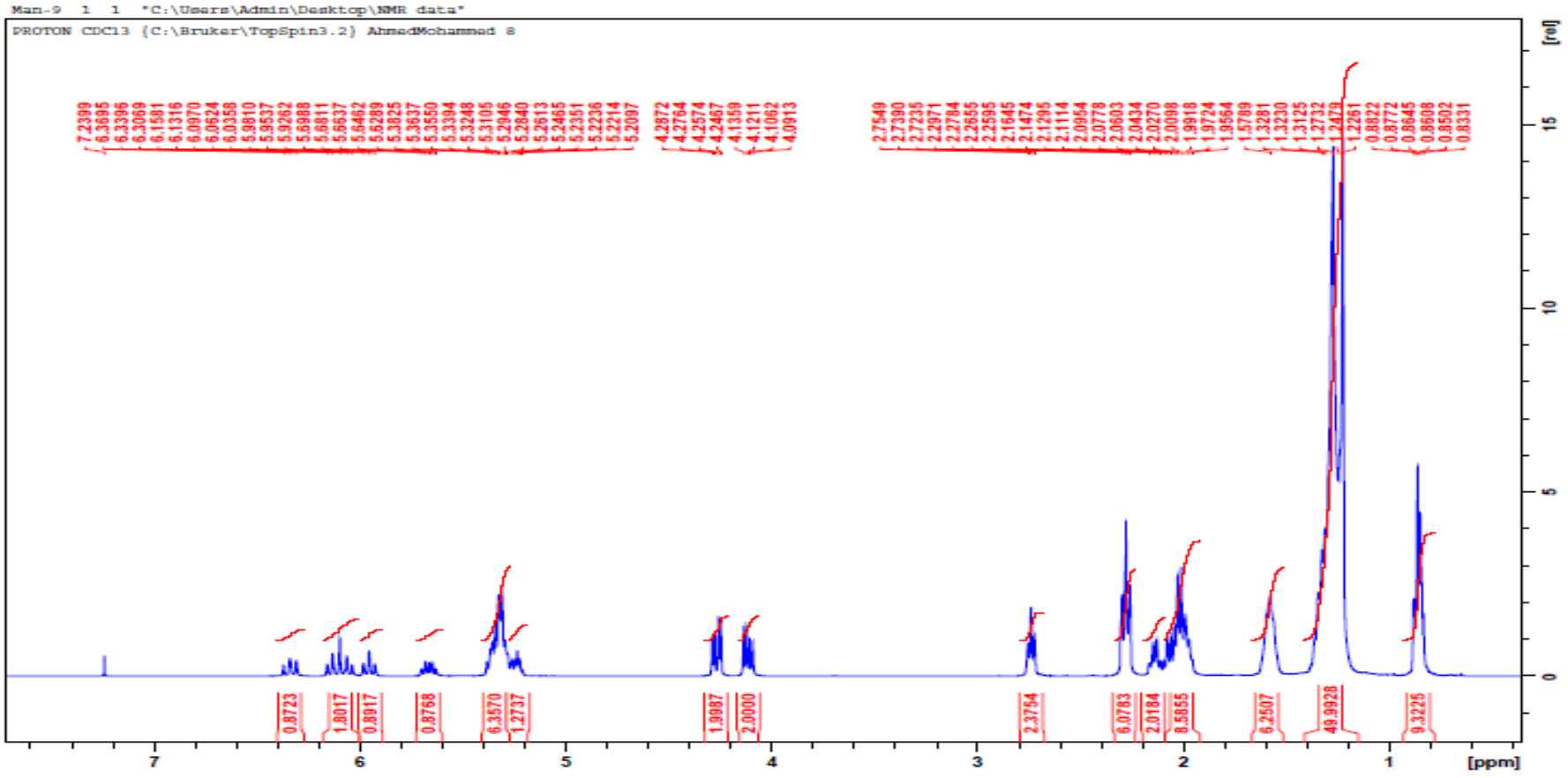


Figure 43: 1H NMR spectrum (400 MHz, CDCl3) of cold pressed Manketti nut oil

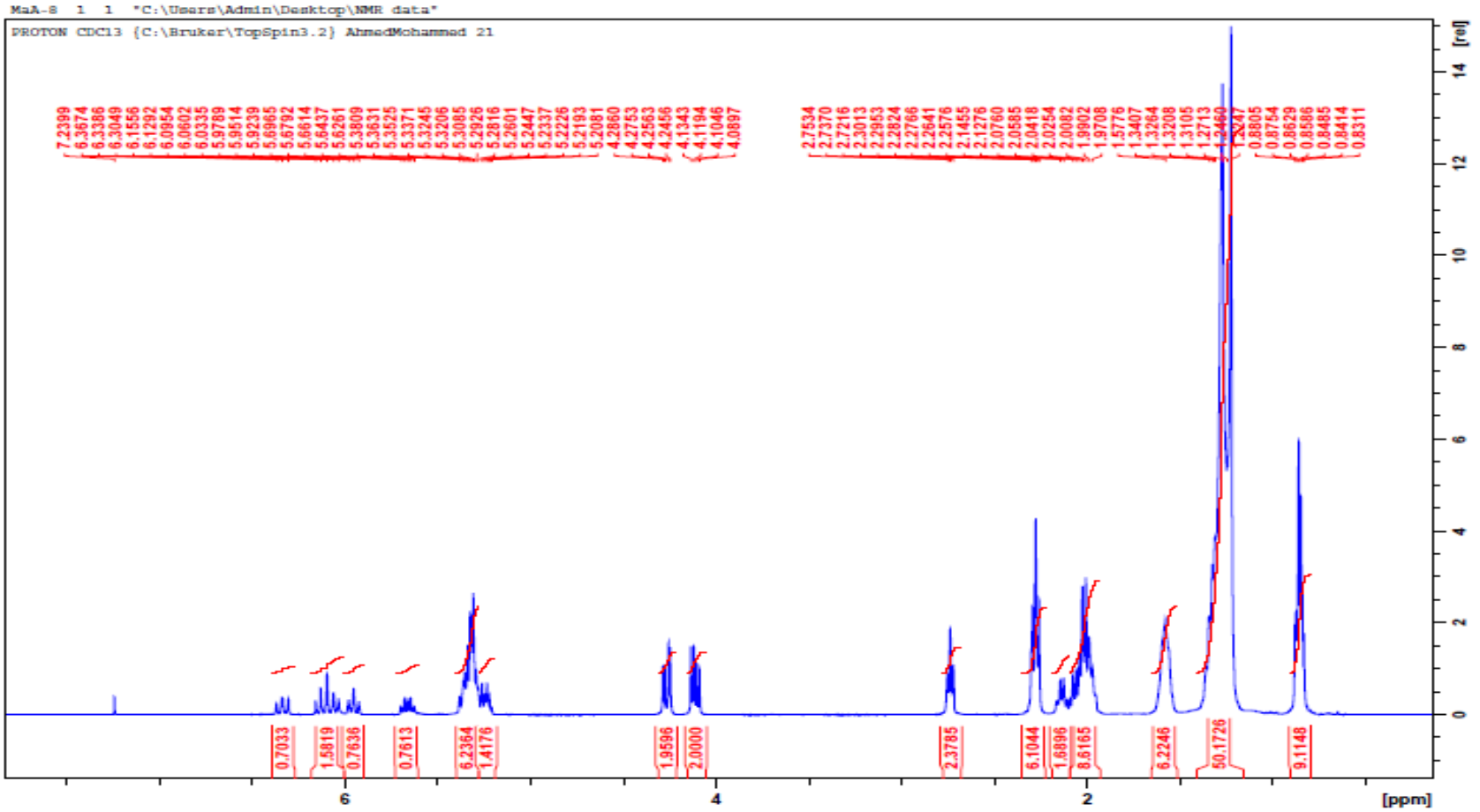


Figure 44: ^{1}H NMR spectrum (400 MHz, CDCl3) of traditionally extracted Manketti nut oil.

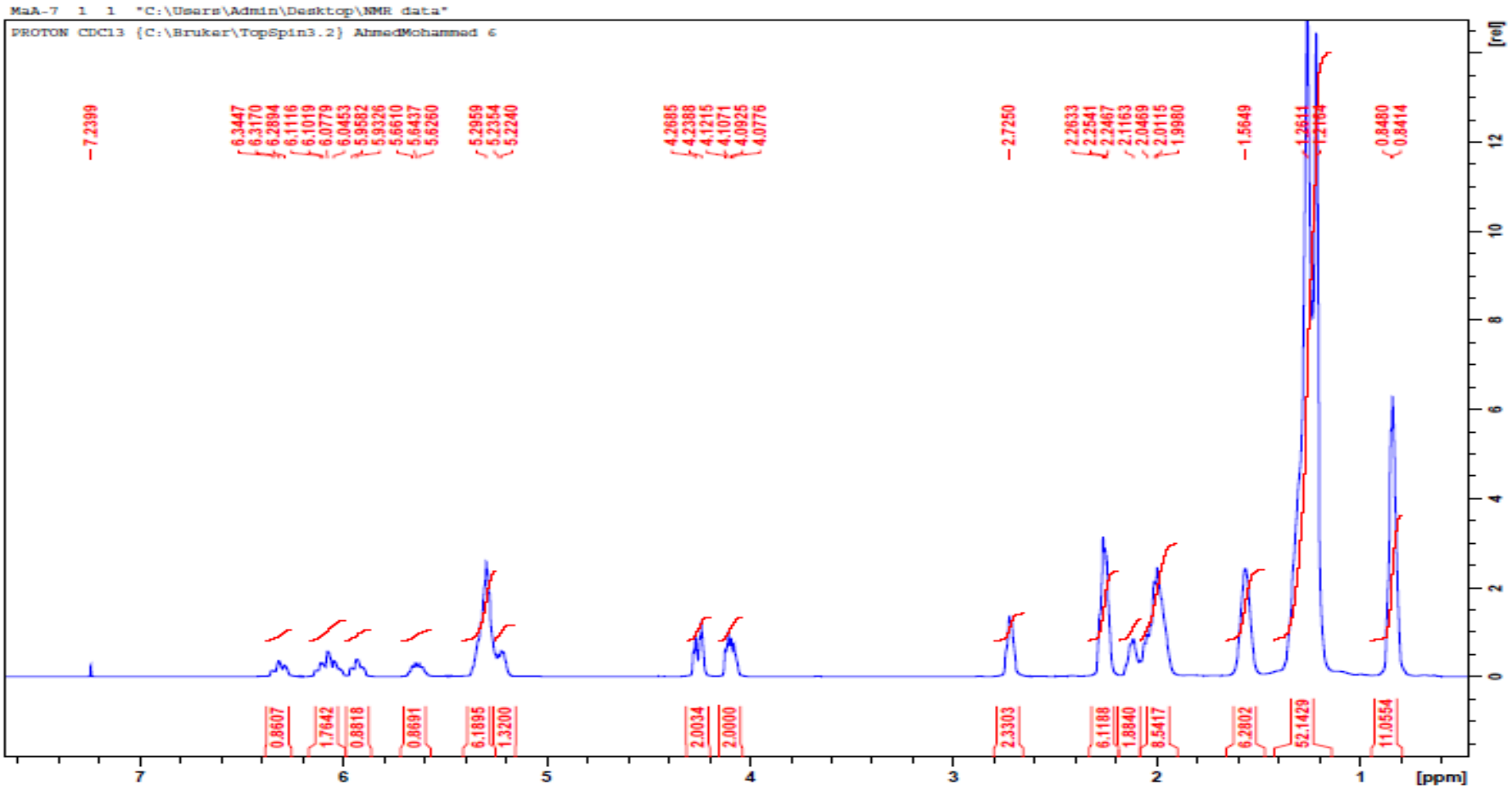


Figure 45: ^{1}H NMR spectrum (400 MHz, CDCl3) of Soxhlet extracted Manketti nut oil.

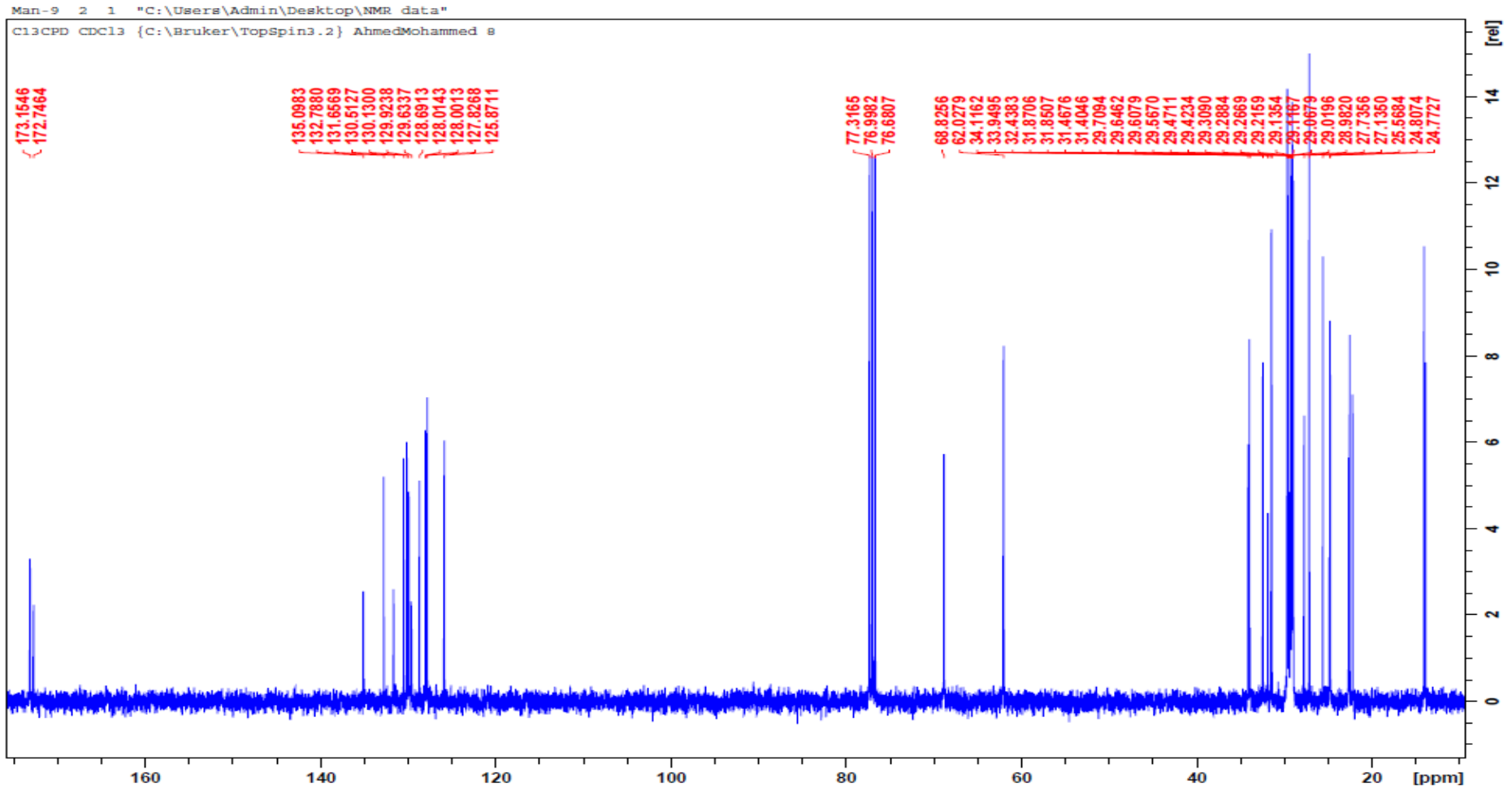


Figure 46: ^{13}CNMR spectrum (100 MHz, $CDCl_3$) of cold pressed Manketti nut oil.

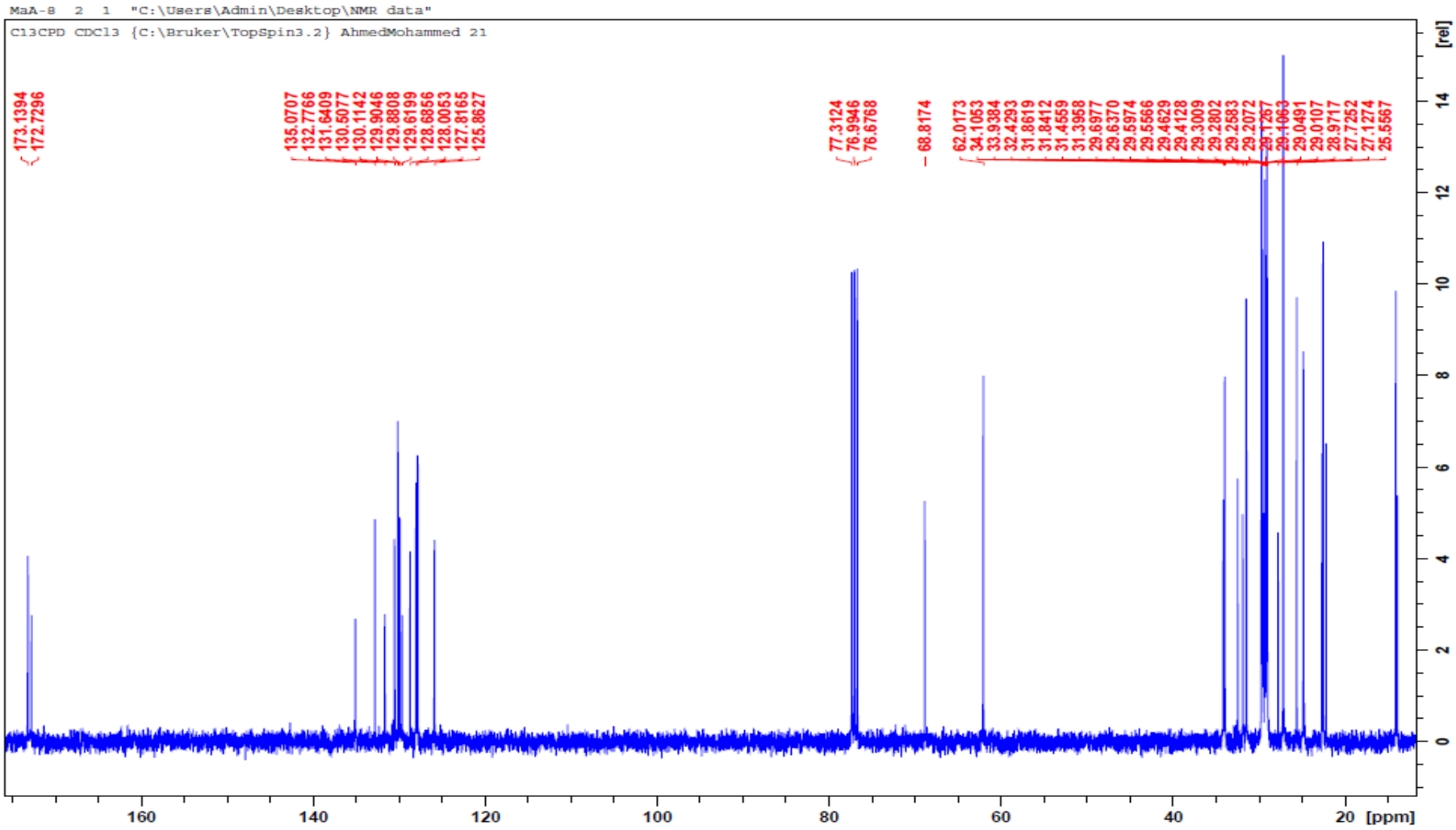


Figure 47: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of cold pressed Manketti nut oil.

