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AN INVESTIGATION INTO THE CHEMICAL COMPOSITION OF VARIOUS SEED OILS FOR APPLICATION IN COSMETIC PRODUCTS



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1385185

A dissertation submitted to the faculty of science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of master of science.

University of the Witwatersrand

Johannesburg

2021

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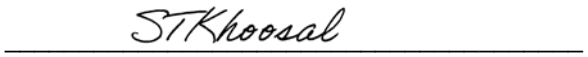
DECLARATION

I hereby declare that this dissertation is a result of my own, unaided work except where acknowledged. It is being submitted in fulfilment of the requirements for the degree Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



Sabrina Tanita Khoosal

____4th_____day of _____June_____2022_____at_____Roodepoort_____

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ABSTRACT

Due to increased consumer awareness, there has been a strong shift in cosmetic product trends towards more natural and/ or greener alternatives in recent years. This is due to several factors such as cases of adverse effects caused by certain synthetic ingredients in products as well as the awareness of synergistic benefits of natural ingredients. Seed oils are one of the solutions being used to satisfy this newly found demand due to their chemical compositions that have cosmetic, nutritional and medicinal benefits (Antignac *et al.*, 2011; Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). Seed oils consist of essential and nonessential fatty acids, antioxidants, and vitamins, amongst other phytochemicals. This makes seed oils useful as emollients, active ingredients or as carriers for other active ingredients (Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). The seed oils focused on in this study are from underutilised plants native to, or highly cultivated in Africa (Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). They are namely *Moringa oleifera (MO), Sclerocarya birrea* (Marula), *Citrullus lanatus* (Kalahari melon), *Schinziophyton rautanenii* (Mongongo), *Adansonia digitate L.* (Baobab), and *Cannabis sativa L.* (Hemp). Other commercially available plant oils included for comparative purposes in the study include avocado oil, olive oil, jojoba seed oil, grapeseed oil and sunflower seed oil. Increasing the commercialisation of these underutilised oils will benefit rural communities involved in harvesting, encourage maintenance of the natural environment, provide more export products and alternative natural ingredients for the cosmetic industry, which will overall benefit our bioeconomy (Zimba *et al.*, 2005; Juliani *et al.*, 2007; Vermaak *et al.*, 2011). Research is therefore needed to contribute to the database of information on these oils to further understand their potential in the natural-based cosmetic sector.

To evaluate the economic importance and potential of these seed oils for cosmetic applications, in-depth profiling of the oils were conducted (Cheikhyoussef, 2018). The oil profiles include physico-chemical properties; namely acid value (AV), saponification value (SV), average molecular weight (AMW), ester value (EV), peroxide value (PV), iodine value (IV), specific gravity and the refractive index. Further characterisation of the oils involved Fourier Transform-Infrared Spectroscopy (FT-IR) analysis, antioxidizing abilities using Ultraviolet-visible spectroscopy (UV-

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Vis), chemical composition using Nuclear Magnetic Resonance (NMR) Spectroscopy, thermal behaviour using Differential scanning Calorimetry (DSC) as well the fatty acid methyl ester (FAME) profile using Gas Chromatography-Mass Spectrometry (GC-MS) as well as studying the effect of sunlight exposure on the various oils.

AV is a reflection of the total acidity of the oil sample; therefore a lower value indicates less degradation and therefore higher quality of the oil and vice versa, the lowest AV amongst the oils of interest was obtained by Baobab oil (0.40 ± 0.01 mg KOH/g oil). Peroxide value (PV) is a chemical characteristic used to determine the extent of oxidative deterioration of the oil at the time of analysis, the lowest PV from the oils of interest belonged to Baobab oil. AV and PV both confirm the stability and high quality of Baobab making it very important to the cosmetic industry as products often have shelf-lives of 12 months. SV is a measure of the chain length of fatty acids present in the oil and is used to calculate an average molecular weight (AMW). The specific SV range optimal for soap making is 188 to 199 mg KOH/ 1g oil (Francis and Tahir, 2016). Therefore from the oils studied, Plush Organics Moringa and Hemp seed oil having SV of 189.23 ± 0.95 mg KOH /1g oil and 195,05 ± 0.56 mg KOH /1g oil respectively, would be most suitable for application in soap making.

IV is related to the degree of unsaturation such that the higher the IV the more unsaturated the oil. The IV obtained for the extracted MO oils (65.93-74.99 g/100g) was lower than that obtained for Baobab seed oil (91.30 g/100g) and Kalahari melon seed oil (131.90 g/100g). Relative IV from ^{1}H NMR data for Lefakong farming cold-pressed MO oil and Soxhlet extracted MO oil (68.54-69.49 g/100g) was lower than that obtained for Baobab seed oil (76.44 g/100g) and Kalahari melon seed oil (126.36 g/100g). From relative fatty acid percentages, the TUFA% results for the extracted MO oils (33.93-64.33%) is lower than the TUFA% obtained for Baobab seed oil (65.61%) and Kalahari melon seed oil (72.24%). Multiple parameters therefore confirm the result that Kalahari melon seed oil was the most unsaturated followed by Baobab seed oil and MO oil was the least unsaturated from this sample group.

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From the effect of sunlight exposure study there was a clear general trend amongst the oils as they deteriorated with time. Many of the selected oils lost their original colour and became pale to translucent; many also lost their original earthy and nutty smells and formed acidic and rancid odours which are clear signs of deterioration. Studies conducted on the thermal properties of selected oils using DSC showed multiple endothermic and exothermic peaks, indicating that there are multiple transition temperatures experienced by each oil due to their unique triacylglycerol profiles. There were no major differences between the Soxhlet extracted MO and cold-pressed MO, indicating that the extraction method used does not affect the thermal properties of oils. NMR results gave insight to the chemical composition of the oils and their degree of saturation which was confirmed with GC-MS fatty acid analysis. Fatty acid analysis indicated that Olive oil had the highest relative percentage of Oleic acid making it highly stable and able to withstand high temperatures hence its application in cooking. The highest relative percentage of Palmitic acid belonged to Grapeseed oil and the highest relative percentage of Linoleic acid belonged to Kalahari melon seed oil. This would indicate that Grapeseed oil and Kalahari melon seed oil are very valuable to skin formulations focussed on protecting the skin barrier from dying and damage. Radical scavenging activity of the oils indicate antioxidant content of the oil and therefore log term shelf-life of the oil. Results indicated that Kalahari melon seed oil and Mongongo had the highest antioxidant content which would make them ideal additives to antioxidant type cosmetic products. Trial application of oil enhanced cosmetic bases show a clear improvement in performance of products and an overall preferred richness in texture and feel.

Overall, this study gave an in-depth insight into the chemistry behind the behaviour and properties of these various oils. This gives a deeper understanding of their potential application in cosmetic formulations.

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PRESENTATION

Participation in the National Young Chemist's Symposium 2021 – **MSc Flash Talk Presentation**

Title: An investigation into the chemical composition of various seed oils for application in cosmetic products

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In loving memory of my mother

Meena Khoosal

1968-2015

All that I am, or hope to be, I owe to my angel mother

-Abraham Lincoln

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ABBREVIATIONS

ANOVA	Analysis of Variance
AOCS	American Oil Chemists' Society
AMW	Average molecular weight
AV	Acid value
13C NMR	Carbon 13 Nuclear Magnetic Resonance
DPPH	1,1-diphenyl-2-picrylhydrazil
DI	Deionised
EV	Ester value
FA	Fatty acid
FAMEs	Fatty acid methyl esters
FFA	Free fatty acid
1H NMR	Proton Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectrometry
IV	Iodine value
MO	*Moringa Oleifera*
MUFA	Monounsaturated fatty acid
nd	not detected

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PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R2	Coeffcient of determination
RI	Refractive index
SAFA	Saturated fatty acid
SG	Specific gravity
SV	Saponification value
TAG	Triacylglycerides
TFA	Trans Fatty Acids
TUFA	Total Unsaturated Fatty Acids

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

Seed oils were historically used for cosmetic purposes, however, during the last century, convenient synthetic or petroleum-based substitutes, such as mineral oil and silicones, have replaced them (Vermaak *et al.*, 2011). Synthetic based cosmetics have the advantage of lower cost and a longer shelf-life but have the disadvantages of at times causing adverse side effects, allergic reactions, and producing waste that is harmful to the environment. Whereas natural-based cosmetics have the advantage of being more environmentally friendly and causing fewer side effects, but have the disadvantage of costing more and having a shorter shelf-life (Chen, 2009). Due to the potential adverse effects of the synthetic materials, there is currently a growing trend to replace them with natural oils, butters and waxes in the cosmetic and pharmaceutical industries (Shackleton *et al.*, 2006; Vermaak *et al.*, 2011; Hetta, 2016).

In modern years there has been an increase in consumers awareness towards ingredients used and the validity of performance claims made on cosmetic products. This has led to a higher global demand for more high-quality natural-based cosmetic products (Vermaak *et al.*, 2011; Hetta, 2016). Seed oils are one of the solutions being used to satisfy this problem due to their chemical composition that have cosmetic, nutritional and medicinal benefits (Antignac *et al.*, 2011; Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). The seed oils are used as emollients, active ingredients or as carriers for other active ingredients (Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). The seed oils focused on in this study include Moringa, Marula, Kalahari melon, Mongongo, Baobab, and Hemp seed oil. These oils are native to, or highly cultivated in Africa, which has rich biodiversity (Vermaak *et al.*, 2011; Dangarembizi *et al.*, 2015). These plants are used by locals for food, medicine, cosmetics, fuel etc. (Zimba *et*

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al. 2005; Vermaak *et al.*, 2011). Increasing commercialisation of these oils will benefit rural communities involved in harvesting, encourage maintenance of the natural environment, provide more export products and alternative natural ingredients for the cosmetic industry which will overall benefit our bioeconomy (Zimba *et al.* 2005; Juliani *et al.*, 2007; Vermaak *et al.*, 2011). These non-traditional seed oil sources, if managed correctly, can satisfy the ever-growing demand for this important renewable commodity by supplementing the worlds current vegetable oil supplies (Mitei *et al.*, 2008; Janaki, 2015).

Moringa oleifera (MO) is currently one of the most attractive plants under study in many disciplines due to its versatility and high nutritional value (Mehta *et al.*, 2011). In a study by Özcan and co-workers, it was concluded that variations in MO oils nutritional value are likely due to differences in plant species, locations, climatic factors, harvesting times, cultivation conditions and extraction processing. Thus, to determine the best extraction method of MO oil for cosmetic purposes, a comparative study had to be conducted on the same MO source using different extraction methods. Cold-press extraction is one of the most attractive green extraction methods compared to traditional Soxhlet extraction. Previous extraction of MO oil have shown that in general, fatty acids, tocopherol and sterol contents recovered using cold-press extraction were higher in comparison to those obtained using Soxhlet extraction but resulted in lower yields (Özcan *et al.*, 2019).

To assess the economic importance and potential of these seed oils for cosmetic applications, an in-depth profiling of the oils needed be conducted (Cheikhyoussef, 2018). The profile of oils mainly includes physico-chemical properties; namely acid value (AV), ester value (EV), saponification value (SV), average molecular weight (AMW), iodine value (IV), peroxide value (PV), density, specific gravity and the refractive index. The triglycerol analysis using nuclear magnetic resonance spectroscopy

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(NMR), antioxidizing abilities using ultraviolet-visible spectroscopy (UV-Vis) as well as the tocopherol, major sterol content and most importantly the fatty acid profile using gas chromatography-mass spectrometry (GC-MS).