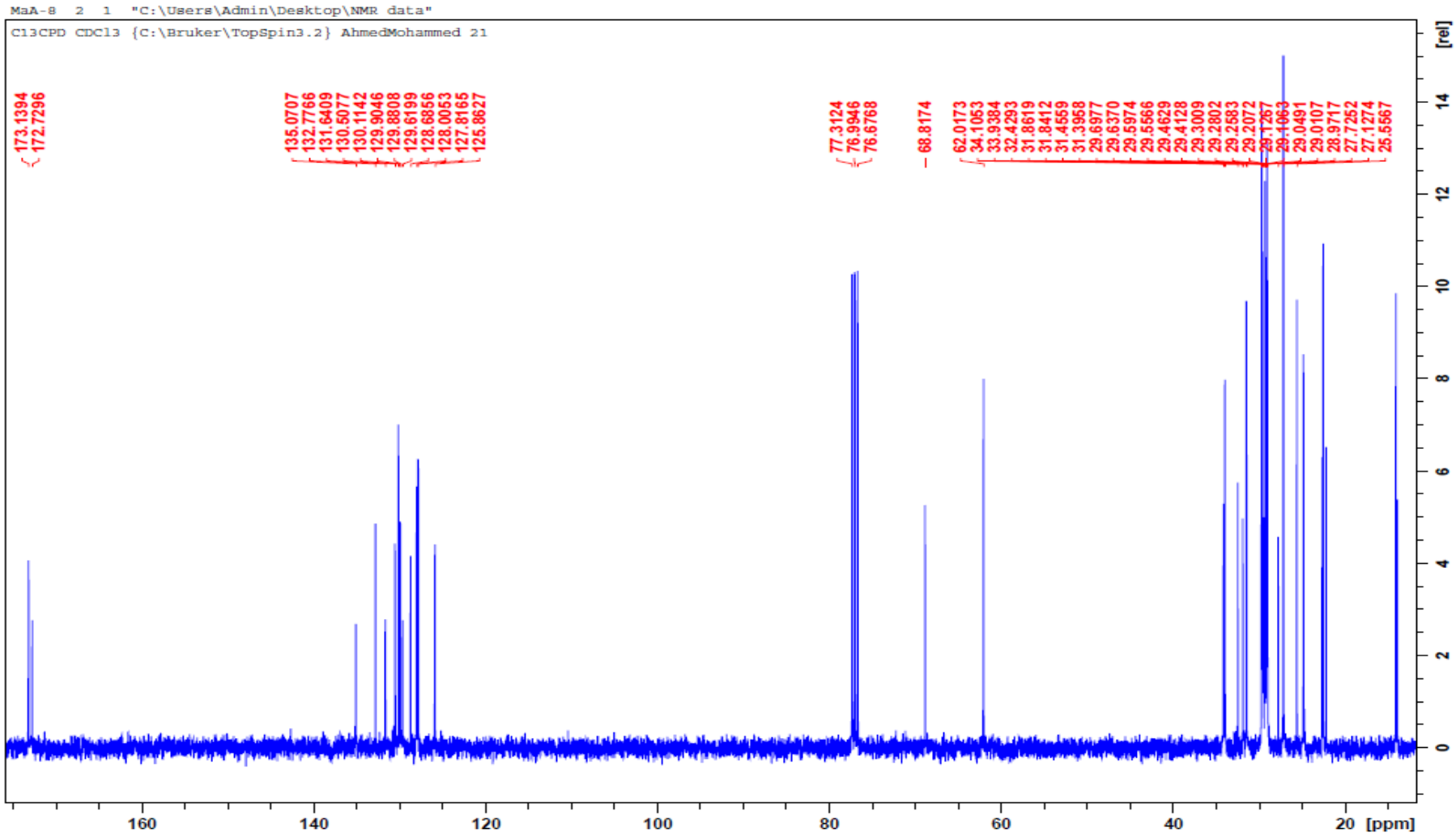


Figure 48: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of traditionally extracted Manketti nut oil.

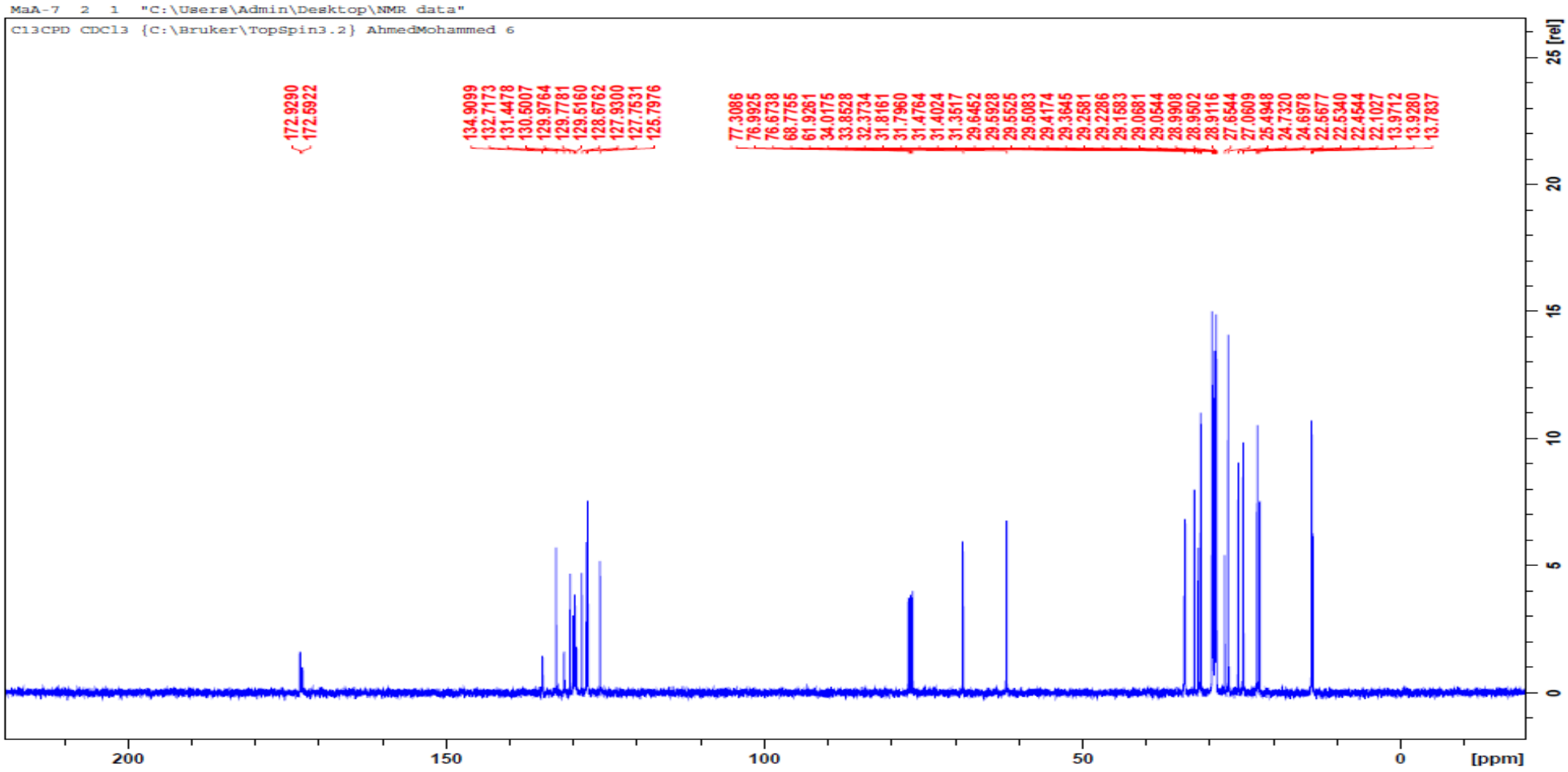


Figure 49: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Soxhlet-extracted Manketti nut oil.

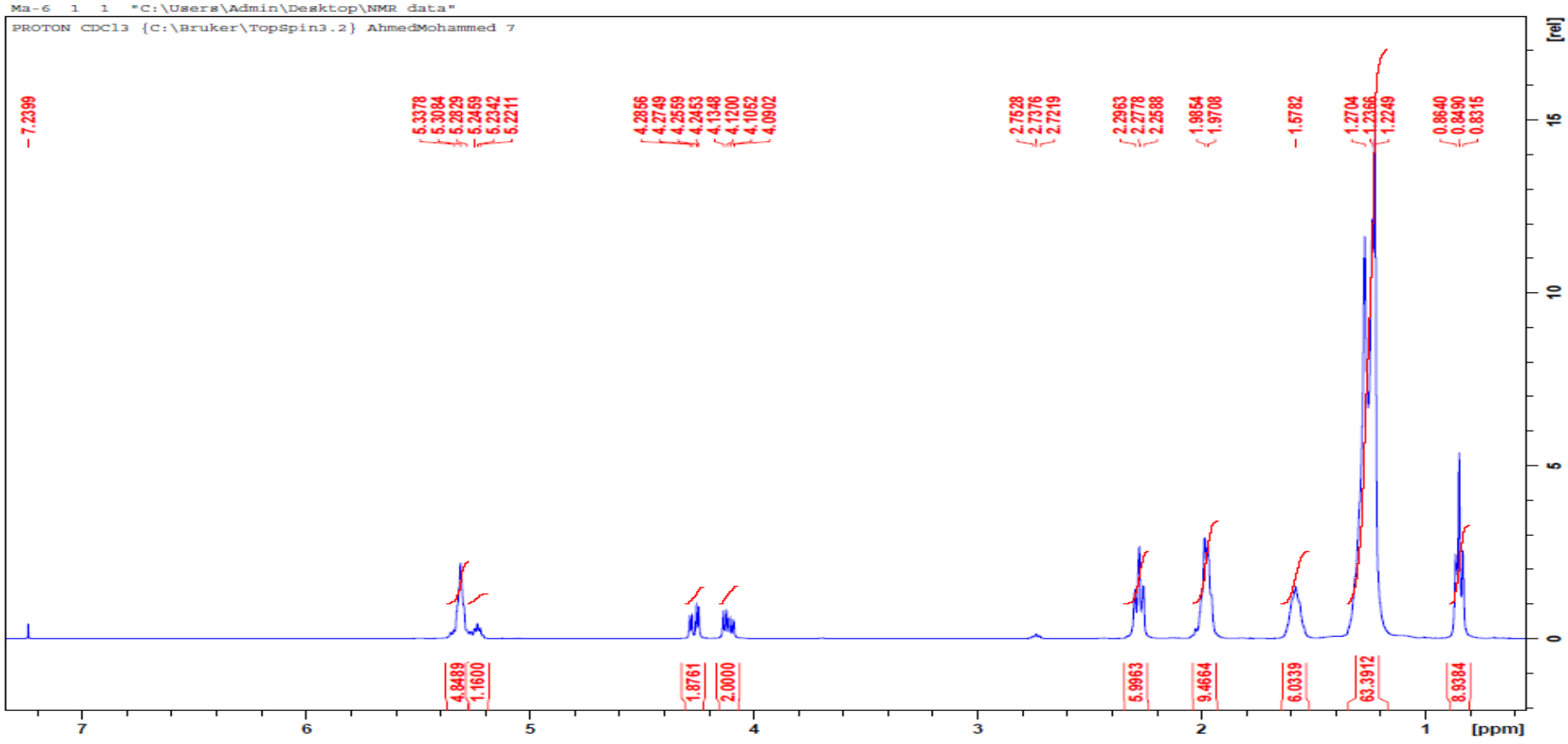


Figure 50: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of cold pressed Marula nut oil.

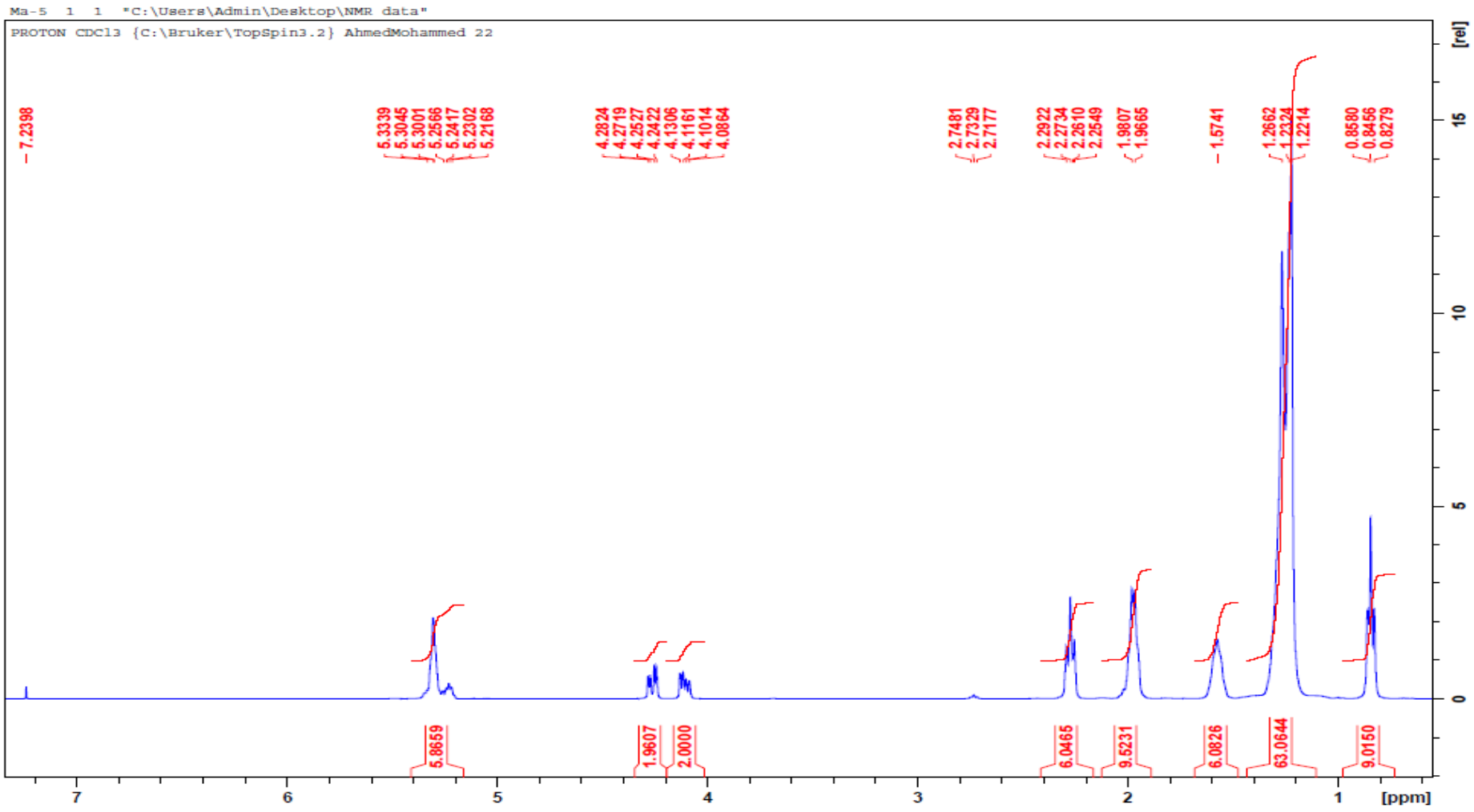


Figure 51: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of traditionally extracted Marula nut oil.

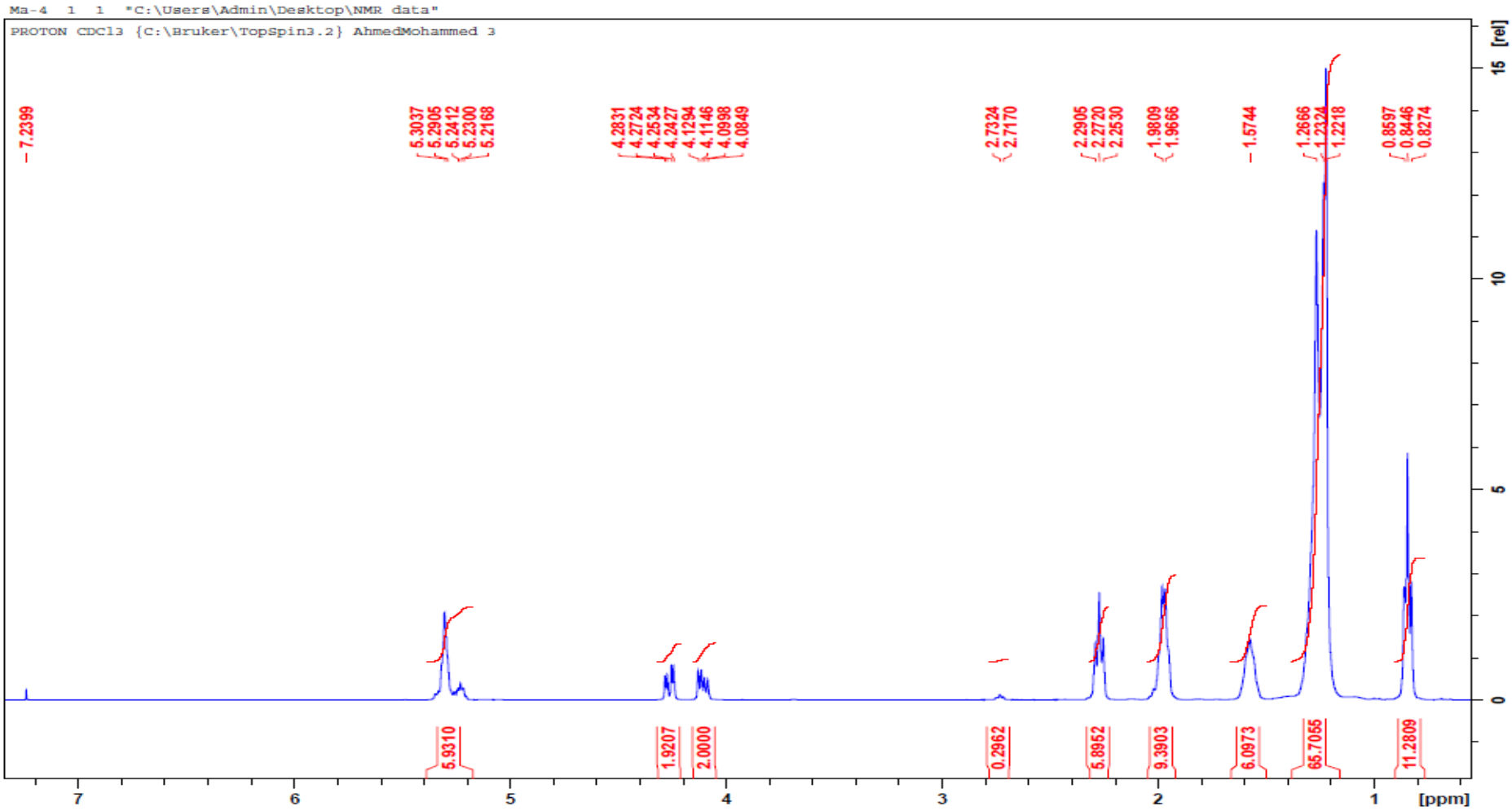


Figure 52: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Soxhlet-extracted extracted Marula nut oil.

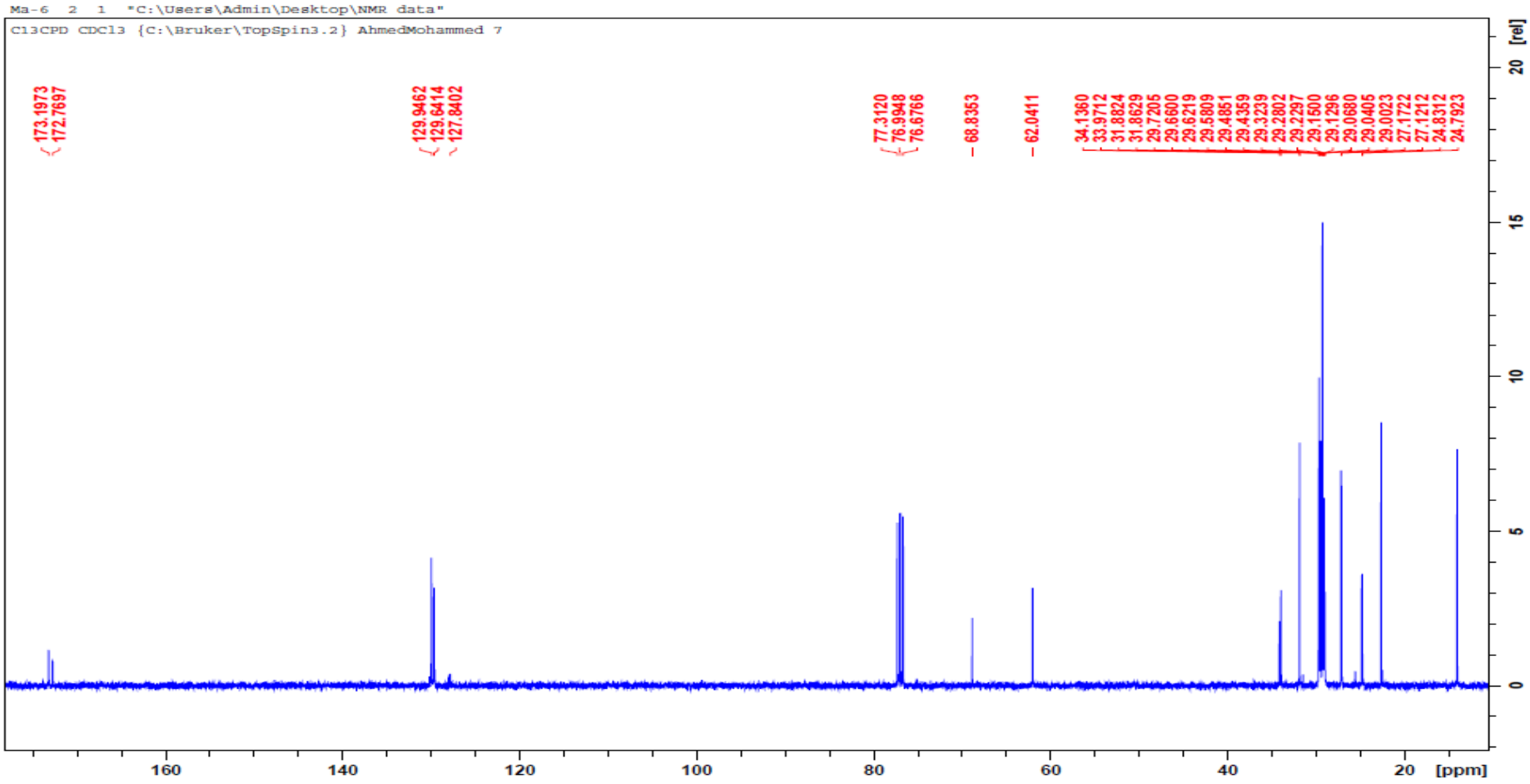


Figure 53: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of cold pressed Marula nut oil.

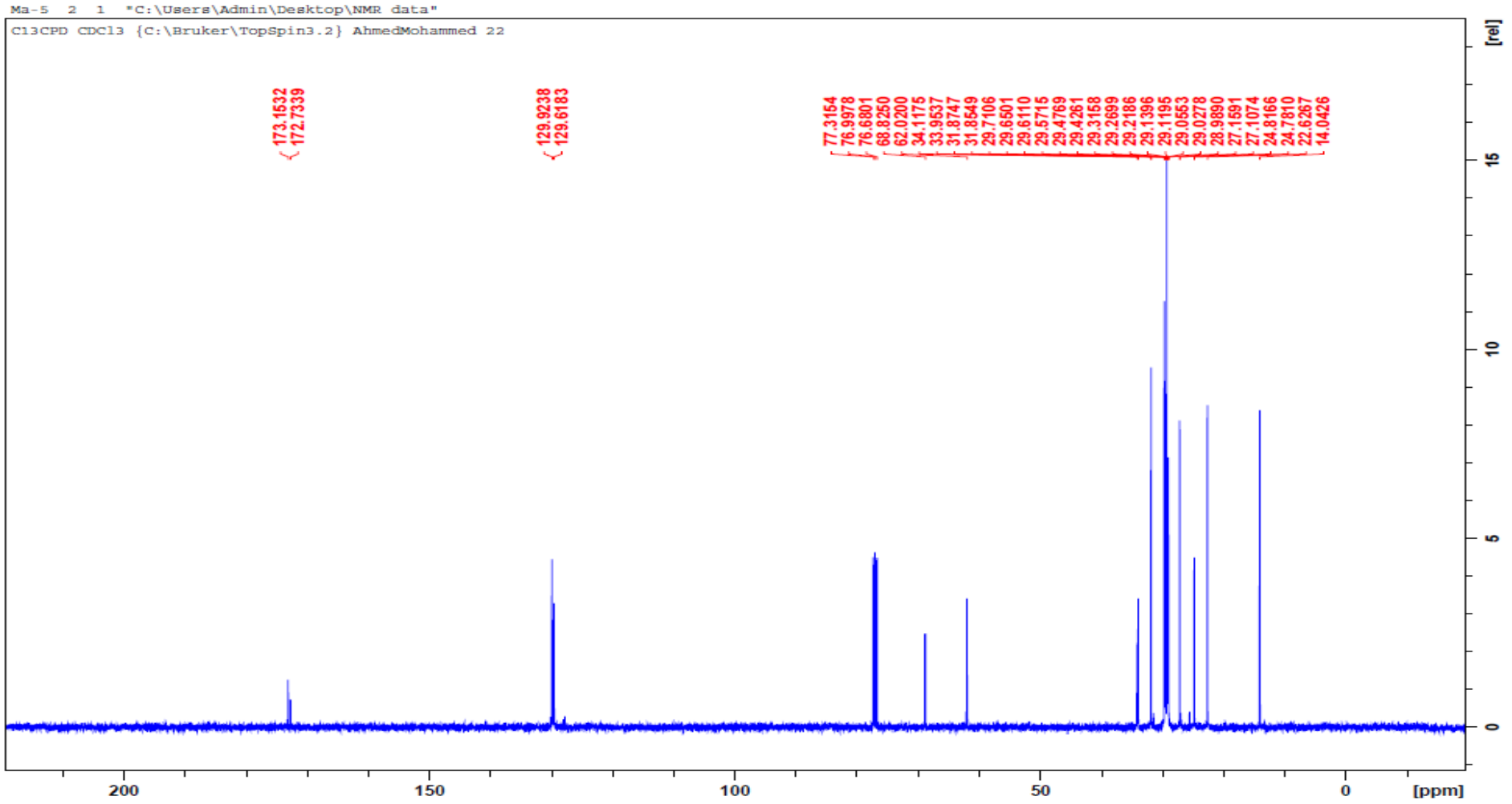


Figure 54: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of traditionally extracted Marula nut oil.

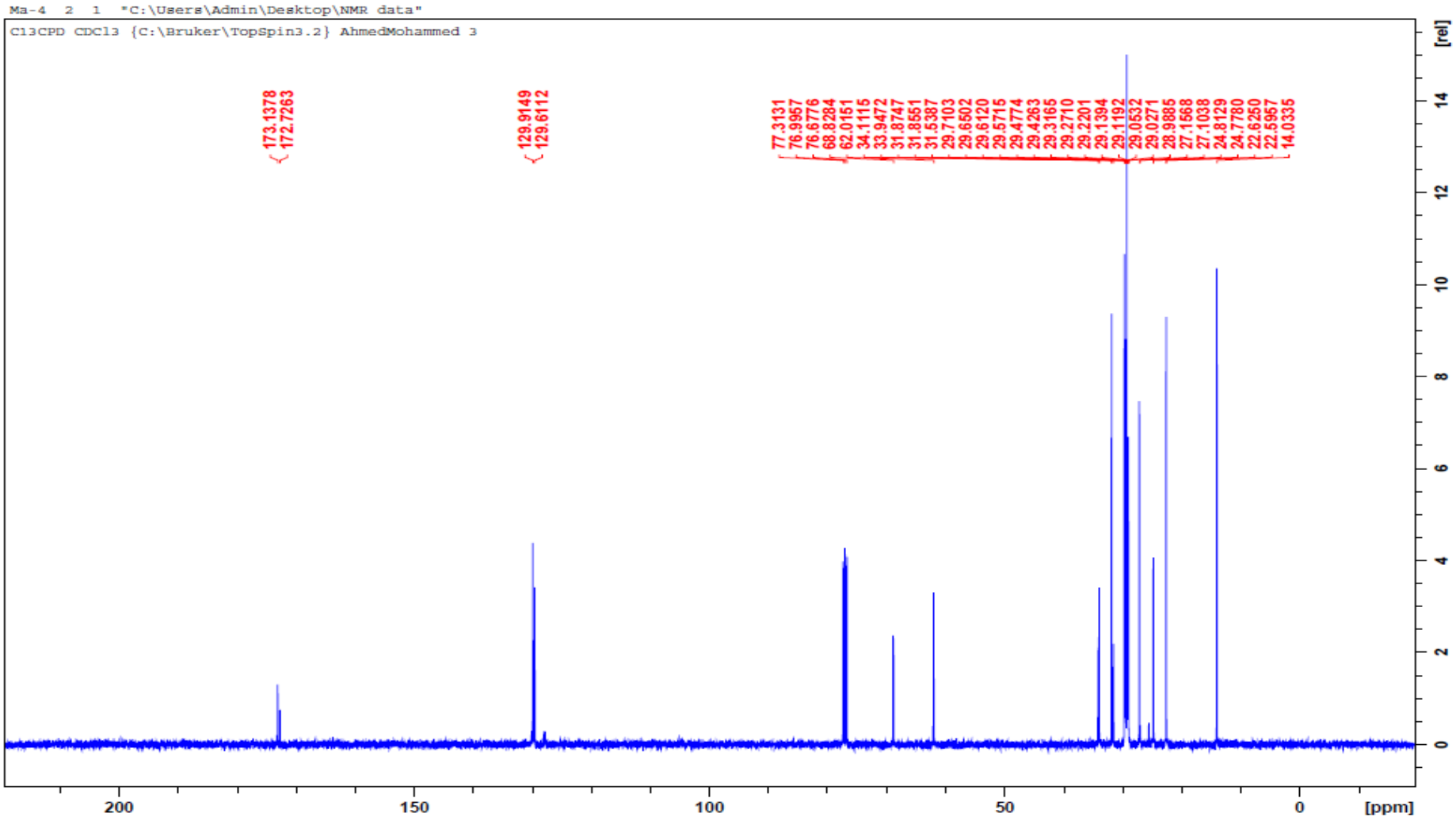


Figure 55: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of Soxhlet-extracted Marula nut oil.

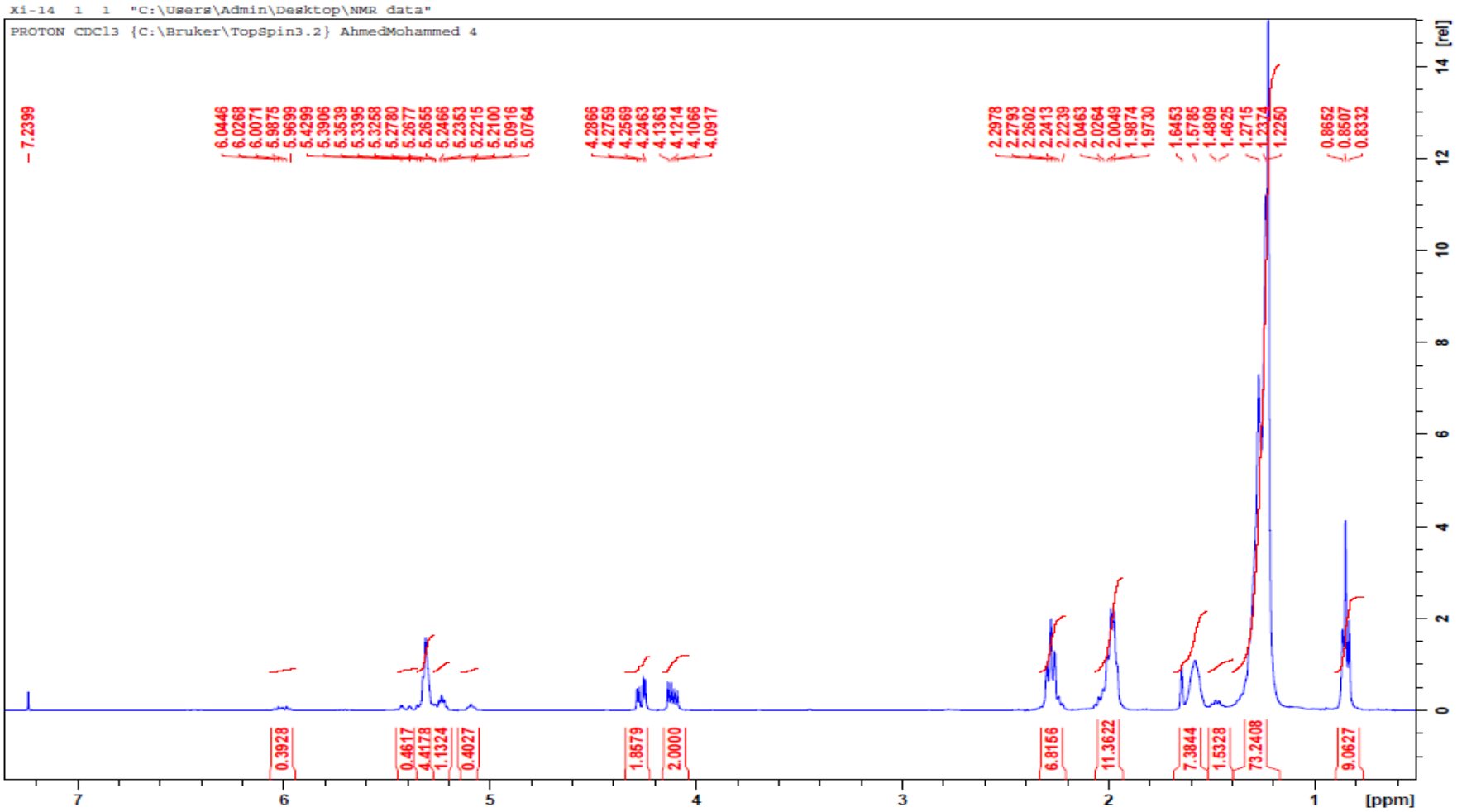


Figure 56: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of cold pressed *Ximenia* nut oil.