The increase in demand for natural-based cosmetics is currently not being met by industry which results in high prices for the limited range available. An increase in the availability of natural-based cosmetics will result in lower prices allowing more people to access this alternative. Further research needs to be done on the potential of renewable seed oils to attract industries to grow the natural-based cosmetic sector in Africa and benefit our bioeconomy (Górnaś and Rudzińska, 2016). Although researchers have already published information on numerous oils covered in this study, it is often difficult to compare data from various sources due to the high variability between techniques used for analysis (Tan and Che Man, 2000). Hence the need for this broad study that looked at MO oil extracted by four different methods, as well as profiling these oils alongside underutilised African oils in comparison to common vegetable oils such as olive and sunflower oil.

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CHAPTER 2: LITERATURE REVIEW

2.1 Natural oils in cosmetics

Natural oils are high in fatty acids, which are carboxylic acids with a long aliphatic chain. When three fatty acids are combined with glycerol they form triglycerides which are a component of fat (Leite and Maia Campos, 2018). Fats with a high unsaturated fatty acid content are usually in liquid form whereas those with a high percentage of saturated fatty acids are predominantly in solid form at room temperature. Seed oils are largely made up of unsaturated fatty acids but may contain some saturated fatty acids (SAFAs) such as palmitic, myristic and stearic acid. Unsaturated fats are categorised as monounsaturated fats, made up of omega 9 fatty acids or monounsaturated fatty acids (MUFAs), and polyunsaturated fats, which are made up of either omega 6 fatty acids or omega 3 fatty acids also known as n6 polyunsaturated fatty acids (PUFAs) or n3 PUFA respectively. The most important fatty acids in cosmetic formulations include palmitic (SAFA), oleic (MUFA) and linoleic (PUFA) acid (Lautenschläger, 2003). Palmitic acid is an important component of the natural skin barrier, it is used as a skin permeation enhancer for co-administered molecules (Vermaak *et al.*, 2011; Hetta, 2016). Oleic acid enhances antioxidative ability and is effective as an absorption enhancer. Linoleic acid is not produced in the human body naturally and is, therefore, the most frequently used in cosmetic formulations. A deficiency of this fatty acid leads to water loss from the skin's epidermis resulting in dryness and scaling, it may also cause hair loss and cracking of nails (Hetta, 2016). Linoleic acid in cosmetic formulations is used to prevent disorders of the skin barrier, lower trans epidermal water loss which therefore moisturises the skin, treat sunburns and regenerate epithelial tissue as it is a membrane-forming phospholipid (Lautenschläger, 2003; Hetta, 2016).In general, a deficiency of these important fatty acids from the skin may lead to erythema, inflammation, poor wound healing and dryness (Brenner,

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2004). These fatty acids are known for their properties in isolation, it is believed that using the crude natural oil sources as a carrier for other active ingredients may have valuable synergistic effects (Vermaak *et al.*, 2011; Hetta, 2016). Figure 1 below shows some of the major fatty acid's chemical structures.

Oils that contain natural antioxidants such as vitamin E have a substantially longer shelf life (Lautenschläger, 2003). An antioxidant is a preservative that reduces the rate of oxidation in oils which is a chemical process that occurs on exposure to oxygen (Muda *et al.*,2017). Vitamin E includes eight types of molecules, four tocotrienols and four tocopherols, which exhibit vitamin E activity. The purpose of vitamin E in cosmetic formulations is therefore to extend its shelf-life and to stop chain propagation in lipid peroxidation by scavenging lipid peroxyl radicals which defends the cell membrane against destruction. This in turn prevents the signs of photoaging (Muda *et al.*,2017). Figure 3 below shows the chemical structures of the four tocopherols. Important plant sterols or phytosterols found in the unsaponifiable fraction of seed oils include stigmasterol, β-sitosterol and campesterol. Sterols molecular structures are made up of rings, some containing unsaturated bonds, as well as branched hydrocarbon tails. When absorbed from the diet, phytosterols reduce the uptake of cholesterol in the body and are transferred to the skin where they make up an important component of the skins surface lipids (Puglia and Bonina, 2008). It is reported that phytosterols repair skin damage by having anti-inflammatory and antipruritic properties. Sitosterol has emulsifying effects due to its hydroxyl group that is attached to four lipophilic carbon rings. Phytosterols have been used in creams, conditioners and lipsticks due to their valuable properties (Svensson and Brinck, 2003; Puglia and Bonina, 2008). Figure 2 below shows the chemical structures of the major phytosterols of interest in this study.

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Due to factors such as environmental elements, ultraviolet radiation, air pollution and natural ageing processes; the barrier properties of the skin and hair may be altered which results in damage. The skin is made up of three layers the deepest being the hypodermis, followed by the dermis above it and finally the top layer the epidermis. Skin damage can result in dry, flaky and rough skin that is easily irritated as well as stretch marks, wrinkles or thin and flaccid skin (Muda *et al.*,2017). Oils incorporated into cosmetic skin formulations such as moisturisers, therefore, protects the skin by reducing water loss. The hair shaft structure is made up of three layers, the innermost layer being the medulla, followed by the cortex and finally the cuticle which protects the two inner layers. In the case of damaged hair, the cuticle layer is not properly orientated in a flat and tightly overlapped configuration and therefore cannot protect the inner layers as effectively. Hair damage results in dry and dull hair that easily breaks (Muda *et al.*,2017). Oils incorporated into cosmetic hair formulations such as leave-in conditioners penetrate the hair shaft to enhance lubrication and therefore protect the hair from breakage (Gavazzoni Dias, 2015). In general, oils leave a transparent, water-repellent film on the surface of the skin and hair which reduces friction, adds shine and increases the softness of the skin and hair (Schueller and Romanowski, 2003; Muda, Aziz and Aziz, 2017).

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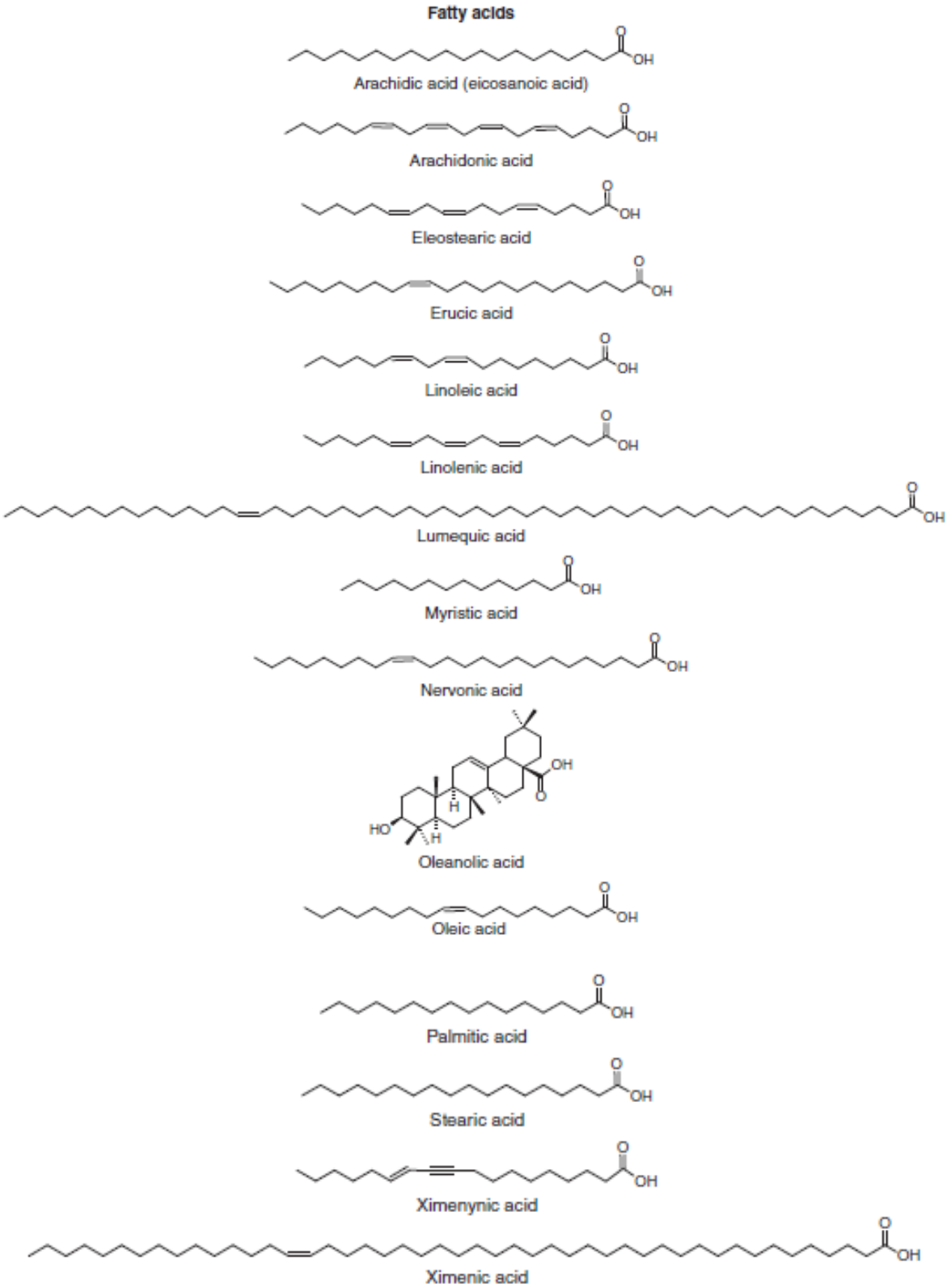

Figure 1: Chemical structures of important fatty acids (Vermaak *et al.*, 2011)

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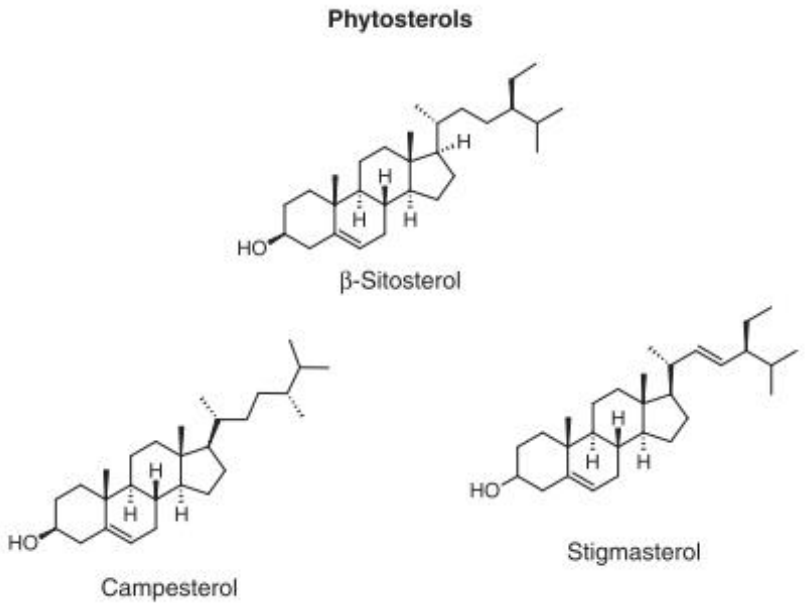

Figure 2: Chemical structures of important phytosterols (Vermaak *et al*., 2011)