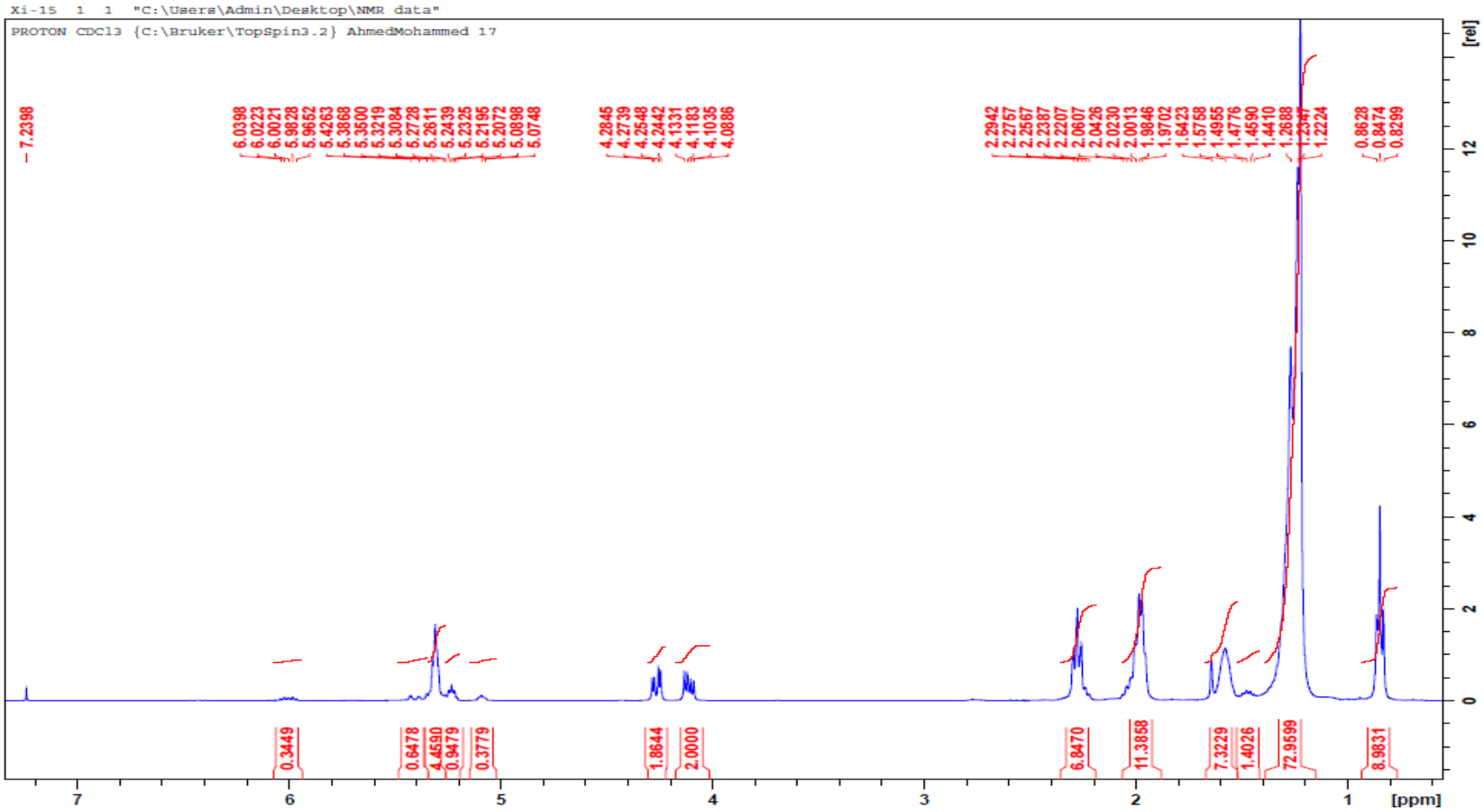


Figure 57: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of traditionally extracted *Ximenia* nut oil.

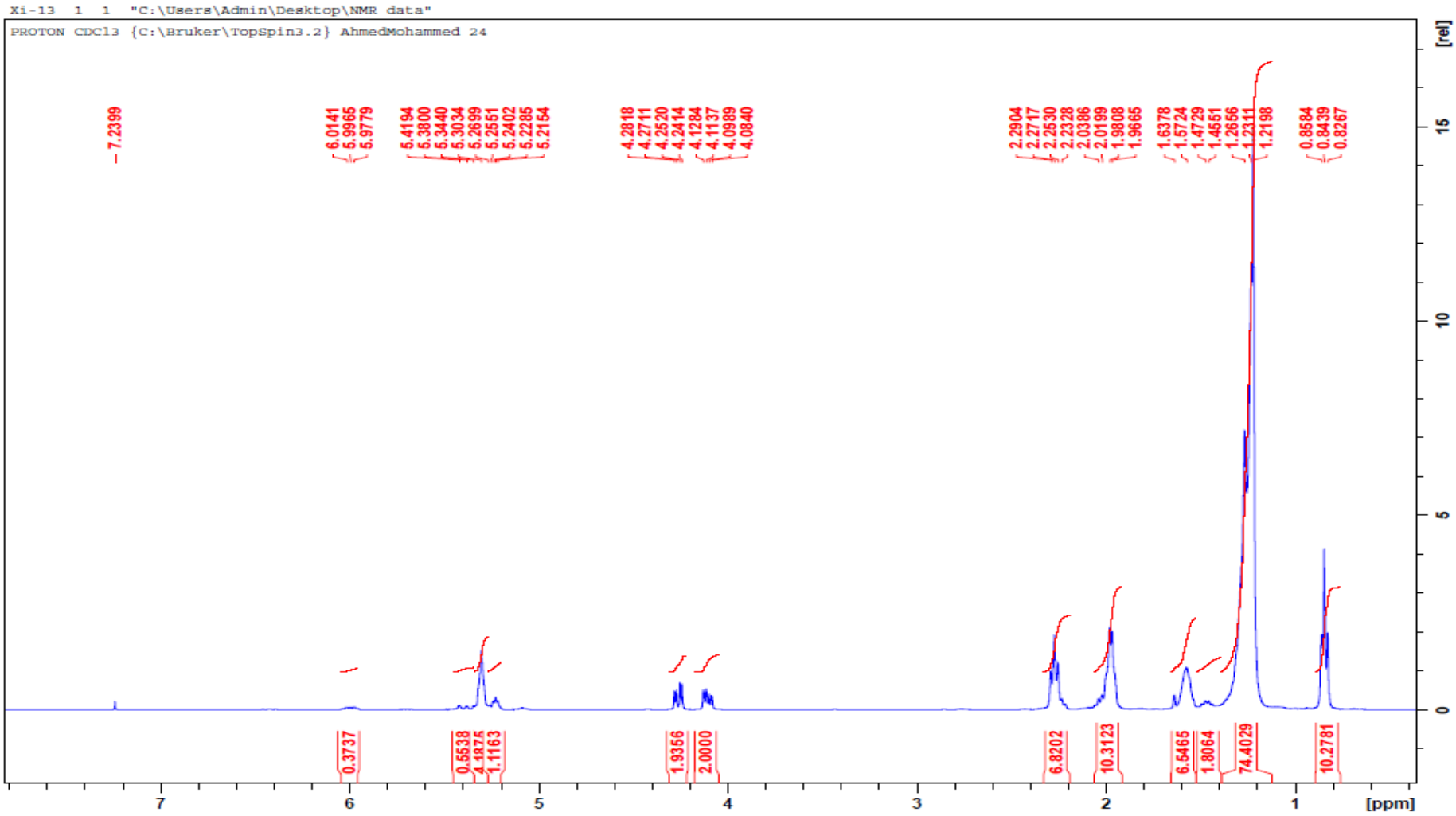


Figure 58: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Soxhlet-extracted *Ximenia* nut oil.

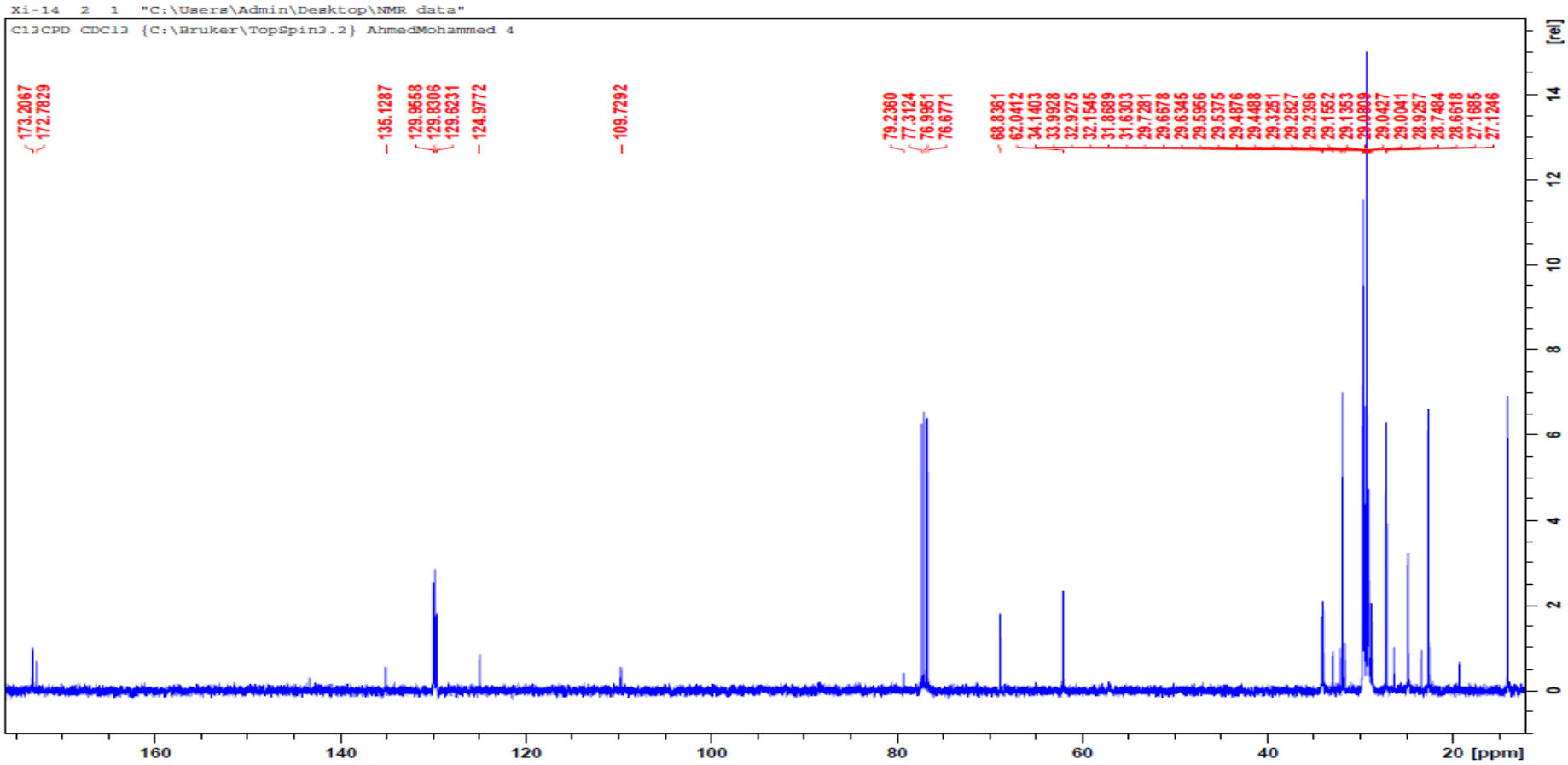


Figure 59: ^{13}C NMR (100 MHz, $CDCl_3$) spectra of cold pressed *Ximenia* nut oil.

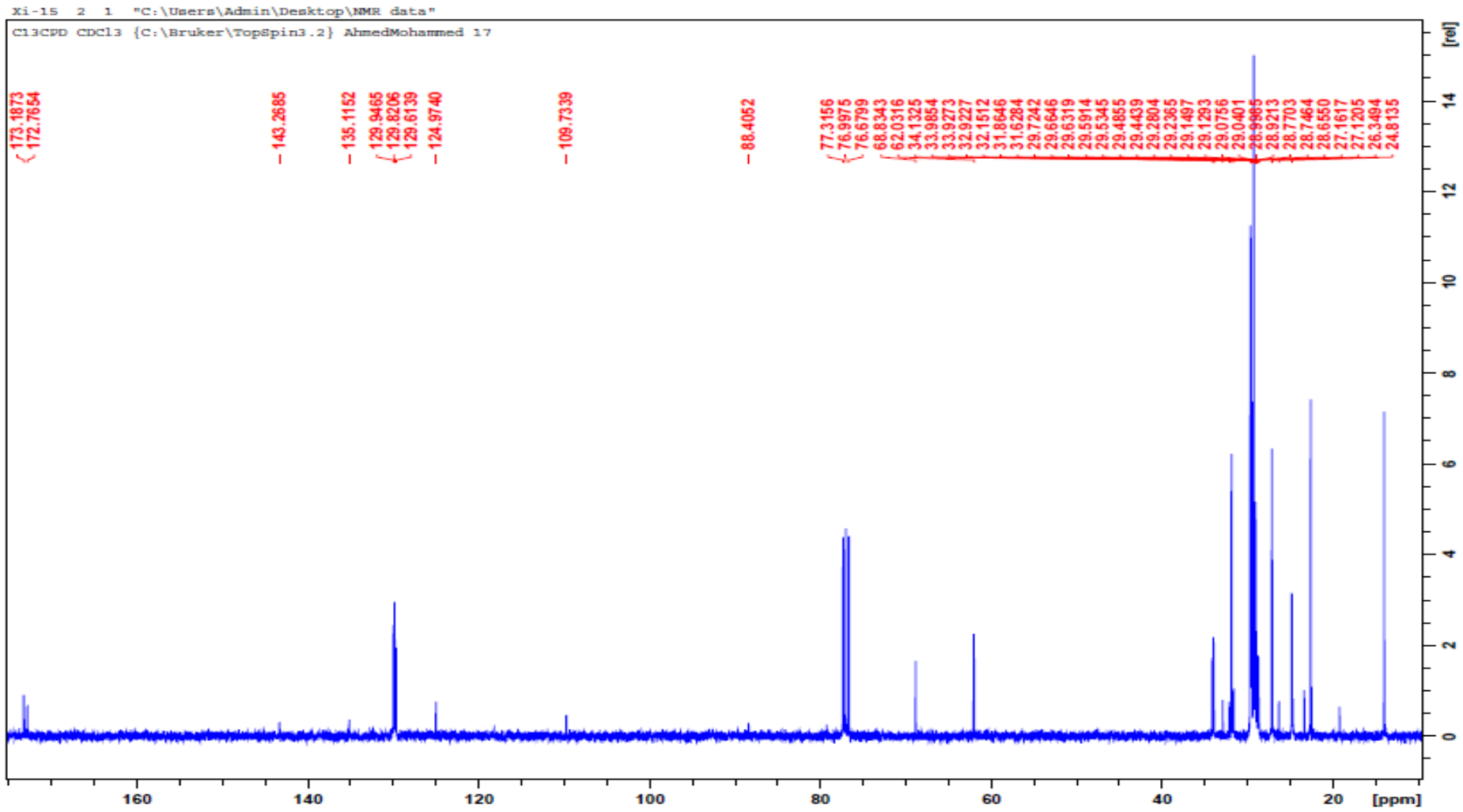


Figure 60: ^{13}C NMR (100 MHz, $CDCl_3$) spectra of traditionally-extracted *Ximenia* nut oil.

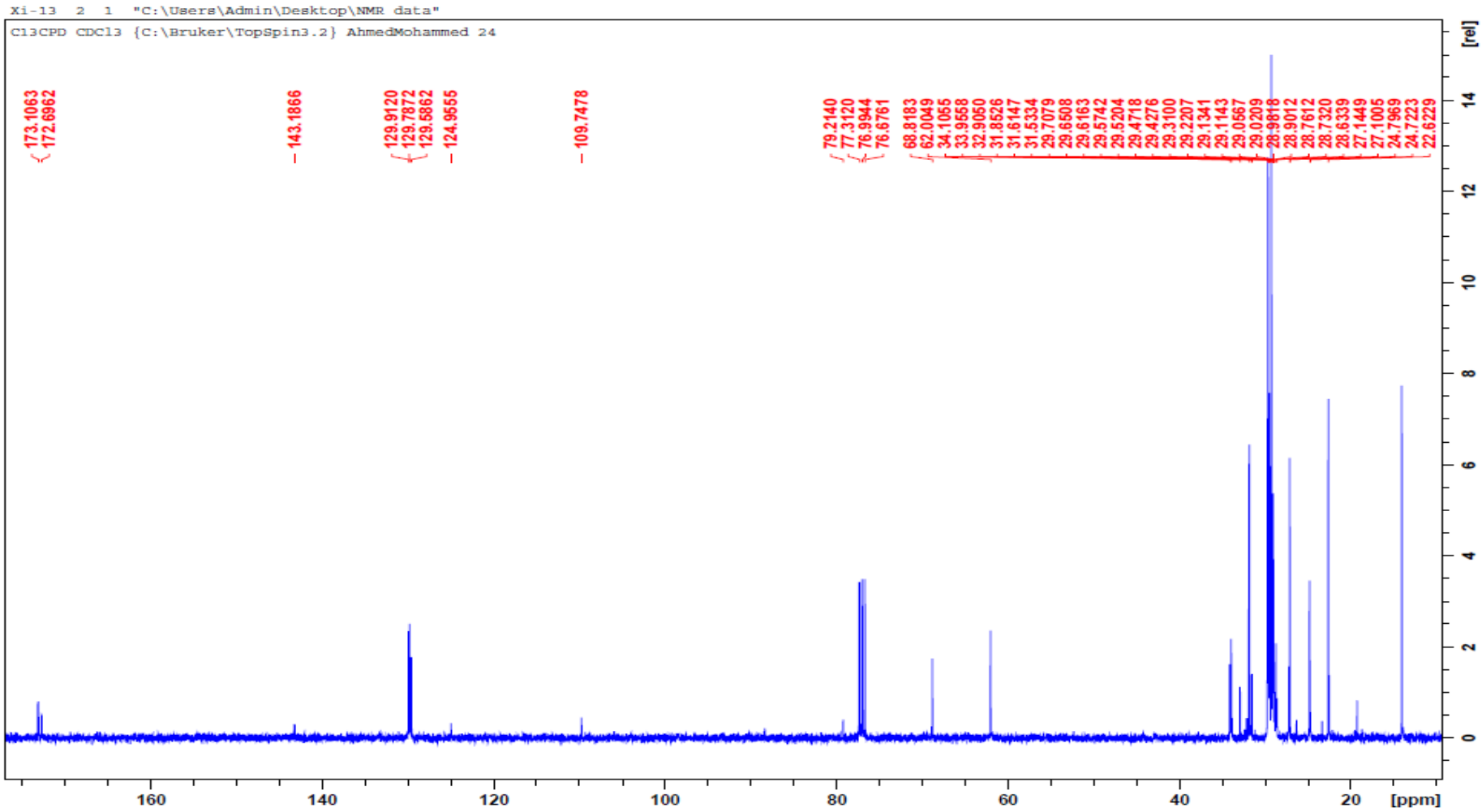


Figure 61: ^{13}C NMR (100 MHz, $CDCl_3$) spectra of Soxhlet-extracted *Ximenia* nut oil.

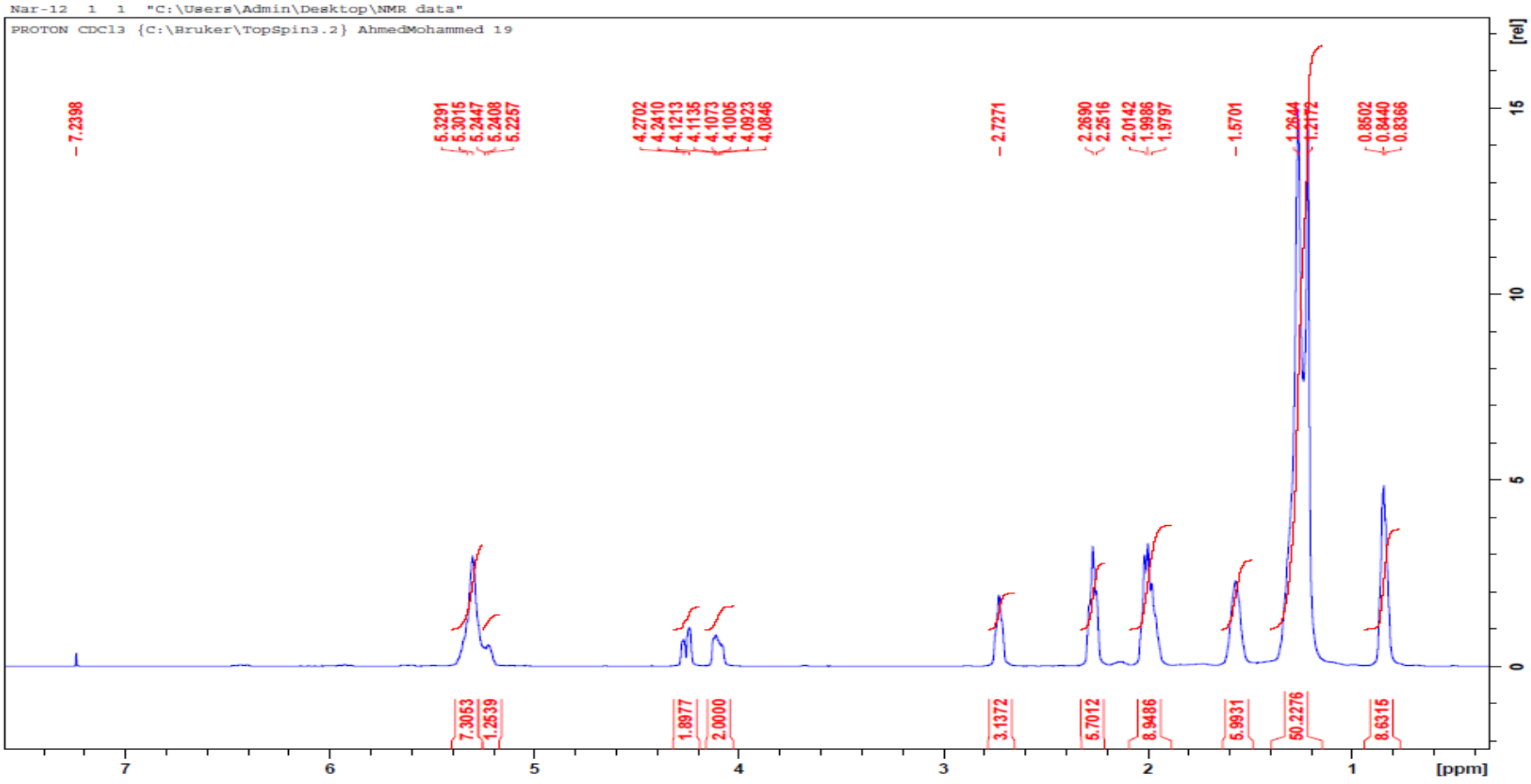


Figure 62: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of cold pressed !Nara seed oil.

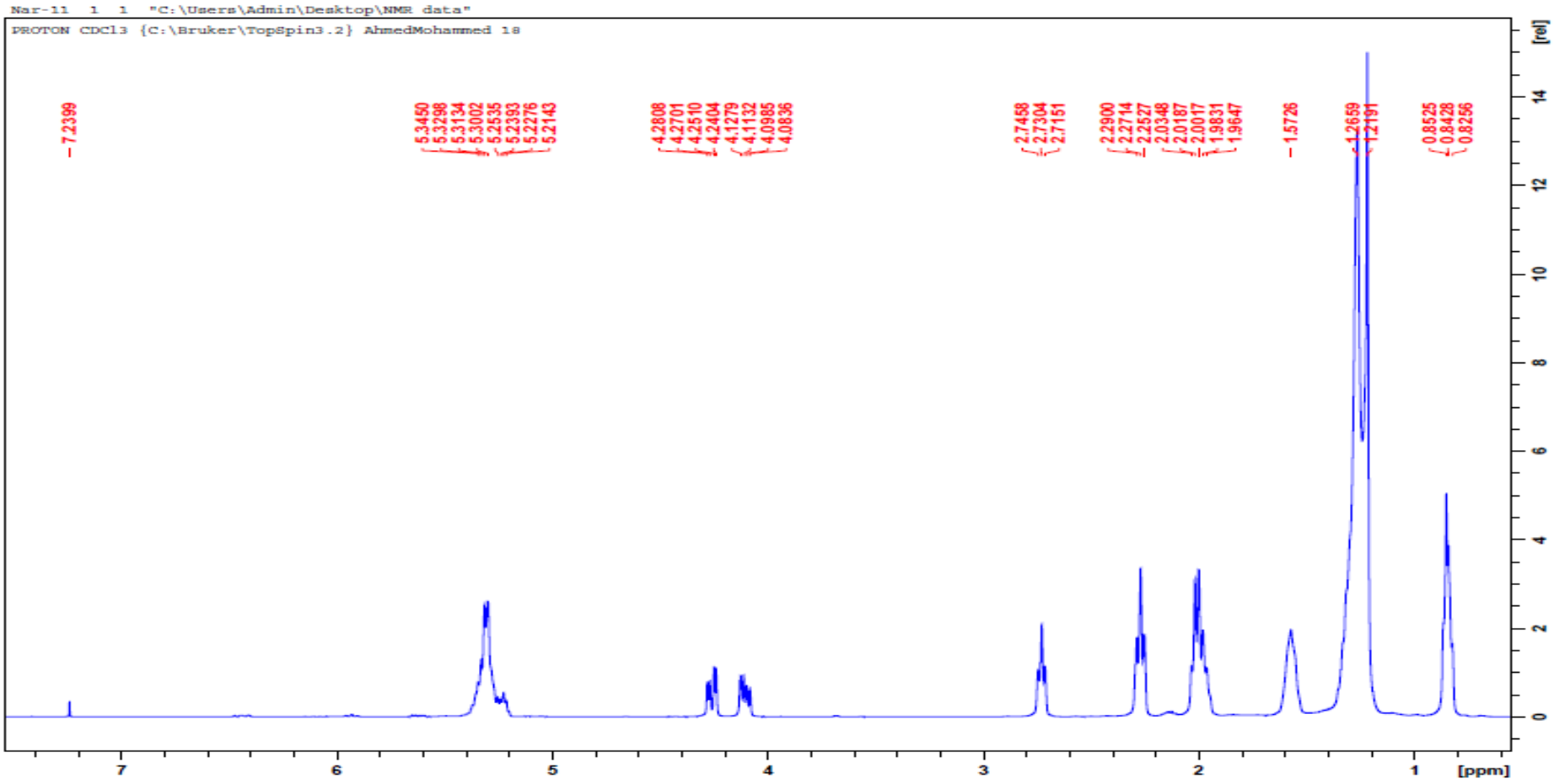


Figure 63: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Soxhlet-extracted !Nara seed oil.

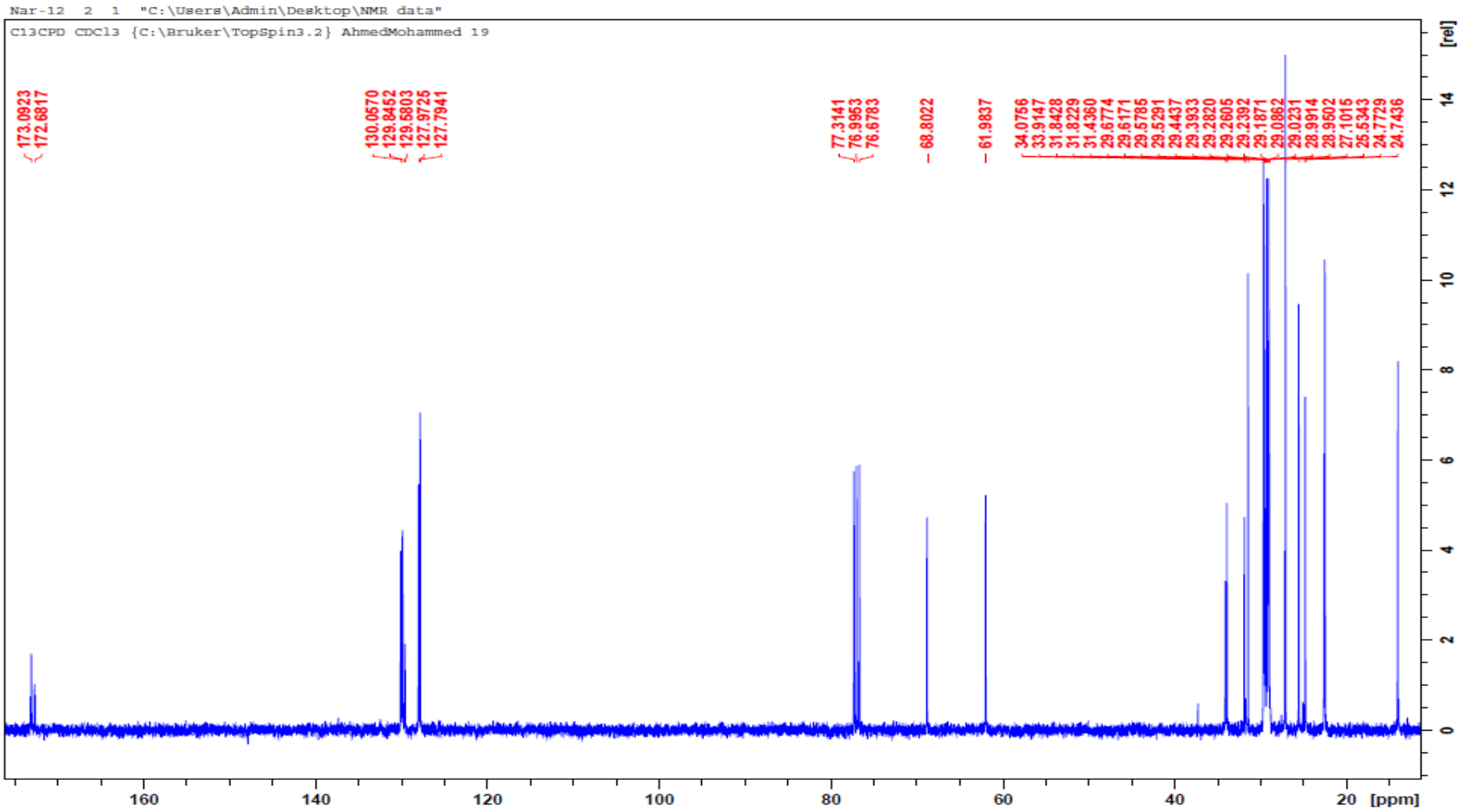


Figure 64: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of cold pressed !Nara seed oil.

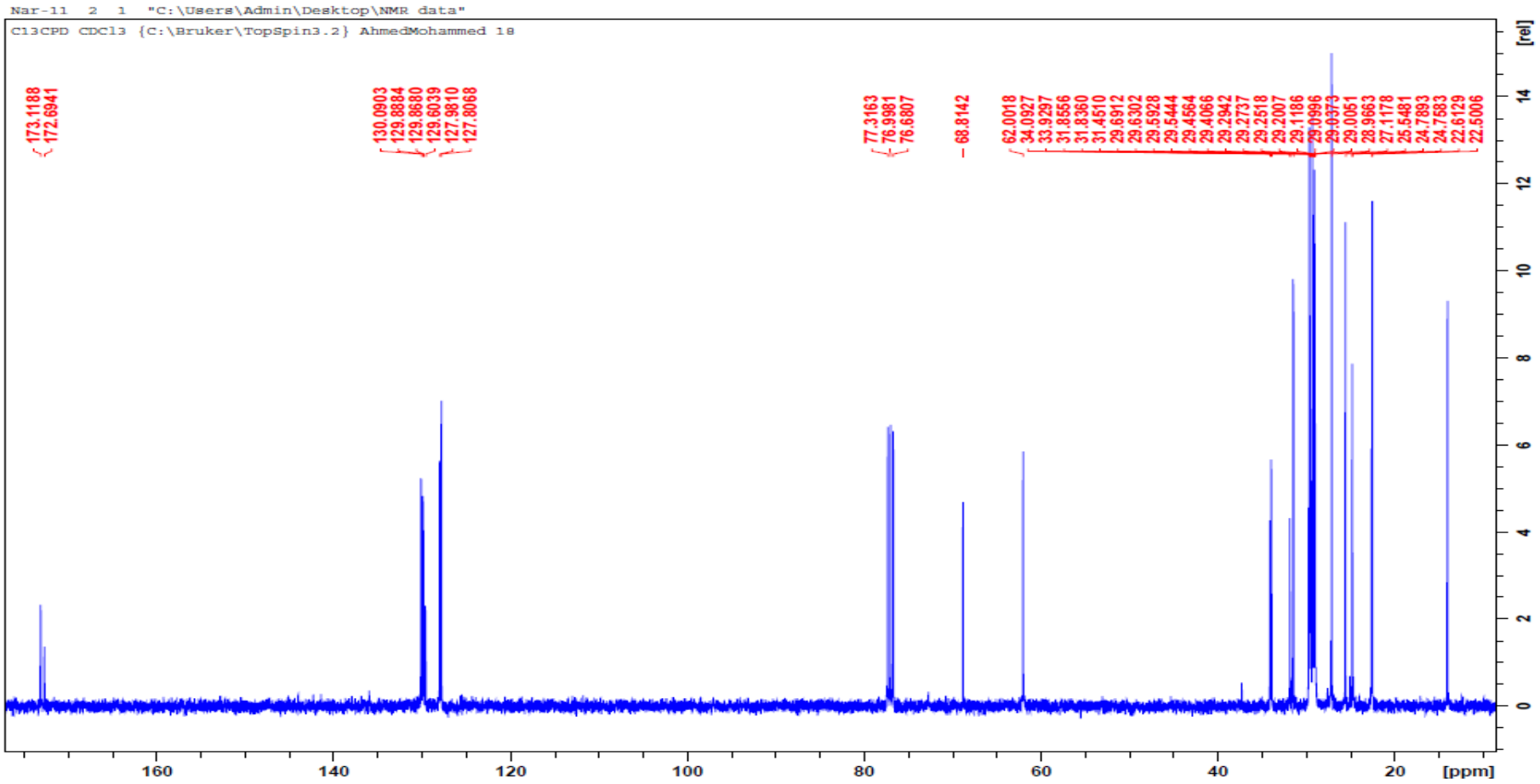


Figure 65: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of Soxhlet-extracted !Nara seed oil.

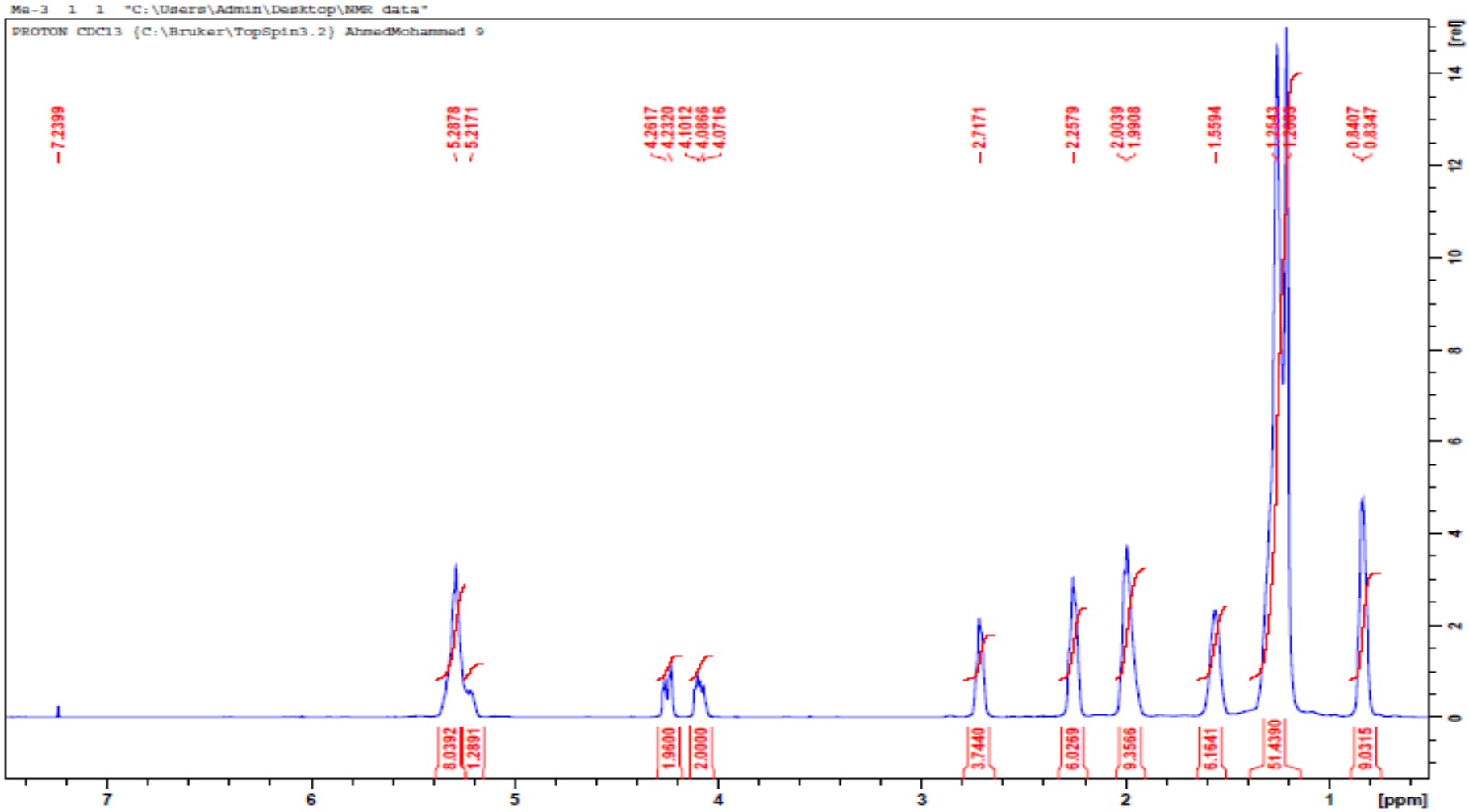


Figure 66: ^{1}H NMR (400 MHz, CDCl3) spectrum of cold pressed Melon seed oil.