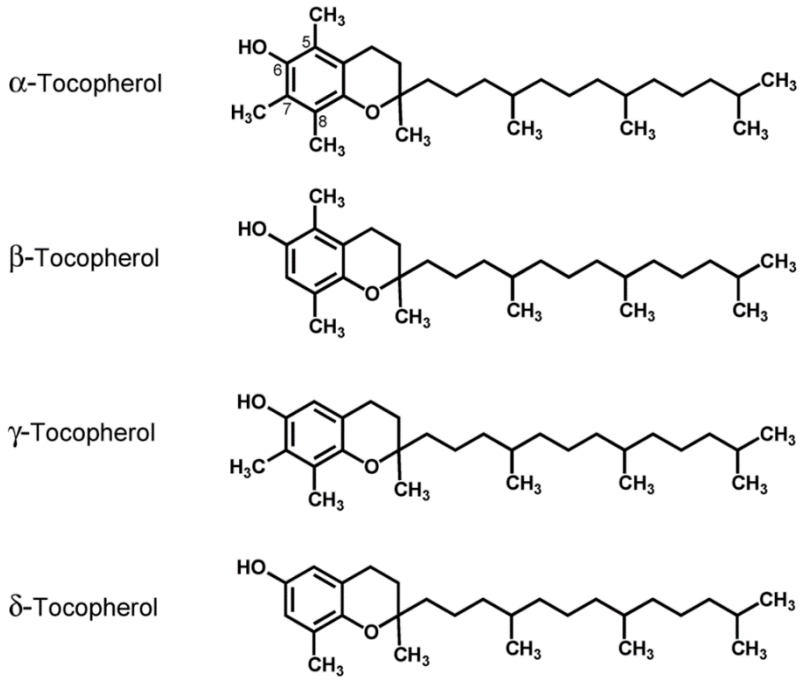

Figure 3: Chemical structures of tocopherols (Constantinou, Papas and Constantinou, 2008)

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2.2 Profile and characteristics of the seed oils

2.2.1 Moringa (*Moringa oleifera*)

The *Moringa oleifera* (MO) tree, also known as the Drumstick or Malunggay tree is one of thirteen Moringa species. It is native to the Himalayas in India but is also found and utilised in most parts of Asia and Africa (Sengupta and Gupta, 1970; Lalas and Tsaknis, 2002; Dhakad *et al.*, 2019). This tree is found in dry tropics making it resilient to drought. MO trees produce fragrant white flowers and bear fruits in the form of long green pods that contain multiple seeds. These seeds are globular with leaf-like wings (Leone *et al.*, 2016). The MO tree has traditionally been used for cooking oil, food, cosmetics, and medication. All parts of this plant, namely the leaves, fruits, flowers seeds and roots, are edible. This makes for a good source of crude fibre, minerals, protein, and nutrients (Verma and Nigam, 2014; Dhakad *et al.*, 2019). The chemical profile and quality of the seed oil are comparable to olive oil and may be used as a cheaper alternative (Lalas and Tsaknis, 2002; Zhao and Zhang, 2013; Nadeem and Imran, 2016). MO oil, also known as Ben oil, has a fatty acid composition that includes a high level of MUFAs and a lower level of PUFAs (Mani, Jaya and Vadivambal, 2007). The MUFAs include mainly oleic acid, which has good oxidative stability that allows for longer storage, high-temperature frying, use in medicine and water treatment (Anwar and Bhanger, 2003). Saturated palmitic and stearic acid are present as well as the PUFAs linolenic and linoleic acids (Lalas and Tsaknis, 2002). MO oil has a high vitamin E content making the oil resistant to oxidative degradation once refined (Koheil *et al.*, 2011). Other vitamins present include vitamin A and B. The seed oil has high pharmaceutical value as it is anti-inflammatory, antithrombotic, antihypertensive, antioxidizing, hepatoprotective, antibacterial, antifungal, antiseptic, antiepileptic and vasodilating (Cicero and Gaddi, 2001; Janaki, 2015; Nadeem and Imran, 2016; Ghafar *et al.*, 2017). MO oil can also be used in treatment to lower

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cholesterol levels, treat venomous bites, rheumatism, gout, skin damage such as cuts and burns as well as skin conditions such as eczema and psoriasis (Koheil *et al.*, 2011; Ojiako and Okeke, 2013; Ghafar *et al.*, 2017). MO oil has high cosmetic value as it is a non-drying and moisturising oil that is easily absorbed by the skin. The oil can thus be used as an active ingredient, a carrier, emollient, or preservative. It is used in a wide range of cosmetic formulations such as cleansers, massage oils, soaps, moisturisers, anti-aging products, lip balm, perfumes, scrubs, hot oil hair treatments and anti-hair fall lotions (Mehta *et al.*, 2011; Warra, 2011; Nadeem and Imran, 2016). Figure 4 below is an illustration of the MO plants components and Table 1 contains a summary of the major physico-chemical characteristics literature values of MO seed oil.

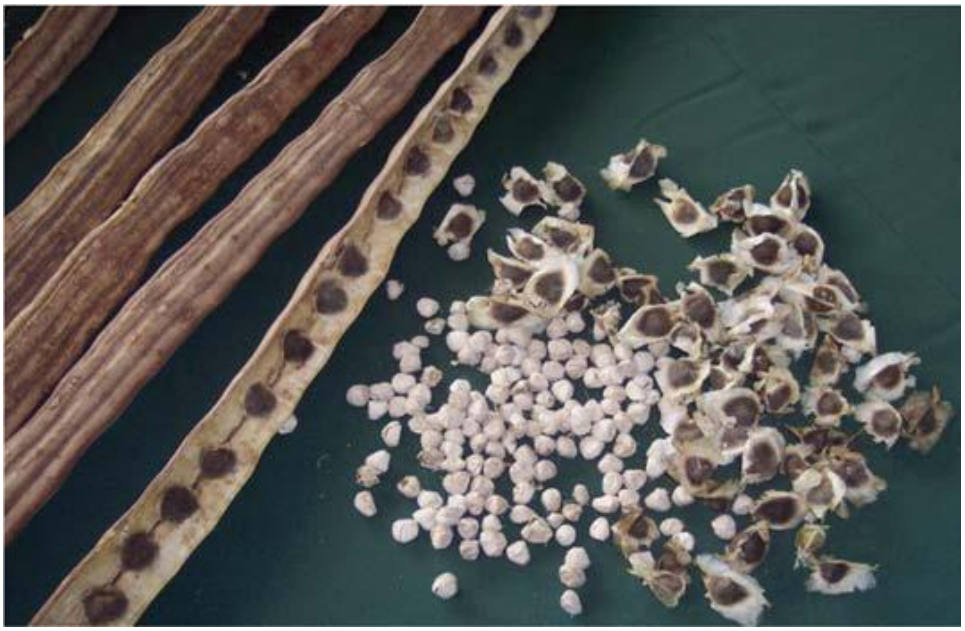

Figure 4: The pods, seeds and kernels of *Moringa oleifera* (Noh, 2018)

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Table 1: Major Physico-chemical characteristics literature values of MO seed oil (Cold-pressed)

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Yellow liquid	(Anwar and Bhanger, 2003; Ogbunugafo *et al.*, 2011; Ruttarattanamongkol et al., 2014)
Acid value (mg KOH/g)	0.19 ± 0.03	(Özcan *et al*., 2019)
Saponification value (mg.KOH/g)	181.3 ± 3.67	(Özcan *et al.,* 2019)
Average molecular weight (g/mol)	928.30	(Özcan *et al.*, 2019)
Ester value (mg KOH/g)	181.11 ± 3.67	Calculated from AV and SV results (Özcan *et al*., 2019)
Iodine value (g/100g)	67.53 ± 1.37	(Özcan *et al*., 2019)
Peroxide value (mequiv/kg)	0.18 ± 0.07	(Özcan *et al.*, 2019)
Density (g/ml) (25 °C)	0.819 ± 0.013	(Özcan *et al*., 2019)
Specific gravity (25 °C)	nd	
Refractive index (25 °C)	1.4359 ± 0.0001	(Özcan *et al.*, 2019)
Highest content fatty acid	Oleic acid	(Özcan *et al.,* 2019)

nd, no data

2.2.2 Marula (*Sclerocarya birrea*)

Marula trees are deciduous trees native to many parts of sub-Saharan Africa such as Ethiopia, Ghana, Nigeria, Zimbabwe, South Africa, Angola, Zambia, Botswana, Namibia, Mozambique etc. (Ngorima, 2006; Borochov-Neori *et al.*, 2008; Russo *et al.*, 2013; Hetta, 2016). They are commonly found in woodland habitats in sandy loam soil (Borochov-Neori *et al.*, 2008; Vermaak *et al.*, 2011; Francis and Tahir, 2016) Marula trees are leafless during winter, produce pink flowers during spring and bear edible fruit during summer (Hetta, 2016). The small Marula fruits are oblong shaped with a thick yellow skin and sticky flesh rich in vitamin C. Normally 2-3 soft edible seed kernels are encapsulated in each fruit (Ogbobe, 1992; Vermaak *et al.*, 2011). Oil extraction is challenging due to the shape of the hard brown seed within the nut (Cheikhyoussef, 2018). Marula has commonly been used for food (nutrition), alcoholic beverage, cosmetics, leather treatment, meat preservation and medicine (Mariod, Matthaus and

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Eichner, 2004; Athar and Nasir, 2005; Kleiman, Ashley and Brown, 2008). The roots, leaves and bark have traditionally been used to treat conditions such as dysentery, fevers, malaria, diarrhoea, rheumatism, sore eyes, infertility, headaches, toothache, body pains and are used in infant's nose drops (Zimba *et al.*, 2005). Extracts have antifungal, antioxidant, antibacterial, astringent, anticonvulsant, antiatherogenic, antihyperglycemic, anti-inflammatory, and aphrodisiac properties (Zimba *et al.* 2005; Francis and Tahir, 2016). Marula oils phytosterol content consists mainly of β-sitosterol and smaller amounts of 5-avenasterol. These phytosterols add antioxidative properties to the oil and also indicate that this seed oil is a potential source of protein (Mariod, Matthaus and Eichner, 2004). It contains unsaturated arachidonic acid which is important in the human diet (Mariod, Matthaus and Eichner, 2004). The seed oil contains mainly oleic, palmitic and stearic fatty acids (Vermaak *et al.*, 2011). It however has a low content of tocopherols making it a poor source of vitamin E. Despite a deficiency in the antioxidant vitamin E, Marula oil is a rich source of oleic acid making it very stable due to the MUFAs antioxidative properties (Mariod, Matthaus and Eichner, 2004). Marula oil is medium-rich and silky to touch with low odour, it penetrates well making it an excellent moisturiser (Zimba *et al*., 2005). This non-irritating oil is ideal for use in cosmetic products as it improves skin hydration, reduces skin redness and relieves skin irritation (Zimba *et al.* 2005; Vermaak *et al.*, 2011; Hetta, 2016). Cosmetic applications of this oil include massage oils, face oil, pomades, soaps, after-sun lotions, aftershaves and makeup removers (Zimba *et al.* 2005; Francis and Tahir, 2016; Cheikhyoussef, 2018). Figure 5 below is an illustration of the Marula fruit and Table 2 contains a summary of the major physico-chemical characteristics literature values of Marula seed oil.

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Figure 5: *Sclerocarya birrea* (Marula) (Hetta, 2016)

Table 2: Major Physico-chemical characteristics literature values of Marula seed oil (Cold-pressed)

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Light yellow liquid	(Cheikhyoussef, 2018)
Acid value (mg KOH/g)	5.16 ± 0.16	(Cheikhyoussef, 2018)
Saponification value (mg.KOH/g)	187.94 ± 0.48	(Cheikhyoussef, 2018)
Average molecular weight (g/mol)	895.52 ± 2.28	(Cheikhyoussef, 2018)
Ester value (mg KOH/g)	182.77 ± 0.63	(Cheikhyoussef, 2018)
Iodine value (g/100g)	85.28 ± 29.30	(Cheikhyoussef, 2018) (Robinson, Lukman and Bello, 2012)
Peroxide value (mequiv/kg)	1.16 ± 2.52	(Cheikhyoussef, 2018) (Robinson, Lukman and Bello, 2012)
Density (g/ml) (25 °C)	nd	
Specific gravity (25 °C)	0.93 ± 0.96	(Cheikhyoussef, 2018) (Robinson, Lukman and Bello, 2012)
Refractive index (25 °C)	1.46 ± 0.003	(Cheikhyoussef, 2018) (Robinson, Lukman and Bello, 2012)
Highest content fatty acid	Oleic acid	(Mariod, Matthaus and Eichner, 2004; Nwabuebo, 2017; Cheikhyoussef, 2018)

nd, no data

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2.2.3 Kalahari melon (*Citrullus lanatus*)

The Kalahari melon is a trailing herb native to Namibia and Botswana. This plant has broad leaves and produces yellow flowers. It bears yellow or green fleshy fruits that vary in size and encapsulate black or pale-yellow seeds. Kalahari melon is commonly found on river banks, dry lakes, or disturbed areas (Vermaak *et al.*, 2011). This plant is commonly used for cooking oil, food (nutrition), water source, cosmetics and medication (Nyam *et al.*, 2010; Lendelvo *et al.* 2012; Cheikhyoussef, 2018). Tar extracted from the seeds are used for the treatment of scabies and skin tanning. Kalahari melon is also used to treat conditions such as herpes lesions, urinary diseases, fever, acne, venereal sores, leg ulcers and for prevention of cancer and coronary heart disease (Cho, 2004; Mariod *et al.*, 2009; Vermaak *et al.*, 2011). Kalahari melon seed oil is known to have a high content of tocopherols (Nyam *et al.*, 2010). This high vitamin E level gives the oil natural antioxidative properties which make the seed oil very stable and thus ideal for industrial, cosmetic, nutritional, and pharmaceutical purposes (Vermaak *et al.*, 2011). The seed oil contains essential fatty acids, the most abundant being linoleic acid followed by oleic, palmitic and stearic acids. Amongst the phytosterols present in the oil, β-sitosterol is the most abundant followed by campesterol and stigmasterol, which adds to the oil's natural antioxidant capacity (Vermaak *et al.*, 2011; Hetta, 2016). This seed oil is typically used in cosmetic formulations as an emollient due to its light texture. The high essential fatty acid content makes this oil very moisturising and nourishing to the skin, therefore, restoring the skin's elasticity (Vermaak *et al.*, 2011). Due to its properties, Kalahari melon seed oil is used in cosmetic formulations such as after-sun skincare, moisturizers, hair lotions, massage oils and soaps (Nyam *et al.*, 2010; Vermaak *et al.*, 2011; Cheikhyoussef, 2018). Figure 6 below is an illustration of Kalahari melon fruit and Table 3 contains a summary of the major physico-chemical characteristics literature values of Kalahari melon seed oil.

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Figure 6: *Citrullus lanatus* (Kalahari melon) (Hetta, 2016)

Table 3: Major physico-chemical characteristics literature values of Kalahari melon seed oil (Cold-pressed)

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Yellow liquid	(Cheikhyoussef, 2018)
Acid value (mg KOH/g)	5.16 ±0.16	(Cheikhyoussef, 2018)
Saponification value (mg.KOH/g)	189.26 ±1.40	(Cheikhyoussef, 2018)
Average molecular weight (g/mol)	889.30 ±6.58	(Cheikhyoussef, 2018)
Ester value (mg KOH/g)	188.16 ± 1.41	(Cheikhyoussef, 2018)
Iodine value (g/100g)	117.64 ± 1.96	(Cheikhyoussef, 2018)
Peroxide value (mequiv/kg)	2.12 ± 0.052	(Cheikhyoussef, 2018)
Density (g/ml) (25 °C)	nd	
Specific gravity (25 °C)	0.922 ± 0.001	(Cheikhyoussef, 2018)
Refractive index (25 °C)	1.4726 ± 0.001	(Cheikhyoussef, 2018)
Highest content fatty acid	Linoleic acid	(Cheikhyoussef, 2018)

nd, no data

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2.2.4 Mongongo/ Manketti (*Schinziophyton rautanenii*)

The Mongongo/ Manketti tree is a large deciduous and dioecious tree. This tree is native to Southern Africa, namely Angola, Namibia, Botswana, South Africa and Zambia, and is found in wooded hill and deep sand habitats (Atabani *et al.*, 2014). It has dark-green leaves and small pale-yellow flowers appearing in spring (Vermaak *et al.*, 2011). This tree bears small egg-shaped green fruits covered in fine hairs, these fruits fall from the tree in autumn and ripen on the ground (Vermaak *et al.*, 2011). The ripe fruit has a sweet soft flesh and relatively large off-white seed. This tree is traditionally a source of food, cosmetics and medicine (Vermaak *et al.*, 2011). The fatty acid profile of the seed oil resembles that of maize oil. It consists mainly of the PUFAs linoleic, linolenic and arachidonic acid, followed by oleic, palmitic, stearic and erucic acids (Vermaak *et al.*, 2011). The seed oil is rich in phytosterols and natural proteins including γ-tocopherol and β-sitosterol, making it nutritious and protective to the hair and skin (Athar and Nasir, 2005; Mitei *et al.*, 2009; Hetta, 2016). The high vitamin E content provides the oil with high oxidative stability and long shelf life, making it useful to the pharmaceutical, food and cosmetic industry (Zimba *et al.* 2005; Juliani *et al.*, 2007; Nyam *et al.*, 2010). The seed oil may be used for the treatment of eczema, atopic disorders, reduction of itching, redness, wrinkles, age spots, scarring, burns and the prevention of keloids (Zimba *et al.*, 2005; Cheikhyoussef, 2018). This is due to the oil being anti-inflammatory, antibacterial, rich in antioxidants and promoting skin renewal (Zimba *et al.*, 2005; Vermaak *et al.*, 2011). Cosmetic formulations using this light texture oil that easily absorbs into the skin include hair lotions, cleansers, aromatherapy products, moisturisers and emollients (Graz, 2002; Juliani *et al.*, 2007; Vermaak *et al.*, 2011; Kivevele and Huan, 2014). Figure 7 below is an illustration of Mongongo fruit and Table 4 contains a summary of the major physico-chemical characteristics literature values of Mongongo seed oil.

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Figure 7: *Schinziophyton rautaneii* (Mongongo) (Hetta, 2016)

Table 4: Major physico-chemical characteristics literature values of Mongongo seed oil (Cold-pressed)

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Light yellow	(Juliani *et al.*, 2007; Cheikhyoussef, 2018)
Acid value (mg KOH/g)	1.83 ± 0.33	(Juliani *et al.*, 2007; Cheikhyoussef, 2018)
Saponification value (mg.KOH/g)	187.66 ± 1.52	(Cheikhyoussef, 2018)
Average molecular weight (g/mol)	896.9 ± 7.30	(Cheikhyoussef, 2018)
Ester value (mg KOH/g)	185.61 ± 1.55	(Cheikhyoussef, 2018)
Iodine value (g/100g)	130.15 ± 1.63	(Juliani *et al.*, 2007; Cheikhyoussef, 2018)
Peroxide value (mequiv/kg)	5.60 ± 4.12	(Juliani *et al.*, 2007; Cheikhyoussef, 2018)
Density (g/ml) (25 °C)	nd	
Specific gravity (25 °C)	0.923 ± 0.003	(Cheikhyoussef, 2018)
Refractive index (25 °C)	1.48 ± 0.00	(Juliani *et al.*, 2007; Cheikhyoussef, 2018)
Highest content fatty acid	Linoleic acid	(Cheikhyoussef, 2018)

nd, no data

2.2.5 Baobab (*Adansonia digitate L.)*

The Baobab tree, native to Madagascar, is commonly found in parts of sub-Saharan Africa such as Malawi, Zimbabwe, Mozambique, South Africa, Benin, Senegal, Cameroon, Kenya, Uganda, Tanzania etc. (Vermaak *et al.*, 2011). This slow-growing deciduous tree is the largest

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succulent in the world found in hot arid regions with low rainfall. Leaves are hand shaped (digitate) and are only present for three months in a year whereas the white hanging flowers, consisting of five petals, are present for only two months of the year (Vermaak *et al.*, 2011; Komane *et al.*, 2017). The seeds are found in the velvety hair covered egg-shaped fruits (Gebauer, et al. 2006). The baobab tree has multiple purposes as each part of the tree; namely the fruit pulp, seeds, leaves, flowers, roots, and bark; have a valuable use. Local communities use this versatile tree for food, medicine, cosmetic purposes as well as for raw materials for other purposes such as fertilizer and clothing. The fruit pulp is especially nutritious as it is high in vitamin C. Baobab is used to treat numerous conditions such as fever, dandruff, diarrhoea, coughs, haemoptysis, dysentery, worms, kidney diseases, burns and other skin conditions as well as scurvy related symptoms (Watt and Breyer-Brandwijk, 1962; Zimba *et al*., 2005; Vermaak *et al.*, 2011; Mahomoodally and Ramjuttun, 2017). These anti-inflammatory, antimicrobial, and anti-viral properties make baobab important to the pharmaceutical industry (Chindo *et al.*, 2010; El-Nagerabi *et al.*, 2013; Komane *et al.*, 2017). This conditioning oil contains many vitamins including vitamins A, D3, E and F (Nkafamiya *et al.*, 2007). Vitamins A and F(polyunsaturated) are responsible for the rejuvenation and renewal of cell membranes, therefore, repairing the skin. Vitamin E is an antioxidant, making the oil very stable and giving the seed oil antiaging activity. The oil contains palmitic, linoleic and oleic essential fatty acids (Andrianaivo-Rafehivola, A.A. Blond *et al.*, 1993; Mahomoodally and Ramjuttun, 2017). These components make baobab oil an excellent moisturizer that can also reduce eczema and psoriasiss (Wren and Stucki, 2003; Athar and Nasir, 2005; Zimba *et al.*, 2005). The major sterols present are β-sitosterol, stigmasterol, and campesterol, which adds to the anti-oxidative properties of the seed oil (Vermaak *et al.*, 2011). The rich seed oil is non-irritating and non-sensitizing which can be used for normal and dry skin (Wren and Stucki, 2003). This oil is highly penetrating and

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can soften dry skin, improve its elasticity and tone without a greasy residue (Wren and Stucki, 2003; Zimba *et al*., 2005; Hetta, 2016). Baobab seed oil is therefore used in bath oil preparations, moisturisers, emollients, massage oils, hot oil soaks and many other cosmetic applications (Wren and Stucki, 2003; Zimba *et al*., 2005; Vermaak *et al.*, 2011) This fatty acid rich oil is therefore very important to the cosmetic industry (Chindo *et al.*, 2010; Komane *et al.*, 2017). Figure 8 below is an illustration of Baobab fruit and Table 5 contains a summary of the major physico-chemical characteristics literature values of Baobab seed oil.