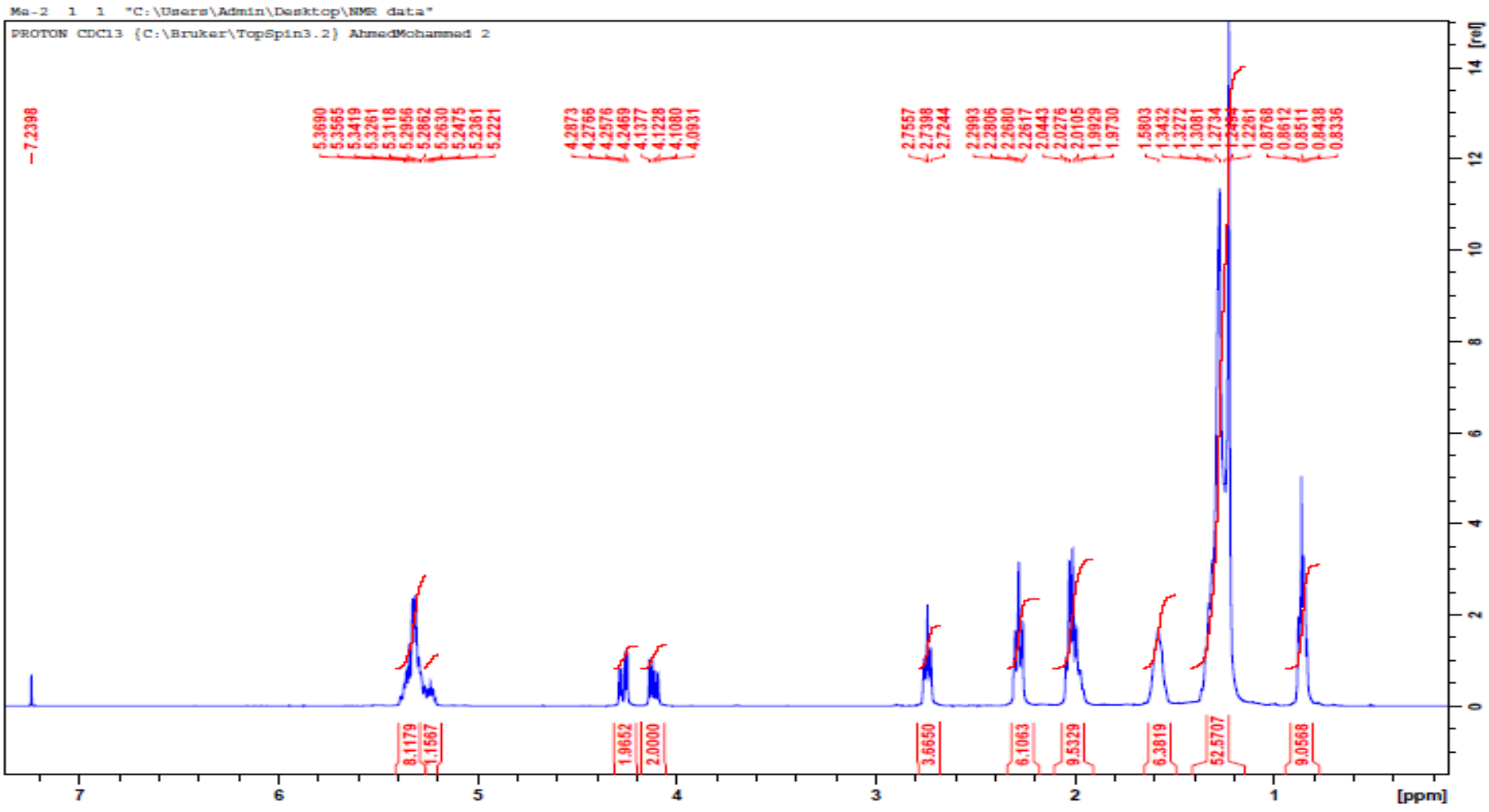


Figure 67: ^{1}H NMR (400 MHz, CDCl3) spectrum of traditionally extracted Melon seed oil.

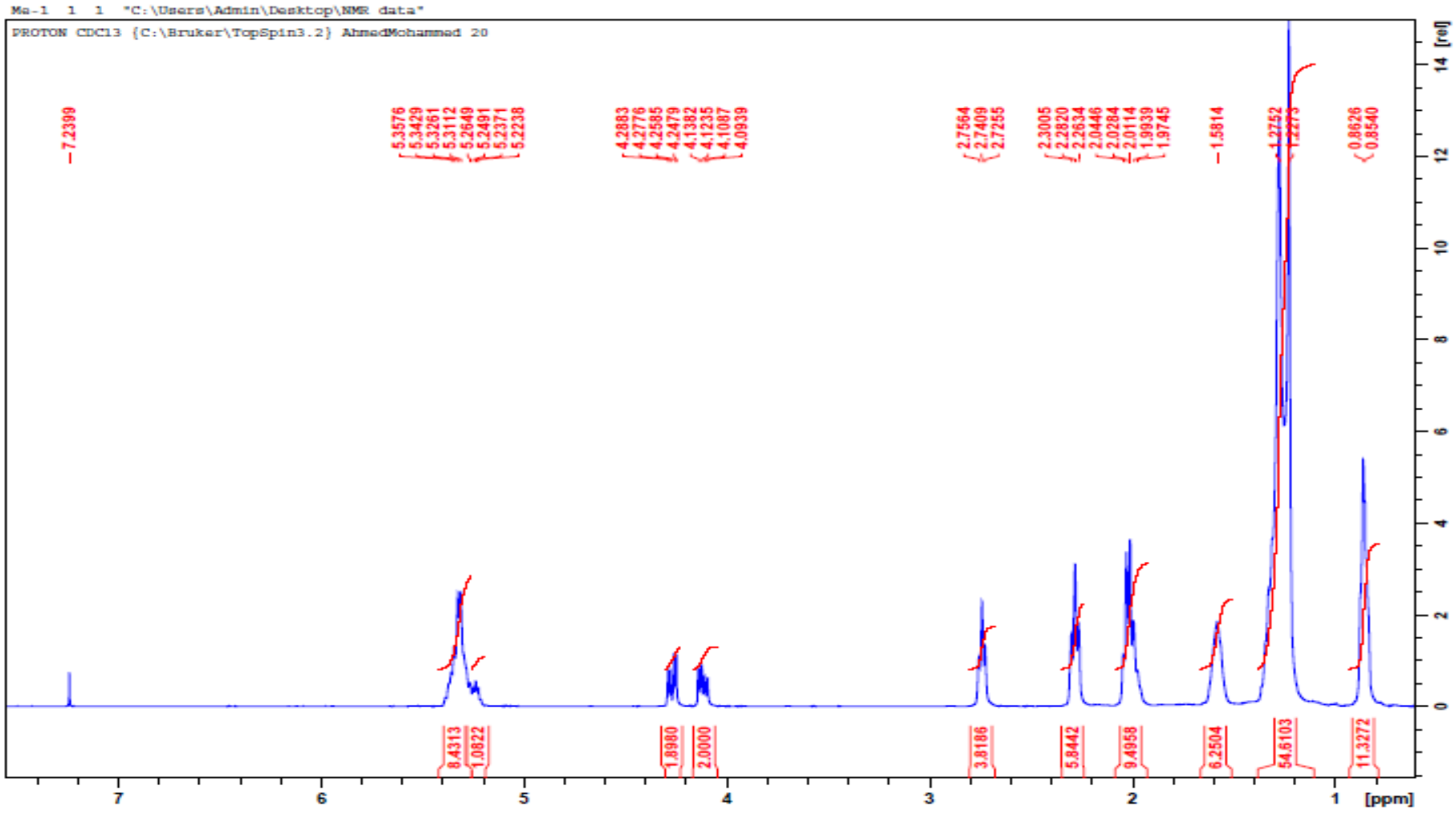


Figure 68: ^{1}H NMR (400 MHz, CDCl3) spectrum of Soxhlet-extracted Melon seed oil.

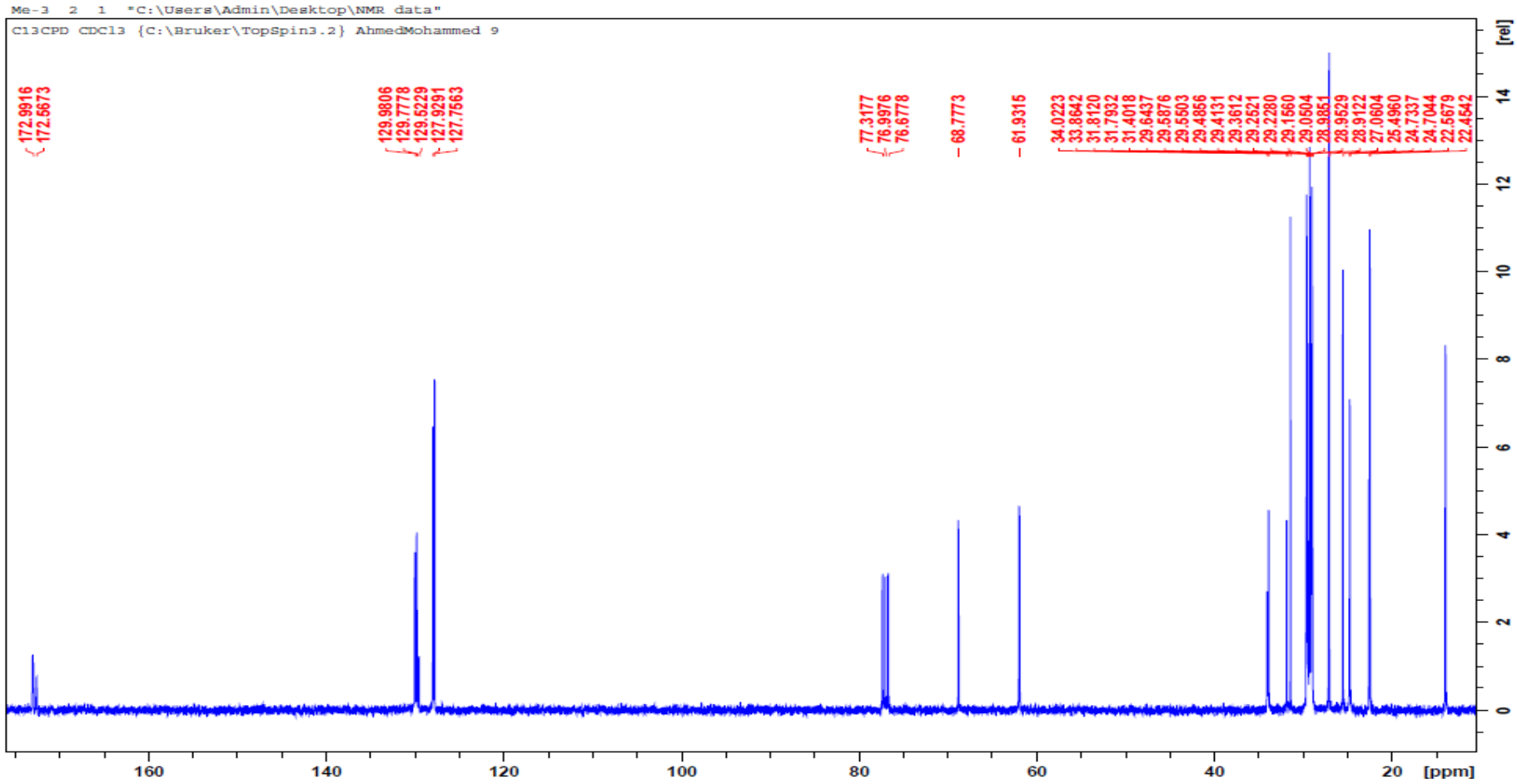


Figure 69: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of cold pressed Melon seed oil.

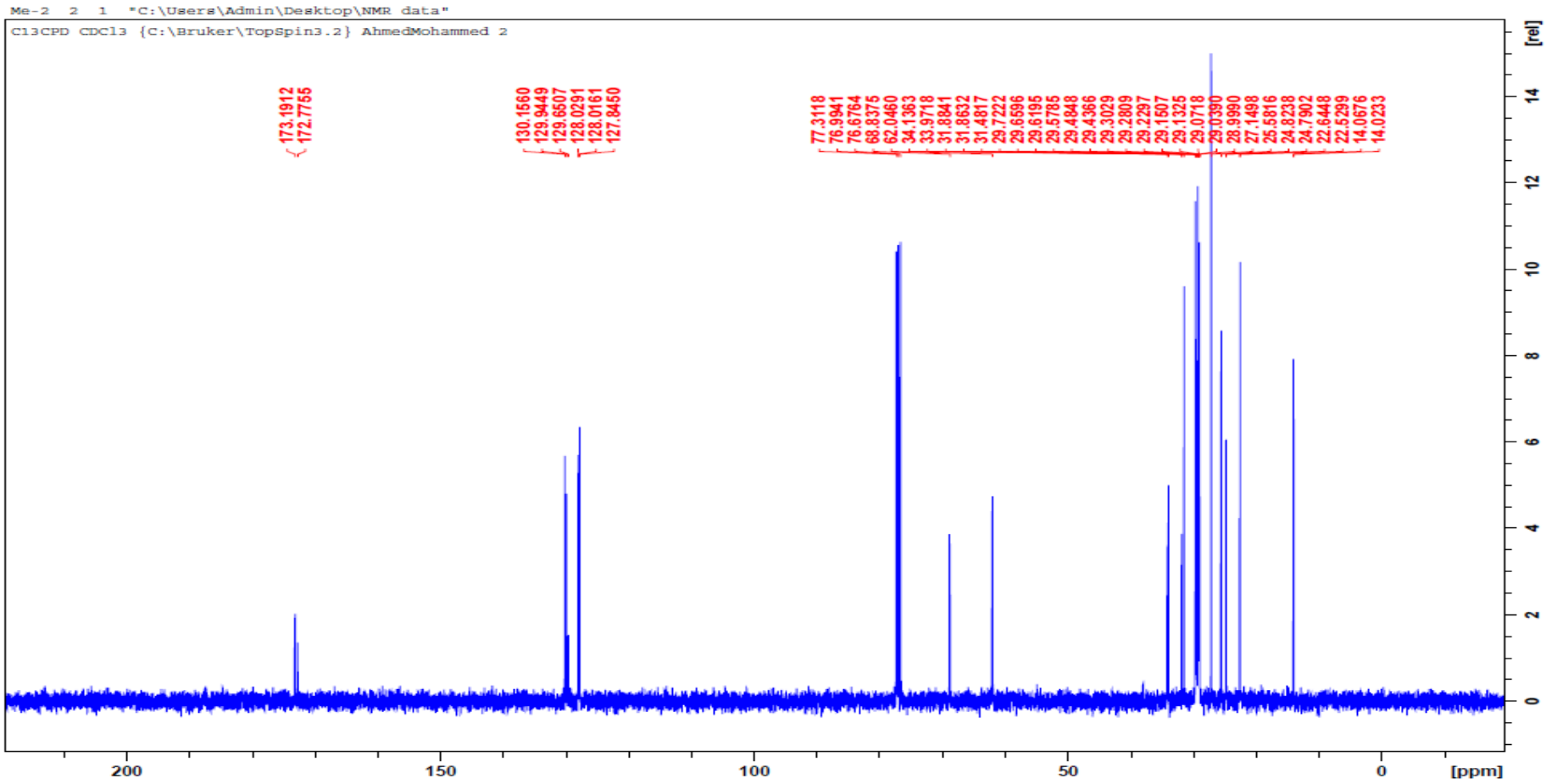


Figure 70: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of traditionally-extracted Melon seed oil.