Figure 8: Adansonia digitatal (Baobab)(Hetta, 2016)

Table 5: Major physico-chemical characteristics literature values of Baobab seed oil

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Yellow liquid	(Cissé *et al.*, 2018)
Acid value (mg KOH/g)	18.827 ± 0.309	(Cissé *et al.*, 2018)
Saponification value (mg.KOH/g)	233.587 ± 0.478	(Cissé *et al.*, 2018)

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Average molecular weight (g/mol)	720.50	(Cissé *et al.*, 2018)
Ester value (mg KOH/g)	214.76 ± 0.36	(Cissé *et al.*, 2018)
Iodine value (g/100g)	99.113 ± 0.528	(Cissé *et al.*, 2018)
Peroxide value (mequiv/kg)	2.091 ± 0.579	(Cissé *et al.*, 2018)
Density (g/ml) (25 °C)	0.911 ± 0.04	(Cissé *et al.*, 2018)
Specific gravity (25 °C)	0.943	(Chindo *et al.*, 2010) Solvent extraction
Refractive index (25 °C)	1.464 ± 2.8	(Cissé *et al.*, 2018)
Highest content fatty acid	Oleic acid	(Muthai *et al.*, 2019)(Zimba *et al.*, 2005)

2.2.6 Hemp (*Cannabis sativa L.*)

Hemp is a part of the Cannabis sativa species which falls under the Cannabaceae family. Hemp is a dioecious, flowering herb with digitate leaves and two lower leaflets. The small flower buds contain and protect many seeds each encapsulated in a shell (Citti *et al.*, 2018). The Hemp plant is very versatile and can be used in food, medicine, paint, cosmetics, fibres and textiles, animal feed etc. The seeds have a relatively high oil content which is very rich in PUFAs mainly linoleic, linolenic, and arachidonic acid. The oil has a favourable ratio of 3:1 omega 3 to omega 6 PUFA, which is optimal for nutrition, however making it chemically unstable (Athar and Nasir, 2005; Smeriglio *et al*., 2016; Citti *et al*., 2018; Tan *et al*., 2018). The far lower MUFA content includes oleic acid and saturated stearic acid. The seed oil contains moderate to high amounts of vitamin E and phytosterols making the oil a source of natural antioxidants (Oomah *et al*., 2002; Mikulcová *et al*., 2017; Citti *et al.*, 2018). Hemp seeds contain no cannabinoids, therefore, any trace amounts of cannabinoids present in hemp seed oil are impurities caused by the extraction process, which is usually cold-pressing *(Vogl et al., 2004; Citti et al*., 2018). Hemp seed oil is used in the treatment of skin conditions such as neurodermatitis and psoriasis (Vogl *et al.*, 2004). The oil's fatty acid profile makes the oil anti-inflammatory, antibacterial, accelerate healing of

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skin wounds and regenerate skin and hair (Tan *et al.*, 2018). Hemp seed oil is ideal for cosmetic applications due to its rich PUFA content giving it emollient and moisturizing properties (Vogl *et al.*, 2004). Hemp seed oil is used in cosmetic formulations aimed at skin protection via lipid barrier restoration as well as hair moisturizing and growth products such as shampoos and hair treatments (Athar and Nasir, 2005; Tan *et al.*, 2018; Paul *et al.*, 2019). Figure 9 below is an illustration of the Hemp plants components and Table 6 contains a summary of the major physico-chemical characteristics literature values of Hemp seed oil.



Figure 9: Hemp seeds (left), a young hemp plant (centre), and a mature flowering hemp plant (Smith, 2018)

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Table 6: Major physico-chemical characteristics literature values of Hemp seed oil (Cold-pressed)

Physico-chemical characteristic	Average literature value	Literature sources
Colour and state (25 °C)	Yellow-green liquid	(Al Jourdi *et al.*, 2019)
Acid value (mg KOH/g)	1.7 ± 0.6	(Mikulcová *et al.*, 2017)
Saponification value (mg.KOH/g)	197.6 ± 4.5	(Mikulcová *et al.*, 2017)
Average molecular weight (g/mol)	851.72	(Mikulcová *et al.*, 2017)
Ester value (mg KOH/g)	195.9 ± 4.46	(Mikulcová *et al.*, 2017)
Iodine value (g/100g)	155.8 ± 1.9	(Mikulcová *et al.*, 2017)
Peroxide value (mequiv/kg)	0.90	(Al Jourdi *et al.*, 2019)
Density (g/ml) (25 °C)	0.908	(Al Jourdi *et al.*, 2019)
Specific gravity (25 °C)	nd	
Refractive index (25 °C)	1.474	(Al Jourdi *et al.*, 2019)
Highest content fatty acid	Linolenic acid	(Al Jourdi *et al.*, 2019)

nd, no data

2.3 Extraction techniques

2.3.1 Cold-press extraction

Cold-press extraction of seed oil involves the application of immense pressure on pre-treated seeds to separate the liquid and solid phase (Zuorro *et al.*, 2014). The cold press machine has one inlet where seeds are fed into, two outlets that release the oil product and a protein-rich by-product known as a seed press cake (Singh and Bargale, 2000). Moringa seed press cakes may be used as a fertiliser (Emmanuel *et al.*, 2011), animal feed and as a natural coagulant in water purification (Ndabigengesere and Narasiah, 1998; Mehta *et al.*, 2011; Oladeji *et al.*, 2017). Process parameters include pressure, feeding rate, restriction dye diameter and rotation speed of propeller (Çakaloğlu, Özyurt and Ötleş, 2018; Chemat *et al.*, 2019). This technique is referred to as cold-press extraction as no additional heat is applied during the process. Care is however taken for some oils to limit the amount of heat generated by friction during the process to prevent oxidation reactions and loss of volatile compounds, such as phenolics (Parry *et al.*, 2005; Nwabuebo, 2017; Çakaloğlu, Özyurt and Ötleş, 2018). Cold-pressing has the

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advantage of being fast, cheap, low-energy and safer as products obtained are free of harmful solvents making it more environmentally friendly (Rotkiewicz, Konopka and Zylik, 1999; Balami, 2007; Ethiopia, 2018; Özcan *et al.*, 2019). The oil produced is high in quality with better nutritional properties than refined oils and very little to no refinement is needed except for washing with water, filtering or centrifugation (Agus Bin *et al.*, 2014; Çakaloğlu, Özyurt and Ötleş, 2018). Cold-pressing, however, has the disadvantages of lower productivity, consistency, yields and selectivity compared to other extraction methods (Çakaloğlu, Özyurt and Ötleş, 2018; Ethiopia, 2018). Figure 10 below illustrates Cold-pressing oil extraction.

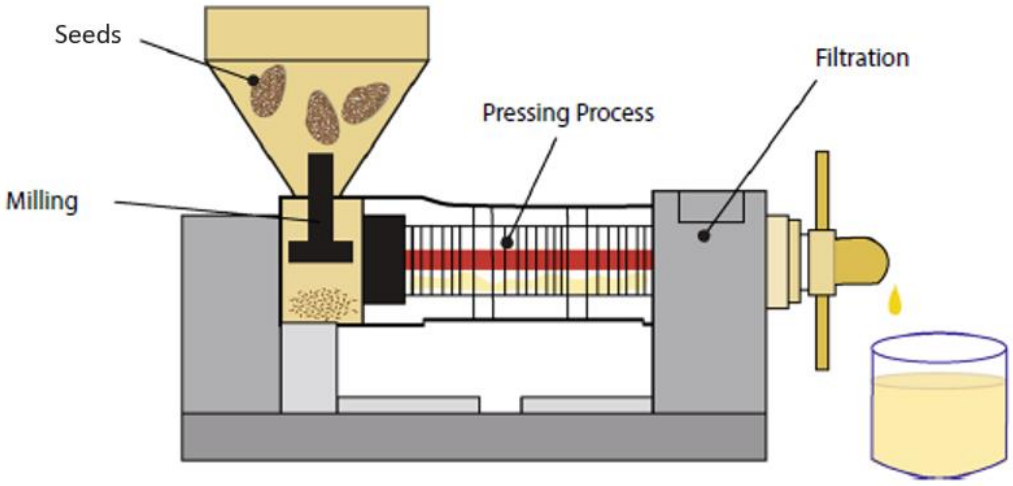

Figure 10: Modified illustration of cold-pressing (Vaughn *et al.*, 2018)

2.3.2 Soxhlet extraction

Soxhlet extraction is one of the traditional extraction techniques to which other extraction techniques are compared (Wang and Weller, 2006). Experimental parameters for this technique include solvent choice (like dissolves like), the contact area of the analyte with solvent (particle size), amount of solvent (solute to solvent ratio), temperature (diffusion rate), the drying time of sample (moisture content), and extraction time (Daroch,

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Geng and Wang, 2013; Balcıoğlu, 2015; Nwabuebo, 2017; Çakaloğlu, Özyurt and Ötleş, 2018) In this study, hexane will be used as the extraction solvent. Hexane is commonly used for extracting vegetable oils for its non-polar nature, efficiency and reliability compared to other solvents such as benzene, toluene and chloroform (Reverchon and De Marco, 2006; Nwabuebo, 2017). The procedure involves extraction by repeated washing (percolation) with an organic solvent, in this case, hexane. The prepared seed sample is placed in filter paper in an extraction chamber situated between a round bottom flask containing hexane, and a condenser. The solvent is heated such that it volatilises and moves into the condenser where it condenses and drips into the extraction chamber. The excess solvent surrounding the sample overflows and drips back down into the solvent flask. During each cycle of the extraction process, a portion of the analyte dissolves in the solvent. After many cycles, the analyte is concentrated in the solvent flask and can then be separated from the solvent using a rotary evaporator (Ethiopia, 2018). Soxhlet extraction has numerous advantages such as high yields, high efficiency, low costs, recyclable solvent, simple equipment and no need for filtration of the oil (Pradhan *et al.*, 2010; Palafox *et al.*, 2012; Çakaloğlu, Özyurt and Ötleş, 2018). The disadvantages, however, are that the solvents are ecologically harmful, toxic and flammable (Bhattacharjee, Singhal and Tiwari, 2007). Recovery and recycling of solvent are difficult and therefore use high amounts of hence residual solvent in the final product is common (Palafox *et al.*, 2012; Nwabuebo, 2017; Çakaloğlu, Özyurt and Ötleş, 2018; Tan *et al.*, 2018). Figure 11 below illustrates the Soxhlet extraction apparatus.

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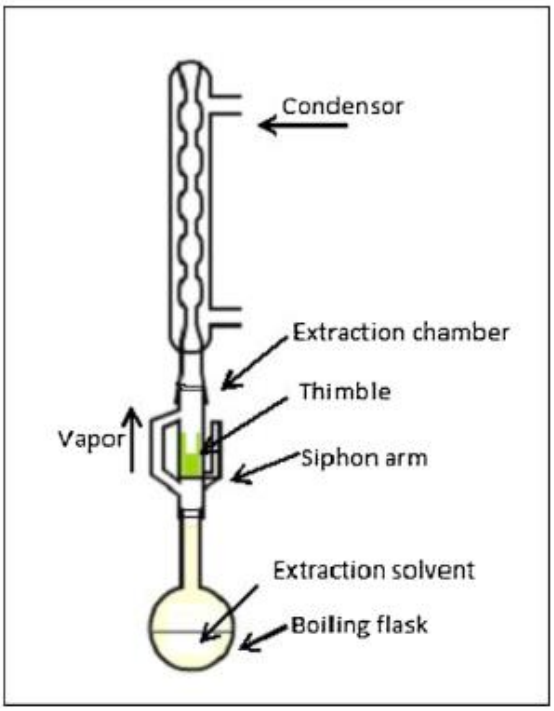

Figure 11: Illustration of Soxhlet extraction apparatus (Bhutada *et al.*, 2016)

2.3.3 Ultrasound-assisted extraction

Ultrasound-assisted extraction is a relatively modern extraction technique that makes use of ultrasound waves to assist the extraction process. Ultrasound waves are above the human hearing range with frequencies over 20 kHz. Propagation of these high-frequency waves above the local tensile strength of the liquid cause a negative pressure to develop which induce vapour bubbles that result in macroturbulence and shear forces as they collapse. Mechanical effects such as cavitation bubbles, mixing, vibration, and pulverization, therefore, take place. Ultimately, this mechanical disruption aids extraction as the cell walls of the sample is agitated and damaged which allows easier permeability of the extraction solvent and therefore intensified mass transfer. Cavitation also results in a chemical disruption by the formation of free radicals that trigger chemical reactions to take place that further aid the extraction process. Ultrasound-assisted extraction is therefore a greener extraction process as the

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mechanism allows for reduced extraction time and lower temperatures ideal for sensitive compounds. Factors that affect extraction efficiency include frequency, solvent type, solvent volume sample size and extraction time (Thirugnanasambandham, 2018; Mwaurah *et al.*, 2020). The ultrasound instrument is either in the form of an ultrasound bath or an ultrasound probe depending on the application as illustrated in Figure 12. Table 7 below contains average literature values for MO oil extraction yields and efficiency.

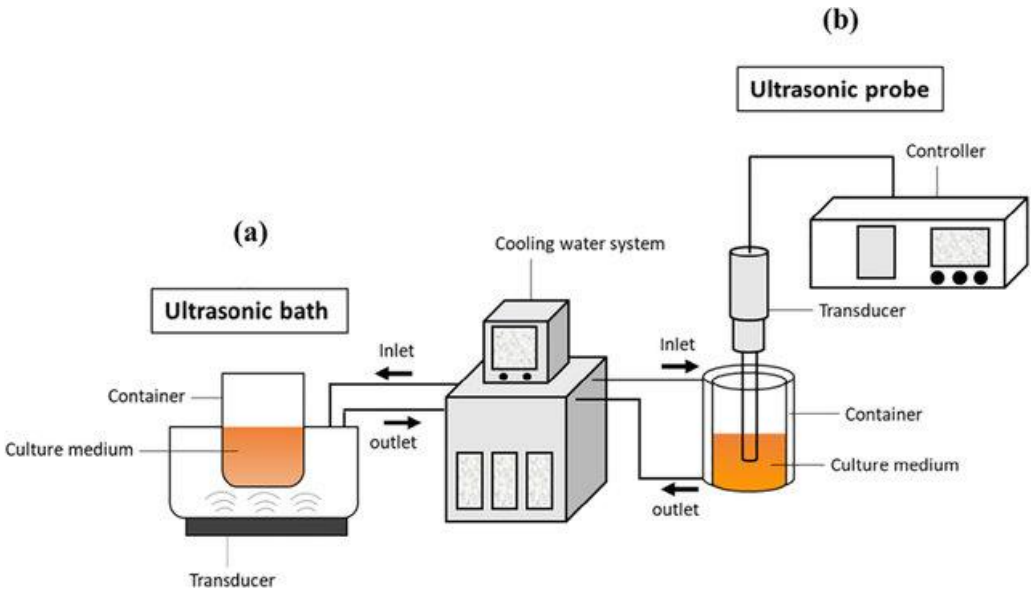

Figure 12: Schematic illustration of an ultrasonic bath and ultrasonic probe(Behzadnia, Moosavi-Nasab and Tiwari, 2019)

Table 7: Average literature values for MO oil extraction yields and efficiency

Method of extraction	% Oil yield Literature values	Literature sources	% Efficiency Literature values	Literature sources
Soxhlet extraction (hexane)	38.3	(Lalas and Tsaknis, 2002)	100.00 ± 1.00	(Nwabuebo, 2017)
	35.30 ± 1.14	(Nwabuebo, 2017)		
	35.3	(Tsaknis *et al*., 1998)		

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	35.7 ± 2.4	(Tsaknis *et al*., 1999)		
	29.12	(Dinesha *et al*., 2018)		
	40.12	(Zhao and Zhang, 2013)		
	40.39 ± 1.15	(Anwar and Bhanger, 2003)		
	41.47	(Ogbunugafo *et al*., 2011)		
	39.16 ± 0.19	(Zhong *et al*., 2018)		
	30.8 ± 2.19	(Abdulkarim *et al.*, 2005)		
	Average: 32.87 ±10.11			
Cold-press extraction	14.43	(Nwabuebo, 2017)	60.91	(Nwabuebo, 2017)
	25.1	(Lalas and Tsaknis, 2002)	65.54	Estimate based on (Lalas and Tsaknis, 2002)
	26.3	(Tsaknis *et al*., 1998)	74.50	Estimate based on (Tsaknis *et al*., 1998)
	25.8 ± 2.6	(Tsaknis *et al*., 1999)	72.27	Estimate based on (Tsaknis *et al*., 1999)
	Average: 22.1 ± 5.67		Average: 68.34 ± 6.23	
Ultrasound-assisted extraction	38.1	(Buddin *et al*., 2018)	59	(Thirugnanasambandham, 2018)
	53.8 ± 0.43	(Mohammadpour *et al*., 2019) M. peregrina oil		
	35.77 ± 0.04	(Zhong *et al.*, 2018)		
	Average: 42.56 ± 9.81			
Shaker method extraction	27.19 ± 0.91	(Nwabuebo, 2017)	77.01 ± 0.55	(Nwabuebo, 2017)

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2.4 Characterization and analytical techniques

2.4.1 Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) can be utilised as both a quantitative and qualitative technique. This combination technique can separate mixtures of volatile and semi-volatile compounds and selectively detect them. The GC instrument separates chemical mixtures into individual compounds using heat. The volatilised compounds are carried through a column with an inert gas, usually helium. As the separated compounds exit from the column opening, they flow into the MS detector. Individual compounds can then be identified by their unique mass using an MS library of known compound spectra. Quantitation is made possible by using calibration curves of known standards. GC-MS can be used for applications such as drug and pesticide detection and quantification (Sneddon, Masuram and Richert, 2007). The solvent selected to dissolve the samples and rinse the column depends on the polarity of analytes present. In some cases, a mixture of solvents is required for complex matrices.

In this study and those similar, GC-MS is used to identify and/or quantify tocopherol, sterols and/or fatty acids. Since these are not volatile compounds, a derivatisation process is done to samples before analysis. For fatty acid analysis in particular many researchers use reference standards to identify and quantify the fatty acids present in a sample. From the total fatty acid concentrations or relative percentages, percentages of saturated fats (SAFA), Trans-fats (TFA), Monounsaturated fats (MUFA), polyunsaturated fats (PUFA), and total unsaturated fats (TUFA) can be determined. This type of data can be used as characterisation references or fingerprint data to which future data can be compared (Danish and Nizami, 2019).

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Cheikhyoussef (Cheikhyoussef, 2018) for example used GC-MS methods described by Du and Ahn (Du and Ahn, 2002) to analyse major tocopherol, sterol and fatty acid content in various Namibian seed oils, namely manketti, marula, Ximenia, !nara and melon seed oil. These compounds' identification and/or quantification are of great importance to many industries. Cheikhyoussef in particular was profiling these seed oils for the food industry and the effect extraction techniques had on quality. 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. 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) (Cheikhyoussef, 2018). Figure 13 bellow illustrates the main components of the GC–MS instrument.

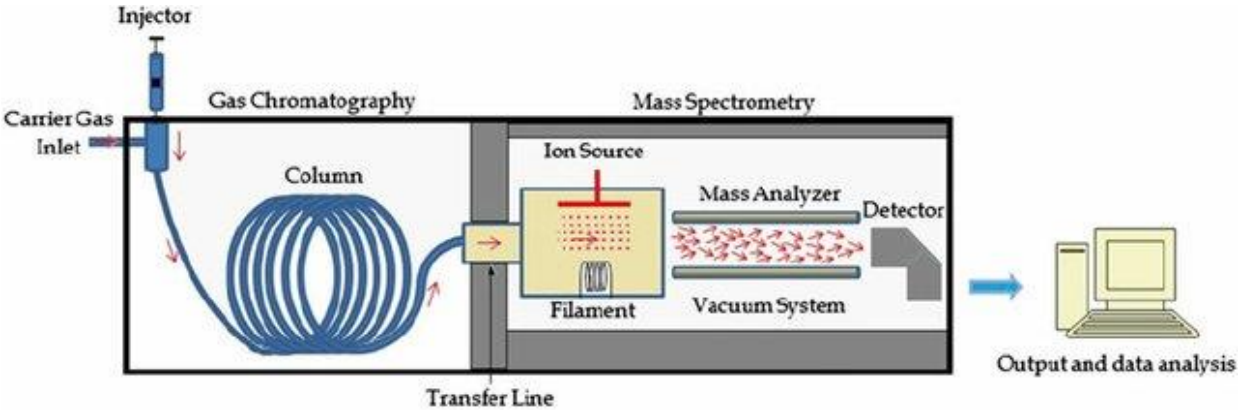

Figure 13: Schematic plot of the main components of the GC–MS instrument (Fullan, 2015)

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2.4.1 Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique that can be used qualitatively or quantitatively. It provides information on atomic environments based on the distinct resonance frequencies displayed by nuclei, in this case, hydrogen and carbon, in a strong magnetic field. The identity, purity and molecular structure of samples can be determined using this technique. NMR is a sensitive non-destructive technique that requires minimal sample preparation. NMR spectroscopic results are in the form of NMR spectra, chemical shift (δ) vs intensity (Sherazi and Mahesar, 2015). NMR is a simple technique that can be used for the fast screening of large numbers of samples and the development of a database of authentic products to detect adulteration (Popescu *et al.*, 2015).

In a study by Popescu and co-workers, ^{1}H and ^{13}C NMR analysis was used for multiple pure and vegetable oil blends to determine the saturated fatty acids, oleic acid, linoleic acid, linolenic acid and iodine value. The major aim being to determine the capabilities of NMR and chemometrics to be used in the quality assessment of vegetable oils in terms of their botanical origin. What was found is that the largest contribution to the total organic signal obtained for an oil sample was given by the 1.30 ppm signal (53%) representing the methylene groups of all the fatty acids, followed by 2.03 ppm, 0.87 ppm and 5.37 ppm signals with contributions of approximately 10%, the lowest signal being given by the 1.02 ppm group (terminal methyl group of linolenic acid) with percentages around 0.68%. The integral positions and intensities give information on the oils' overall saturation and allowed Popescu and co-workers to correctly group the oils with the same level of saturation which correlates to their iodine values. Similarly, Mitei and co-workers used NMR to determine the relative compositions of the saturated, monounsaturated and diunsaturated fatty acids and their average chain lengths. This was done using the relative

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sizes of the NMR signals integrals for the signals corresponding to the allylic, diallylic and methyl protons using Holmback's equations. Other techniques including GC-MS were used in conjunction to confirm fatty acid content. It was found that NMR fairly accurately elucidates the fatty acid content in oils except for some poor resolution of the oleoyl and linoleoyl signals in the 13C-NMR spectrum (Mitei *et al.*, 2008). Figure 14 below illustrates the components of the NMR instrument.