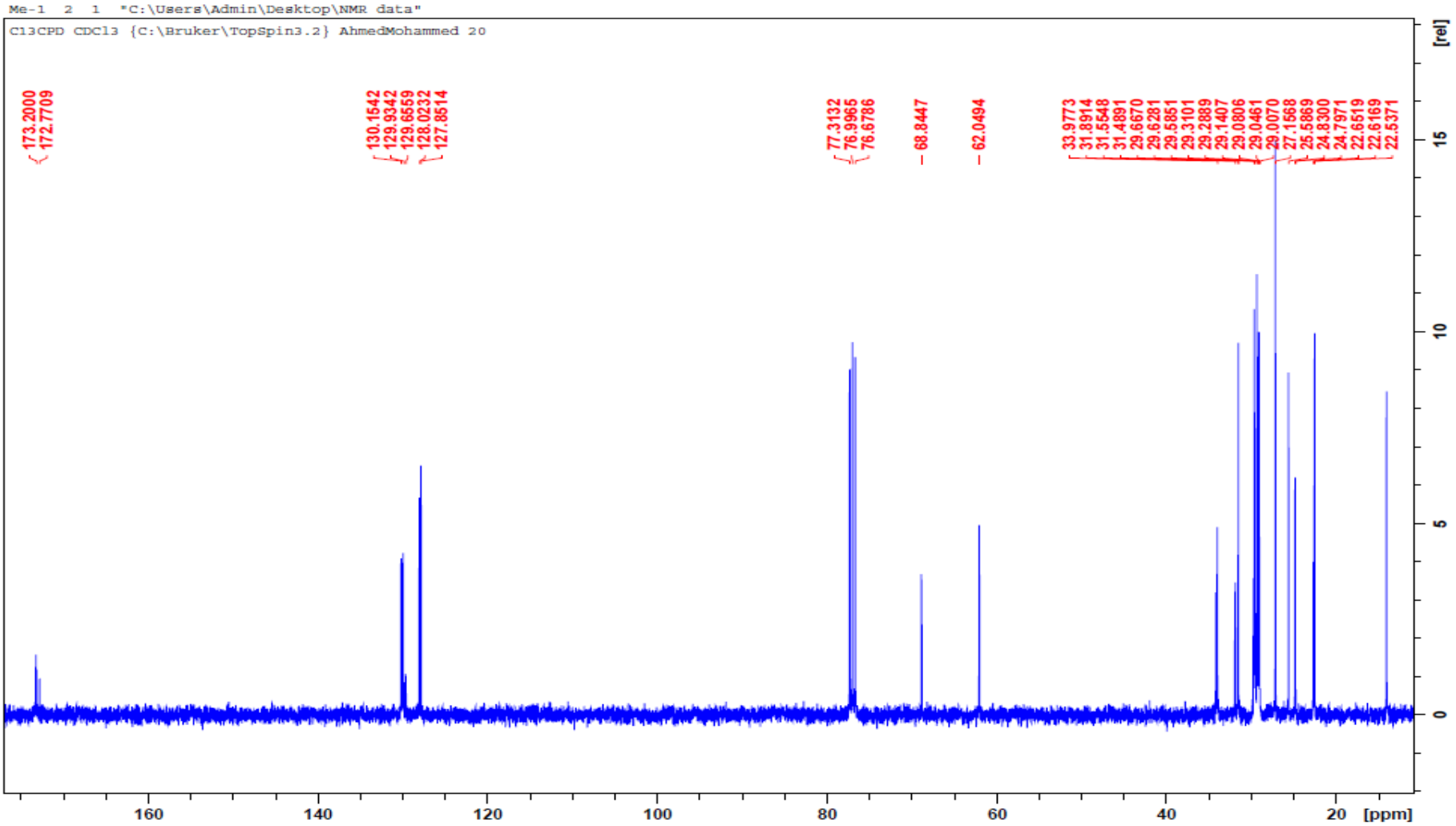
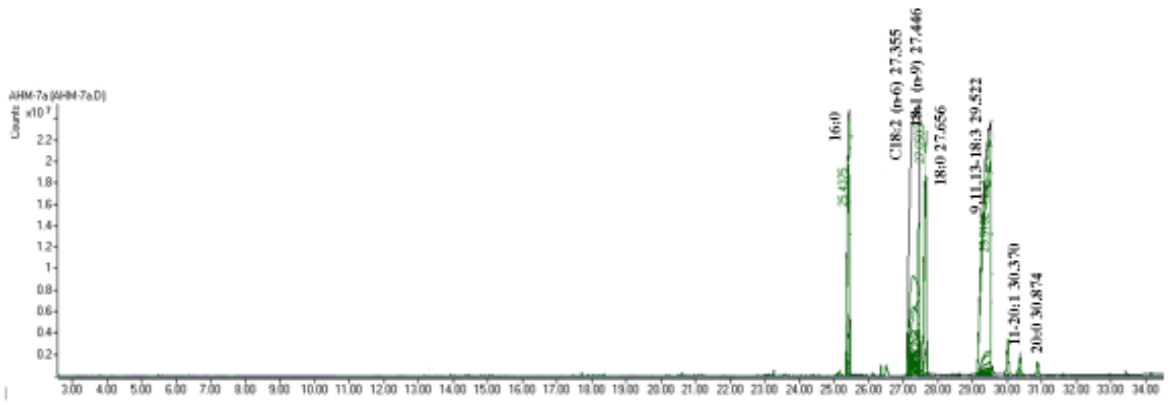
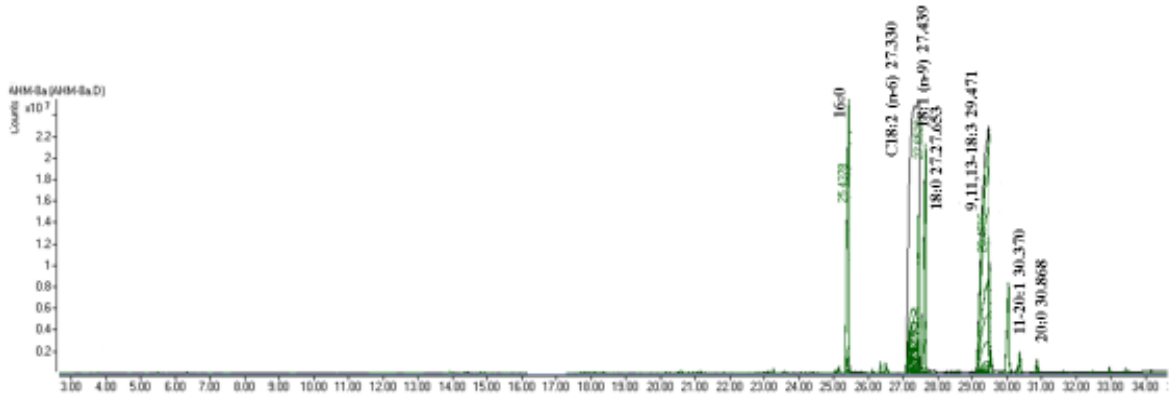


Figure: 71: ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of Soxhlet-extracted Melon seed oil.

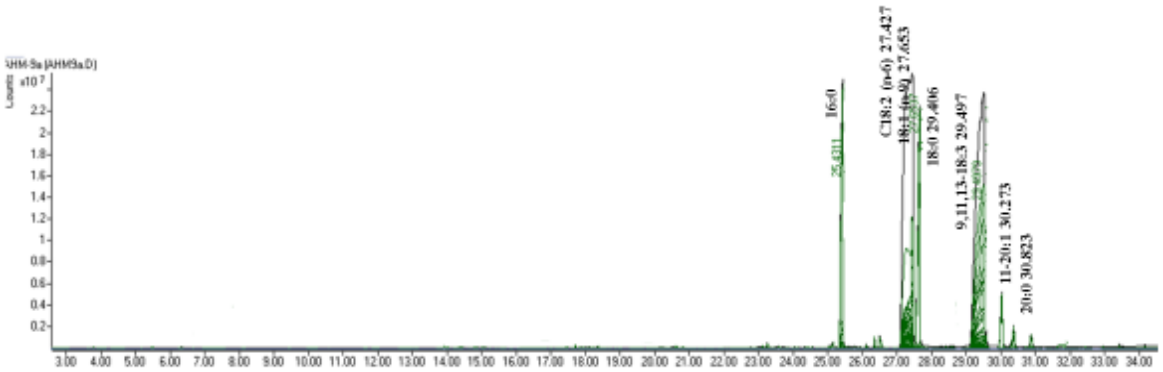
APPENDIX F: Gas chromatograms for fatty acid profile



(a)

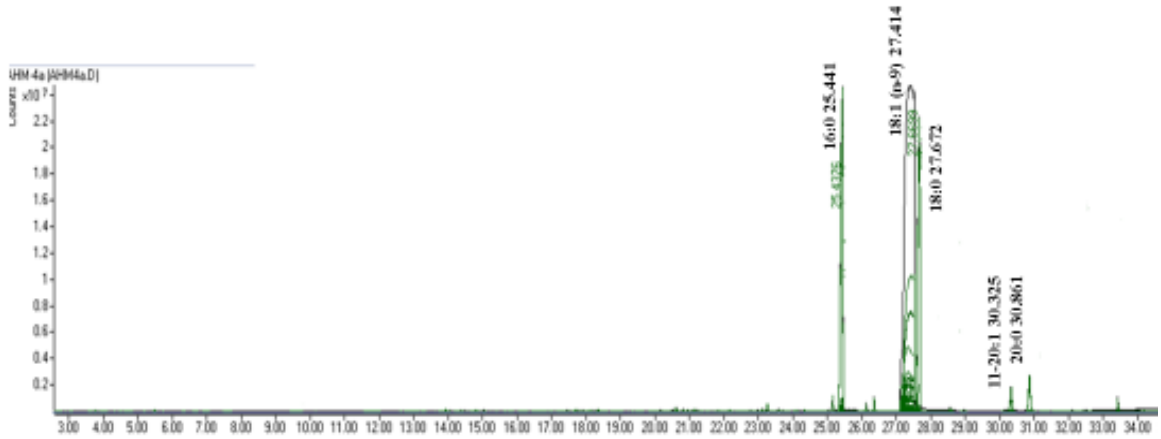


(b)

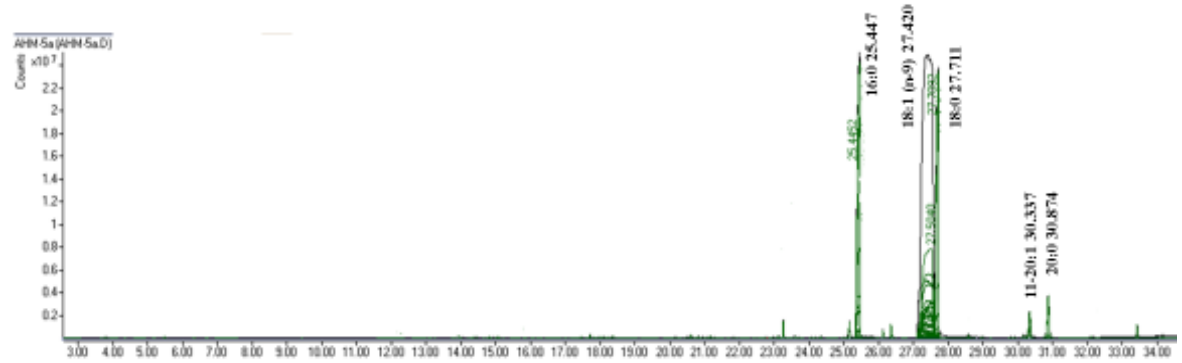


(c)

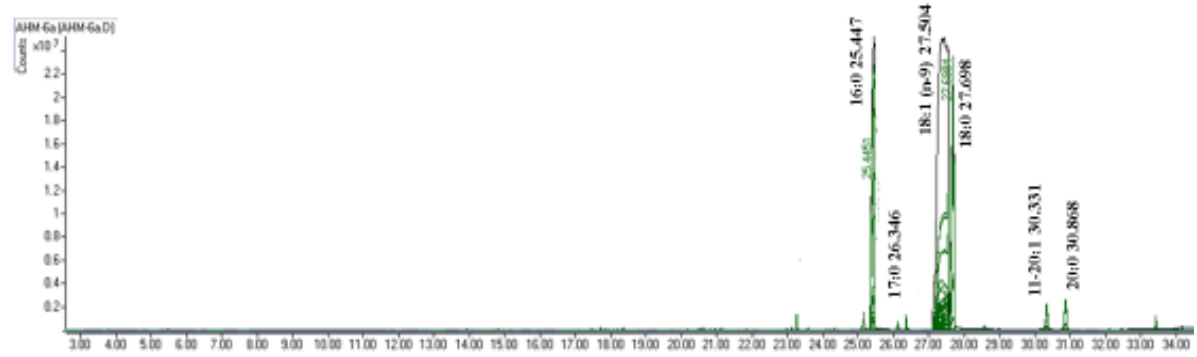
Figure 72 (a-c): Gas chromatograms for Manketti nut oil obtained through Soxhlet extraction (a), traditional extraction (b) and cold pressing (c) method.



(a)



(b)



(c)

Figure 73 (a-c): Gas chromatograms for Marula nut oil obtained through Soxhlet extraction (a), traditional extraction (b) and cold pressing (c) method.

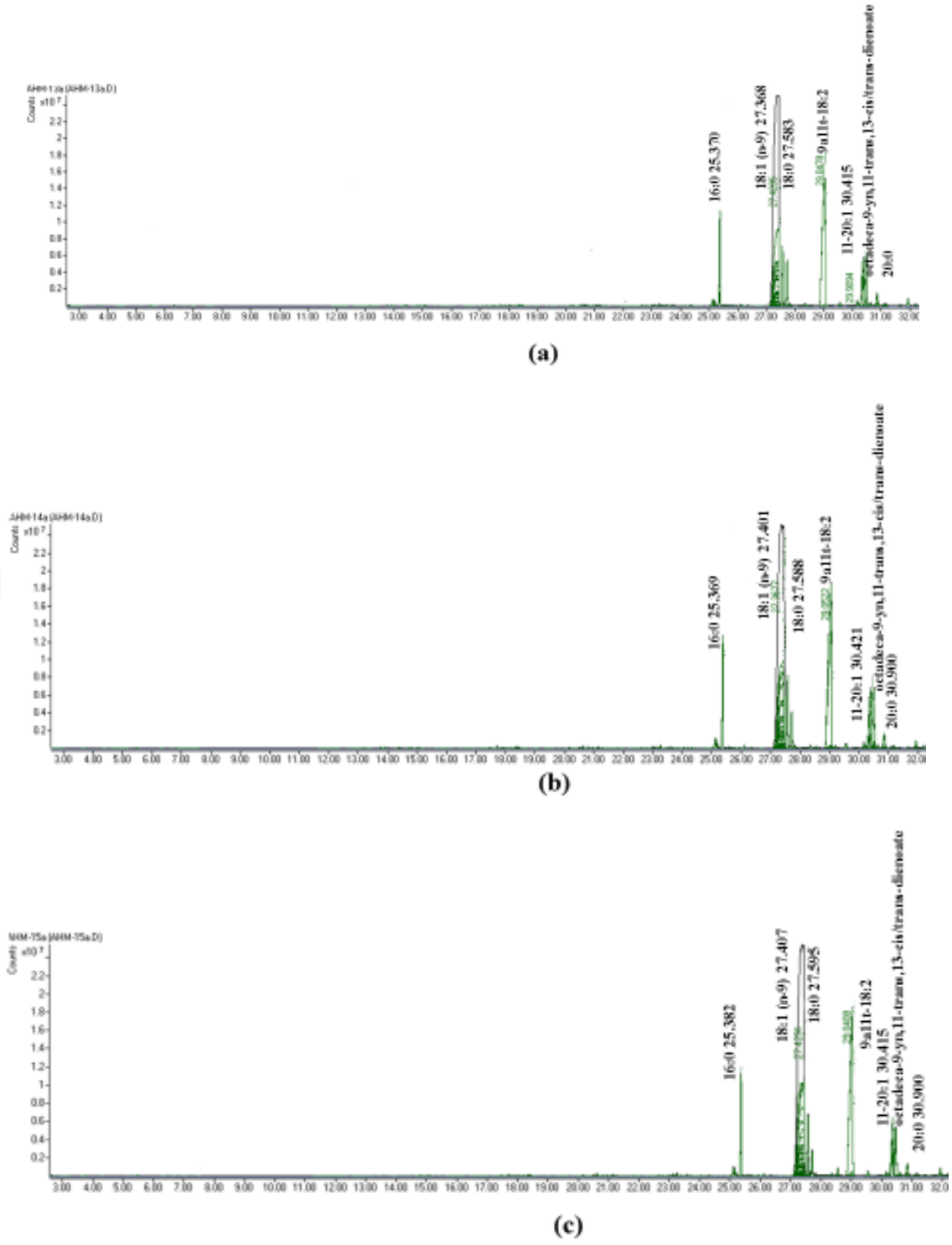
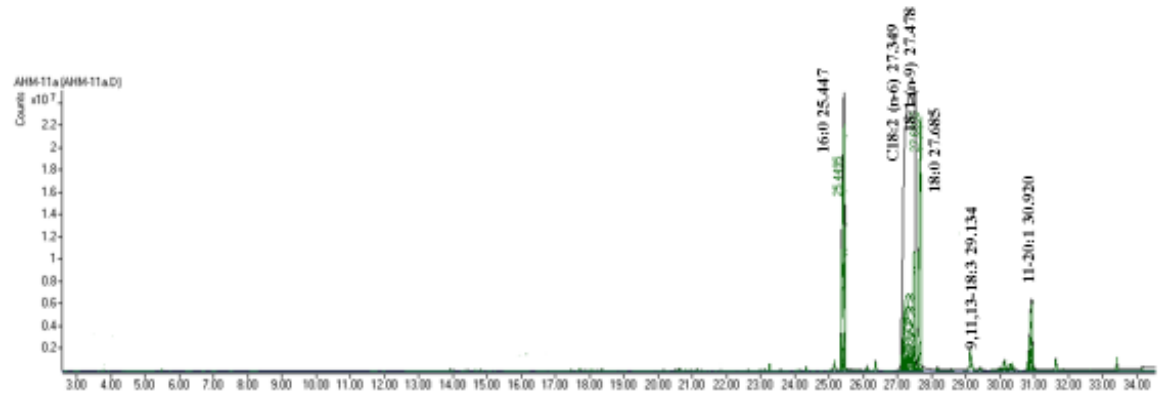
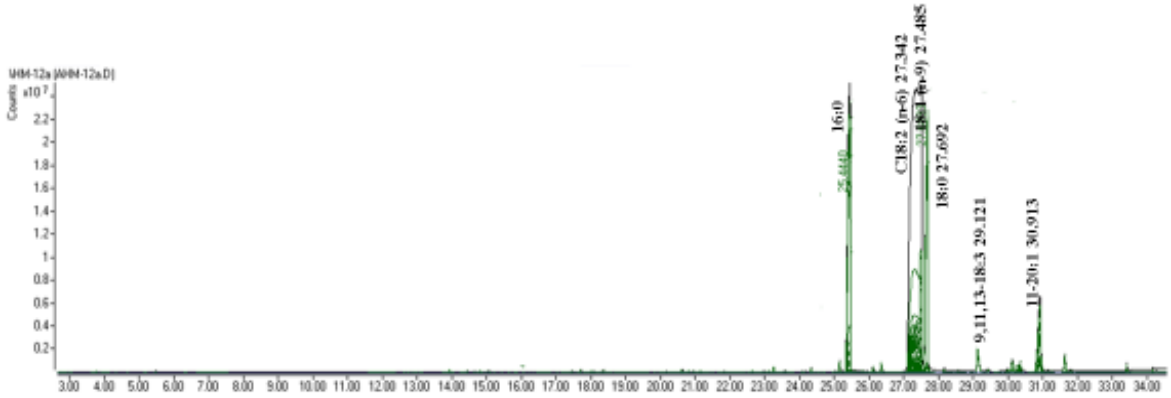


Figure 74 (a-c): Gas chromatograms for *Ximenia* nut oil obtained through Soxhlet extraction (a), traditional extraction (b) and cold pressing (c) method.