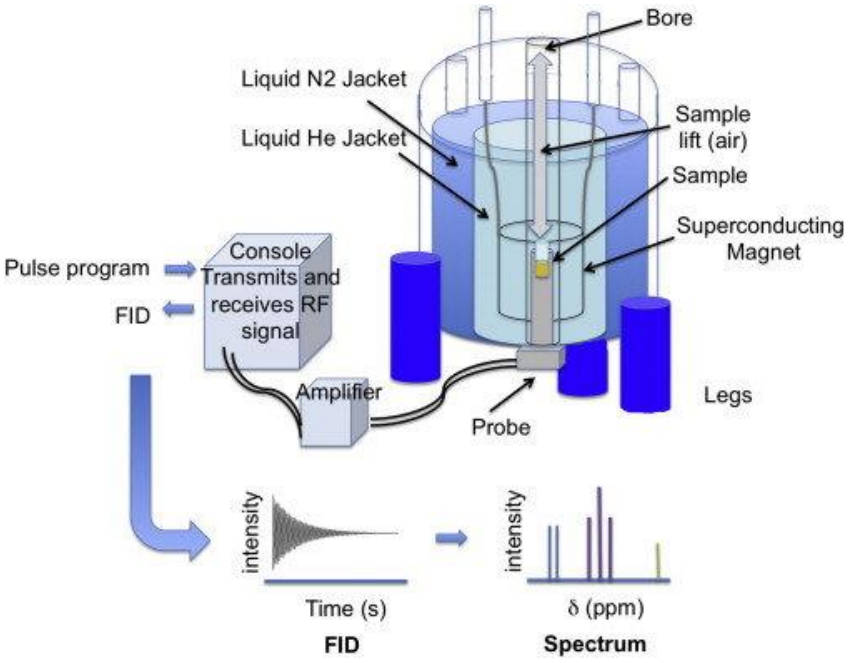

Figure 14: Schematic diagram of an NMR spectrometer(Rankin *et al.*, 2014)

2.4.2 Ultraviolet-visible spectroscopy

Ultraviolet-visible (UV-Vis) spectrometers are one of the most important analytical instruments. UV-Vis spectrometry is known for its simplicity, versatility, speed, accuracy and cost-effectiveness. Spectroscopy is related to matters interaction with light which involves the radiation being absorbed, transmitted, scattered, reflected or it can excite fluorescence. When matter absorbs radiation, there is an excitation of electrons present in the solution producing a distinct spectrum. The principle behind UV-Vis

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follows the Beer-Lambert Law which states that when monochromatic radiation passes through a homogenous solution in a cell, the decreasing rate of the radiation intensity along with the thickness of the absorbing solution is proportional to the concentration of the solution and the incident radiation.

This law is expressed through the equation: $A = \log (I_0/I) = ECL$, where I_0 is the intensity of the incident radiation, I is the intensity of transmitted radiation, C is the concentration of the solute, L is the length of the sample cell and E is the molar absorptivity. UV-Vis spectroscopy is mainly used for the quantitative determination of different compounds in a solution. It may also be used for applications such as determining the purity of a substance and identifying unknown compounds by comparing its spectrum to the spectrum of a reference compound(ThermoSpectronic, 2012).

Nwabuebo (Nwabuebo, 2017) as well as Delfan-Hosseini and co-workers used UV-VIS to study the radical scavenging activity, of various oils extracted in different ways, towards 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. This was done to elucidate their antioxidant properties and therefore their oxidative stability. This simple colourimetric analysis involved monitoring the decrease in absorbance at 517 nm for the prepared oil samples in comparison to a reference sample. In the study conducted by Nwabuebo, % Inhibition, which correlates directly to antioxidant content, was as follows for both marula and moringa seed oil: Aqueous extraction at 37 °C > Aqueous extraction at 60 °C> Screw press> Hexane extraction (shaker)> Hexane extraction (Soxhlet) Hexane (Nwabuebo, 2017). In the study conducted by Delfan-Hosseini and co-workers antioxidant content in Purslane seed oil was found to be in the following order: microwave pre-treatment cold press extracted> Solvent extracted> Cold press extracted. In both cases, it was concluded that the oil extraction method used does indeed affect the oils' antioxidant properties and therefore oxidative stability. According to Nwabuebo, this is

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due to differences in solvent and temperature between the different extraction methods that affect natural antioxidant content present thus leading to a lower scavenging ability/ % inhibition (Nwabuebo, 2017). Figure 15 below illustrates the components of a UV-Vis instrument.

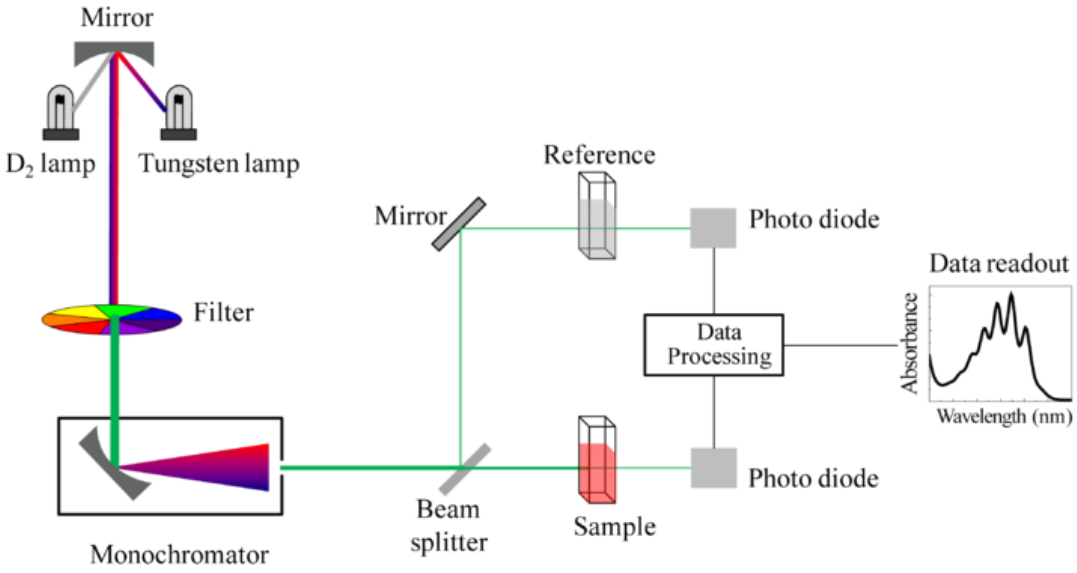

Figure 15: Schematic diagram of a UV-Vis spectrometer(Sobarwiki, 2013)

2.4.3 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique that has been used in different ways over the years to determine and monitor several different characteristics and trends in oils and fats (Tan and Che Man, 2000). It is a fast and sensitive technique that requires very little sample for analysis (*Differential Scanning Calorimetry for Routine Analysis*, 2015). DSC works on the basic principle that both a sample and reference sample are taken through a temperature programme and maintained at the same temperature throughout. It is the energy needed by the system to maintain both sample and reference at the same temperature that allows for the identification and quantification of physical

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transformations that are taking place in the sample by measuring the enthalpies associated with transitions and reactions and the temperatures at which these take place. Differences in heat flow take place when the analyte/sample absorbs or releases heat due to thermal effects such as melting, chemical reactions, crystallization, vaporization, polymorphic transitions, and many other processes (*Differential Scanning Calorimetry for Routine Analysis*, 2015). Due to the complex chemical profiles of oils, they do not have specific melting and freezing/ crystalizing temperature but rather thermal profiles showing associated changes in enthalpy and therefore phase transitions (Tan and Che Man, 2000). By determining the oxidation onset temperature of oils, the thermal stability can be measured and old or used oils can be distinguished from new or fresh oils (*Differential Scanning Calorimetry for Routine Analysis*, 2015).

Mohammadpour and co-workers made use of DSC to study the difference in thermal behaviour between Moringa peregrina extracted in different ways, namely ultrasound-assisted extraction and Soxhlet extraction. Melting profiles of the two samples both produced two exothermic peaks with onset temperatures of −12.5 °C and 6.4 C for UAE as well as −21 °C and 6.2°C for the Soxhlet method. Cooling profiles showed a single endothermic peak for both samples with onset temperatures of -37.4 °C for UAE and -35.9 °C for the Soxhlet extracted oil. This indicated that the UAE sample contained a higher degree of saturated fatty acids. In conclusion, they found that the different extraction techniques had no significant effect on the thermal properties of M. peregrina oil (Mohammadpour *et al.*, 2019).

Nyam and co-workers however used the DSC to compare thermal properties amongst different oils namely bittermelon, Kalahari melon, kenaf, pumpkin and roselle seed oils. Melting and heating profiles indicated that bittermelon seed oil crystallized at a lower onset temperature of -12.10 °C, and melted at a higher onset temperature of

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37.52 °C, in comparison to the other seed oils. A significant difference in melting and cooling profiles could be seen between different oils allowing this technique to be used as an identification analysis for unknown seed oil samples. According to the general trend that samples with a high content of saturated fatty acids produce DSC melting and cooling profiles with higher temperatures as compared to oil samples with a high content of unsaturated fatty acids, bittermelon seed oil contains a higher content of saturated fatty acids compared to the other oils studied (Nyam *et al.*, 2009). Figure 15 below illustrates the components of a DSC instrument.

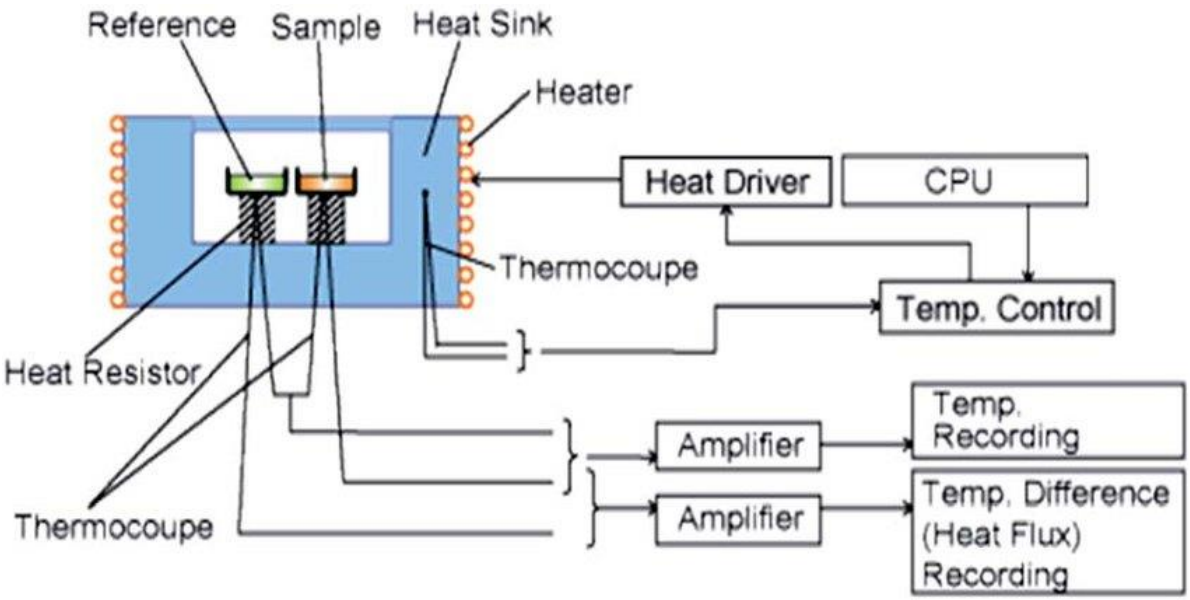

Figure 16: Schematic diagram of DSC(Bibi *et al*., 2015)

2.4.4 Fourier transform- Infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is a simple and fast analytical technique that can give accurate and reliable data. Fragments of structure can be elucidated due to characteristic vibrations, and quantitative information can be determined using peak intensity as a direct relationship to concentration. Characteristic absorption peaks at specific

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wavelength ranges take place due to the unique vibrational or rotational energy transformations a chemical bond in a molecule undergoes from the ground to the excited state when exposed to infrared light. The spectrum produced is thus a series function of absorbed energy responses. Due to these characteristic infrared absorption frequencies, each molecule, except for isomers, has a unique fingerprint spectrum. This can be used to identify molecules and confirm the purity of a sample (Laboratories, 2015; Li *et al.*, 2019).