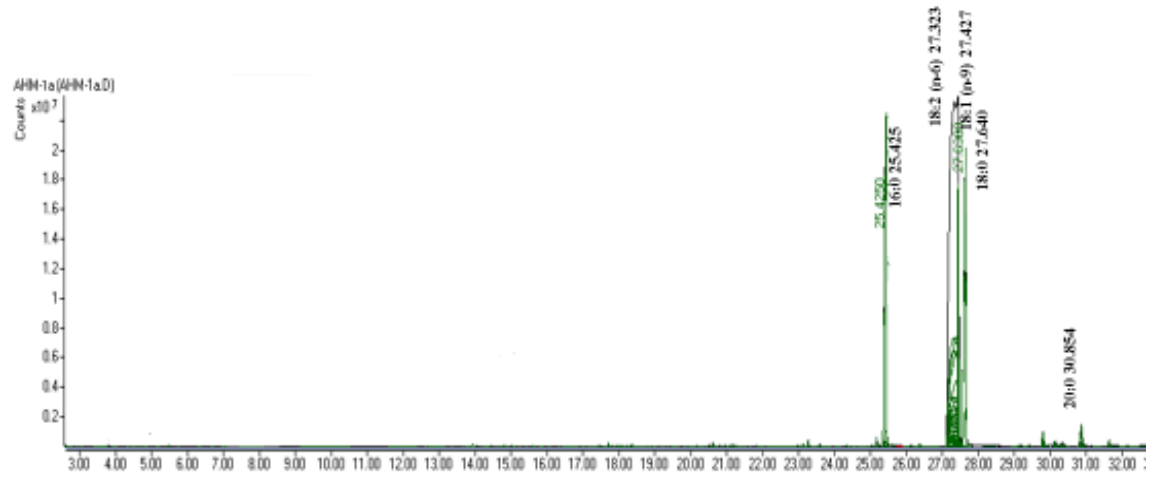


(a)

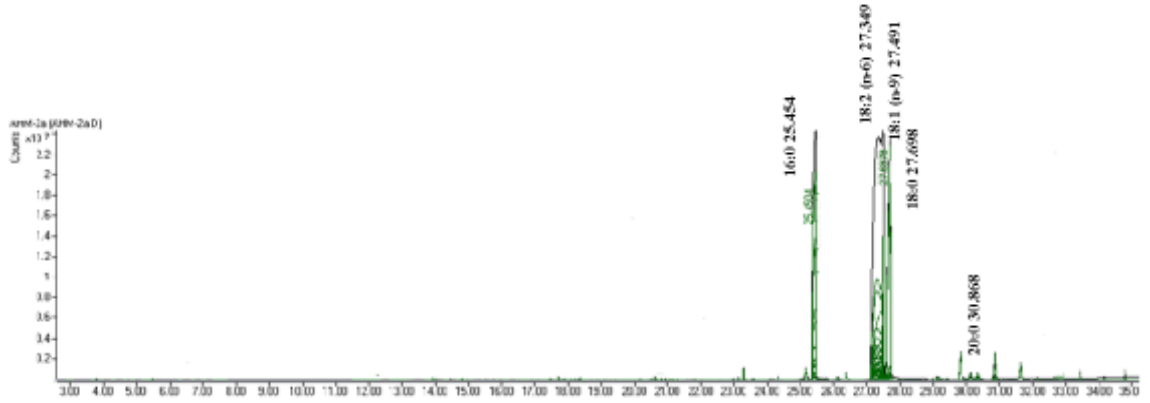


(b)

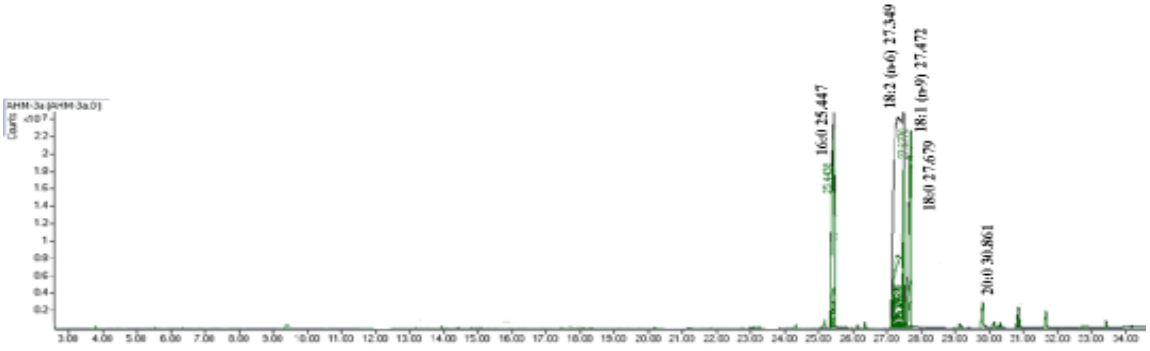
Figure 75 (a-b): Gas chromatograms for !Nara seed oil obtained through Soxhlet extraction (a) and cold pressing (b) method.



(a)



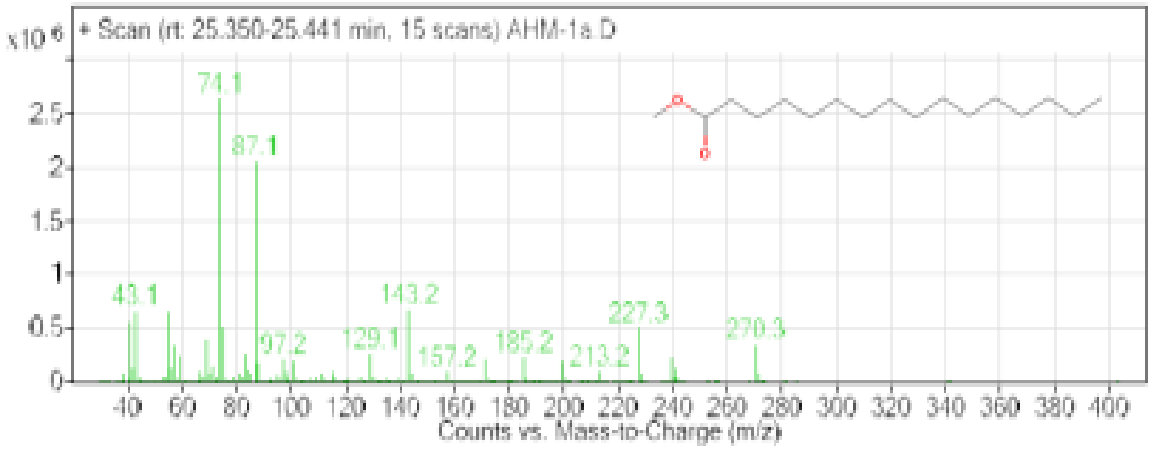
(b)



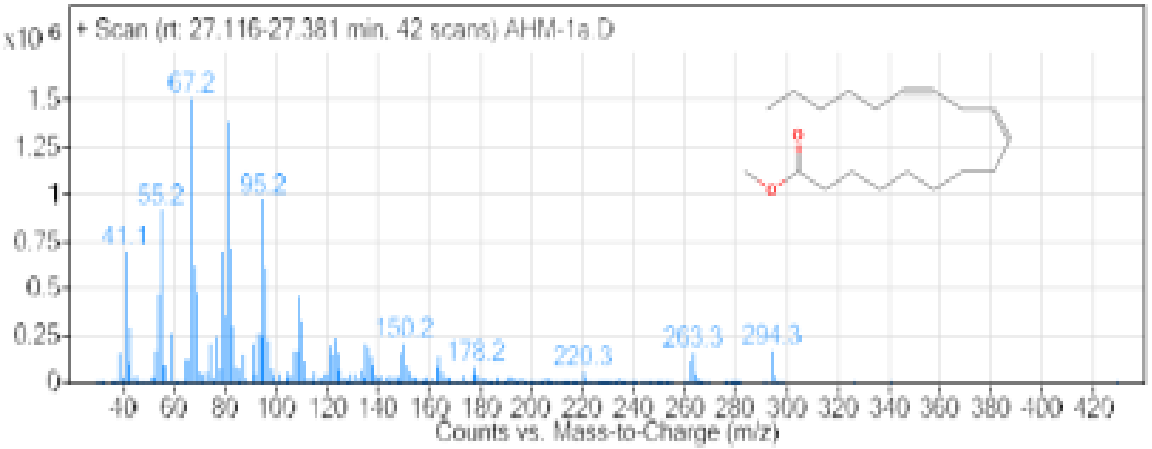
(c)

Figure 76 (a-c): Gas chromatograms for Melon seed oil obtained through Soxhlet extraction (a), traditional extraction (b) and cold pressing (c) method.

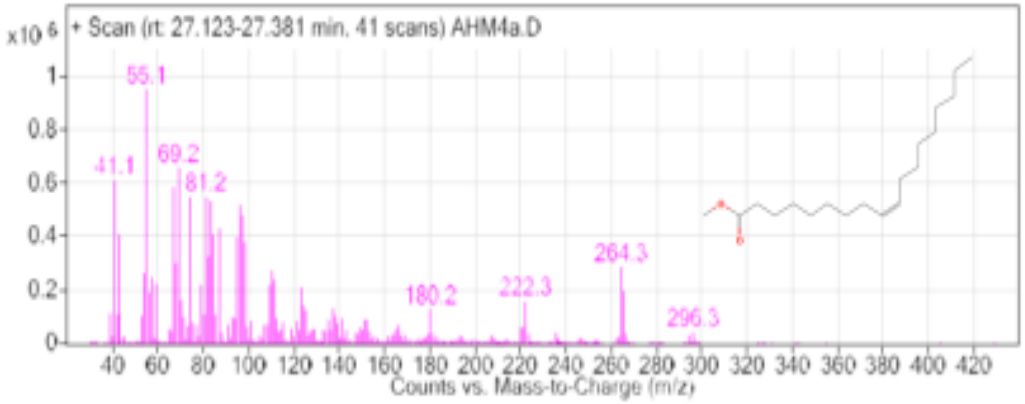
APPENDIX G: Mass Spectra for fatty acid profiling



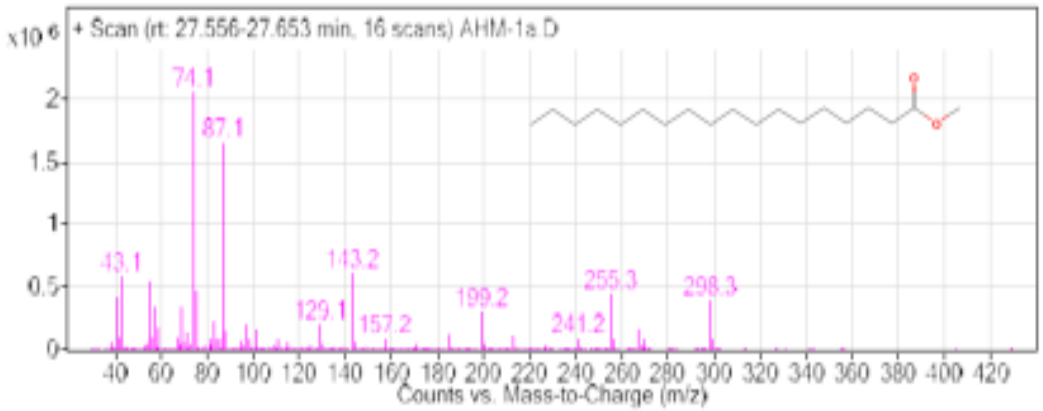
(a)



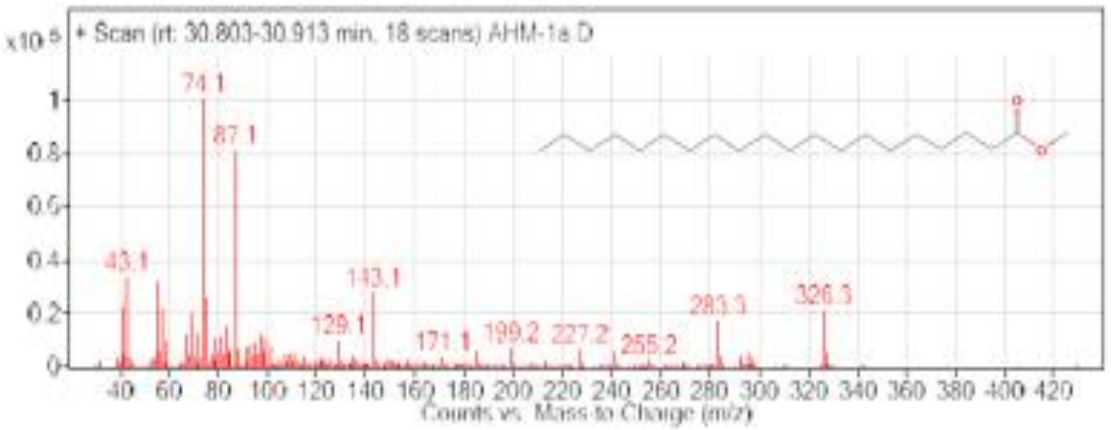
(b)


+ Scan (rt: 27.123-27.381 min, 41 scans) AHM4a.D
x10 6
55.1
69.2
41.1
81.2
180.2
222.3
264.3
296.3
Counts vs. Mass-to-Charge (m/z)

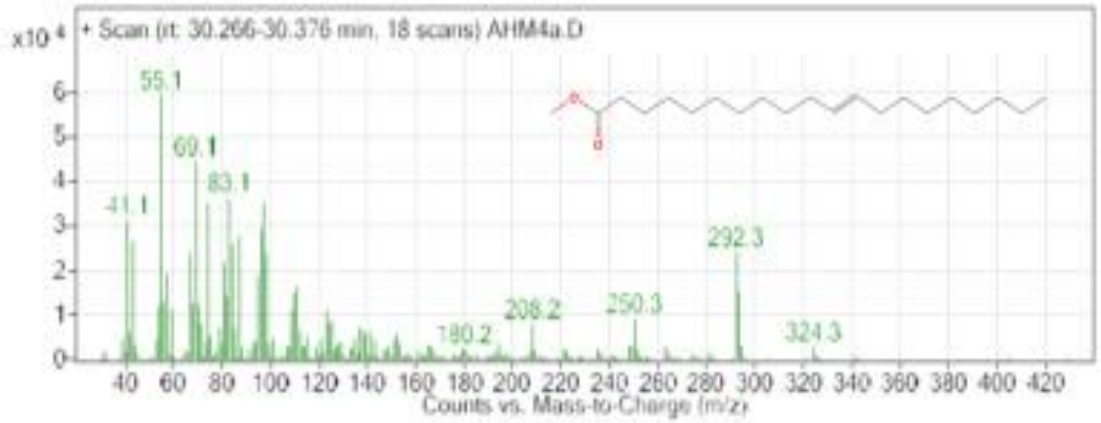
(c)


+ Scan (rt: 27.556-27.653 min, 16 scans) AHM-1a.D
x10 6
74.1
87.1
43.1
143.2
129.1
157.2
199.2
241.2
255.3
298.3
Counts vs. Mass-to-Charge (m/z)

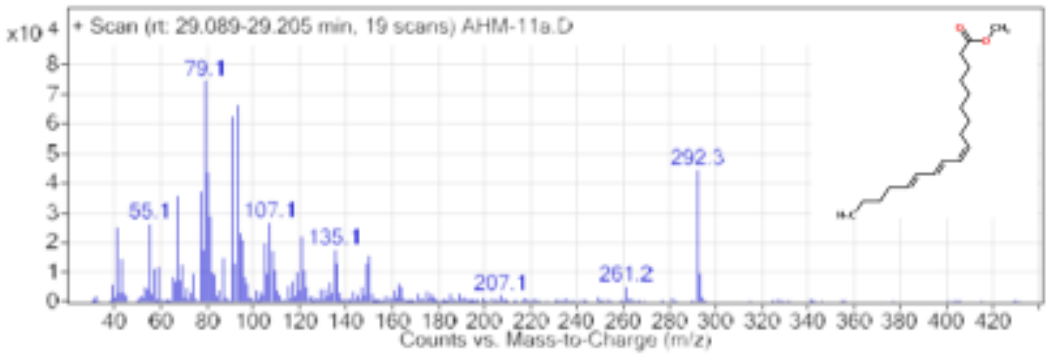
(d)


x10 5
+ Scan (rt: 30.803-30.913 min, 18 scans) AHM-1e.D
74.1
87.1
43.1
143.1
129.1
171.1
199.2
227.2
255.2
283.3
326.3
Counts vs. Mass-to-Charge (m/z)

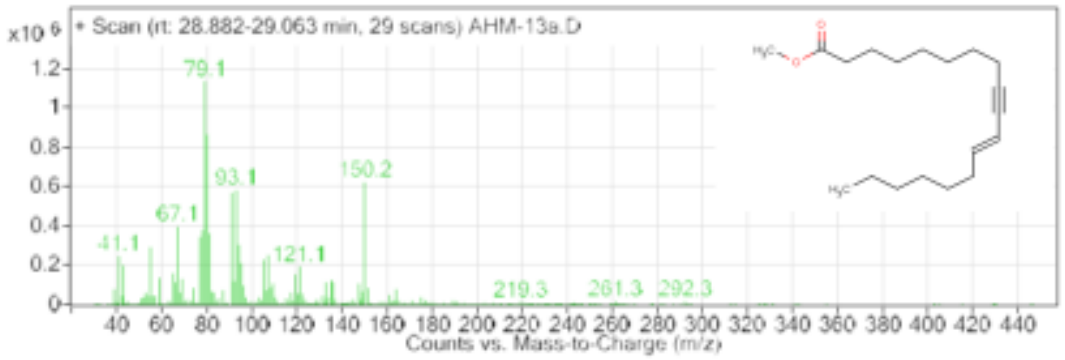
(e)


x10 4
+ Scan (rt: 30.266-30.376 min, 18 scans) AHM4a.D
55.1
69.1
83.1
41.1
292.3
208.2
250.3
180.2
324.3
Counts vs. Mass-to-Charge (m/z)

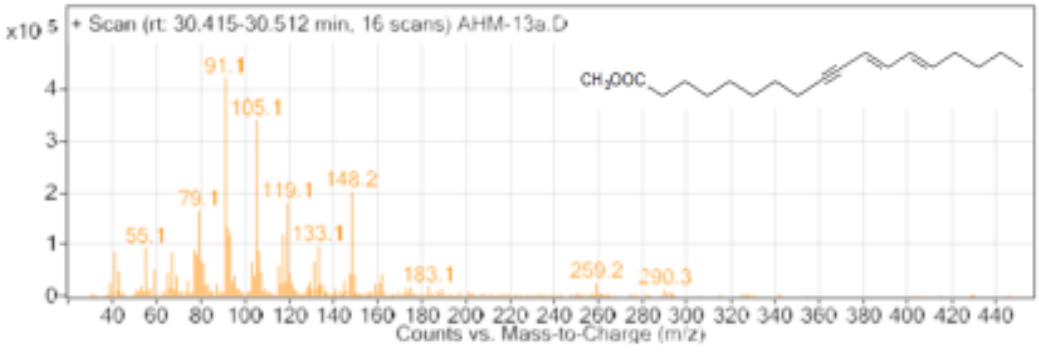
(f)



(g)



(h)



(i)

Figure 77 (a-i): Mass spectra obtained for fatty acid methyl esters during GC-MS analysis (Hexadecanoic acid (a), 9,12-Octadecadienoic acid (b), 9-Octadecenoic acid (c), Octadecanoic acid (d), Eicosanoic acid (e), 11-Eicosenoic acid (f), methyl 9-cis,11-trans,13-trans-octadecatrienoic acid (g), Octadeca-9-yn-11-trans-enoic acid (h), Octadeca-9-yn, 11-trans, 13-cis/trans-dienoic acid (i) (Source for structures on mass spectra: Sigma-Aldrich, 2016).