Oji and Vivian (Oji and Vivian, 2020) used FT-IR to determine the compounds present in coconut, avocado and carrot oil. From the three FT-IR spectra it was evident that all three oils possessed similar major compounds. These functional groups include the O-H group of carboxylic acids aromatics and C-O stretch in esters. The aromatic overtone of ring bends band peak at 1744.4cm-1 was a deep peak for coconut and carrot oil however a narrow peak for avocado oil. Due to the striking similarities in the FT-IR spectra of coconut and carrot oil, it was very clear that coconut oil was used as the base oil in the extraction of carrot carrier oil (Oji and Vivian, 2020).Oladipo and Betiku (Oladipo and Betiku, 2019) used FT-IR as one of the characterisation techniques to assess the difference between *Moringa oleifera* seed oil extracted by Soxhlet extraction using three different solvents namely ethyl acetate, ethanol and n-hexane. The results showed equivalent peaks in all the spectra, indicating that the choice of solvent did not affect the functional groups of the oil samples (Oladipo and Betiku, 2019). Figure 17 below illustrates the components of an FT-IR instrument.

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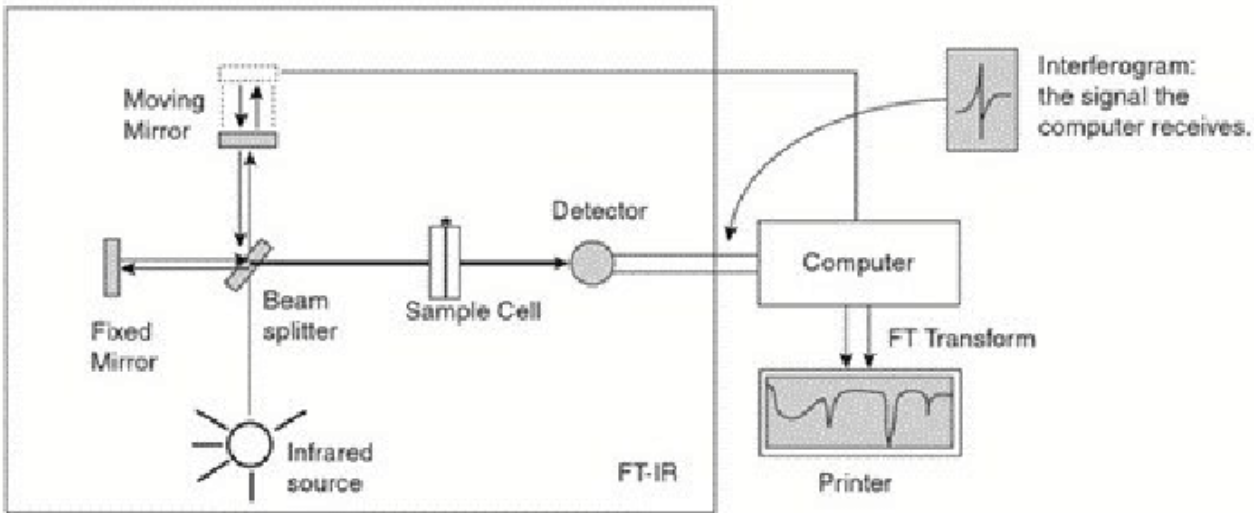

Figure 17: Schematic diagram of FT-IR(Bibi *et al*., 2015)

CHAPTER 3: AIMS AND OBJECTIVES

3.1 Aims

This study aimed to investigate the chemical composition of commercial Moringa, Marula, Kalahari melon, Mongongo, Baobab, and Hemp seed oil for use in cosmetics. This was achieved through the following objectives:

3.2 Objectives

- Extraction of Moringa seed oil using Cold-press, Soxhlet, Ultrasound-assisted and Shaker extraction techniques.
- Determination of the chemical profile of commercial Moringa, Marula, Kalahari melon, Mongongo, Baobab and Hemp seed oils.
- Understand from the chemical and physical composition, the strength of each oil in cosmetic industry applications.
- Trial formulation of a cosmetic product(s) incorporating these commercial seed oils using modified existing formulation.

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3.3 Motivation

The significance of this research study stems from the fact that high variability exists amongst seed oil literature currently available. An in-depth study on the chosen oils using the same seed source for extraction and the same analysis techniques significantly reduced variability and therefore allowed one to make more accurate comparisons between parameters of the different oils (Tan and Che Man, 2000). This broad study looked at MO oil extracted by four different methods, as well as profiling these oils alongside underutilised African oils in comparison to well-known commercial vegetable oils such as olive and sunflower oil. This study aimed to increase the database of knowledge available on underutilised African oils to assess their economic importance. Increasing commercialisation of these oils will benefit rural communities involved in harvesting, encourage maintenance of the natural environment, provide more export products and alternative natural ingredients for the cosmetic industry which will overall benefit our bioeconomy (Zimba *et al.*, 2005; Juliani *et al.*, 2007; Vermaak *et al.*, 2011). These non-traditional seed oil sources, if managed correctly, can satisfy the ever-growing demand for this important renewable commodity by supplementing the world's current vegetable oil supplies (Mitei *et al.*, 2008; Janaki, 2015).

CHAPTER 4: MATERIALS AND METHODS

4.1 Materials and reagents

All reagents and solvents used in this study were purchased from Merck as an analytical grade. Hexane, anhydrous sodium sulphate, ethanol, phenolphthalein, sodium hydroxide, potassium hydroxide, hydrochloric acid, calcium oxide, chloroform, iodine, potassium iodide, sodium thiosulphate, starch, acetic acid, 2, 2-diphenyl-1-picrylhydrazyl, ascorbic acid, ethanol, 5-α cholestane, campesterol, β- sitosterol,

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stigmasterol, $\alpha, \beta, \gamma \; and \; \delta$ tocopherol standards, Supelco 37 components FAMES mix. *Moringa oleifera* (MO) seeds and remaining seed oils were locally sourced. Cosmetic bases were purchased from Nautica organics. Working solutions were prepared by appropriate dilution of the stock solutions with solvent.

4.2 Extraction of *Moringa oleifera* seed oil

4.2.1 Sample preparation

MO seeds were sourced from Lefakong Farming based in Hammanskraal. The seeds were manually cracked and deshelled to obtain the kernel. The kernels were then air-dried over two days to reduce water content. The kernels (~1 kg) were then ground into a fine powder using a domestic blender and stored at -20 °C until extraction. Figure 18 below shows the state of the MO seed during sample preparation.



Figure 18: Illustration of the MO sample preparation steps from A- Whole seed, to B- De-shelled kernel and lastly C-Kernel/seed powder

4.2.2 Cold-press extraction

Cold-press extraction of MO oil was carried out at the Innovation Hub by Lefakong Farming. Whole MO seeds were used for extraction, after which the oil was filtered to remove impurities and stored in an amber glass bottle. In Figure 19 below the MO oil, the cold-pressing process is explained. A- Power was switched on; pressure and heat settings were adjusted. B- Whole MO seeds were added to a feed hopper. C- Milling/

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screw component then fed the seeds into the main component of the machine in a uniform manner. D- A controlled amount of seeds then entered the squeezing/ pressing chamber. E- The MO oil was then pressed out from the whole seeds. F- Crude oil drips out from below squeezing/ pressing chamber into a container fitted with a sieve to catch large seed material. G- Leftover seed material form solid seed cakes that were expelled at the end of the pressing process. H- Seed cake by-product to be used for animal feed. From this point, oil would then be filtered and packaged.

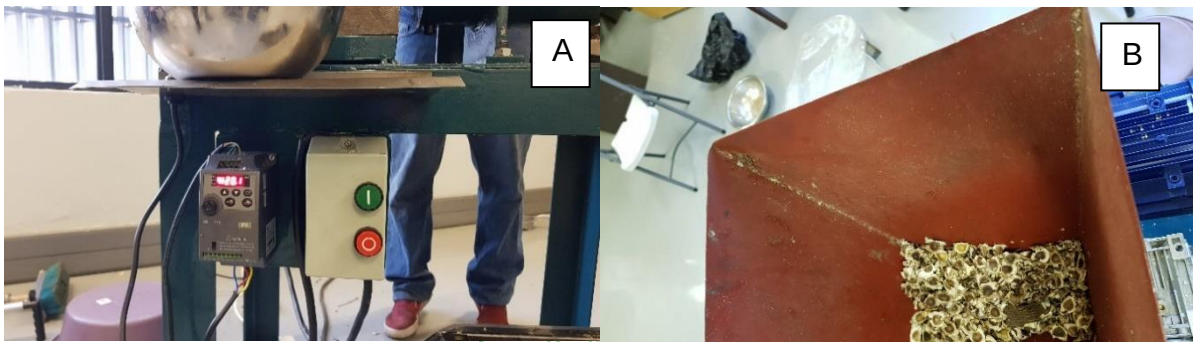













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Figure 19: Illustrations of the MO oil Cold-pressing process (A-H)

4.2.3 Soxhlet extraction

About 45 g of seed kernel powder was placed in filter paper inside a Soxhlet extractor with cotton wool at the base. A 500 mL round bottom flask containing boiling stones and 450 mL of hexane was attached below the Soxhlet extractor and a condenser attached above it. An oil bath was used to boil the solvent at 65 ℃ for six hours of extraction (Zhao and Zhang, 2013). Thereafter the solvent volume was reduced using a rotary evaporator at 65 ℃ (Yang *et al*., 2013; Adeniyi, 2019; Latimer, 2019). The mixture was then dried using anhydrous sodium sulphate and filtered into a pre-weighed 100 mL round bottom flask using filter paper to remove any solid impurities. The residual extraction solvent was then removed by once again using a rotary evaporator at 65 ℃ followed by placing the sample in an oven for four hours at 60 ℃ and by blowing with nitrogen gas. The extracted oil was stored in amber glass bottles and kept at 4 ℃ until further analysis. Figure 20 below illustrates the Soxhlet extraction set-up.



Figure 20: Images of Soxhlet extraction process where A= Soxhlet tube set up and B= Rotary evaporator set up

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4.2.4 Ultrasound-assisted extraction

The extraction method used was based on optimised conditions reported by Mohammadpour and co-workers. About 20 g of MO seed powder was mixed with 360 mL of hexane in a DURAN laboratory glass bottle which was secured with parafilm and foil and shaken for 10 minutes. The bottle was placed into the water bath set at 30 °C and sonicated for 30 minutes. Ice was used to maintain the water bath temperature throughout the extraction process. After extraction, the mixture was centrifuged to separate the seed sample from the solvent and filtered into a 500 mL round bottom flask. The solvent volume was reduced using a rotary evaporator at 65 °C (Yang *et al*., 2013; Adeniyi, 2019; Latimer, 2019). Contents were transferred to a pre-weighed 100 mL round bottom flask and residual extraction solvent was then removed by once again using a rotary evaporator at 65 °C followed by placing the sample in an oven for four hours at 60 °C and by blowing with nitrogen gas. The extracted oil was stored in amber glass bottles and kept at 4 °C until further analysis.

4.2.5 Hexane extraction at ambient temperature (shaker method)

About 120 g of MO seed powder was mixed with 300 mL of hexane in a DURAN laboratory glass bottle which was secured with parafilm and foil and shaken (Labcon 150 RPM) for 8 hours. After extraction, the mixture was centrifuged (Hettich zentrifugen ROTOFIX 32 A 4000 RPM) to separate the seed sample from the solvent and filtered into a 500 mL round bottom flask. The solvent volume was reduced using a rotary evaporator (BUCHI Switzerland Rotavapor RII) at 65 °C (Yang *et al*., 2013; Adeniyi, 2019; Latimer, 2019). Contents were transferred to a pre-weighed 100 mL round bottom flask and residual extraction solvent was then removed by once again using a rotary evaporator at 65 °C followed by placing the sample in an oven for four hours at 60 °C and by blowing with

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nitrogen gas. The extracted oil was stored in amber glass bottles and kept at 4 ℃ until further analysis (de Oliveira *et al.*, 2014; Nwabuebo, 2017).

4.2.6 Determination of percentage oil yield and efficiency

The oil yield was calculated using the following equation:

$$Oil\ yield = \frac{Mass\ of\ extracted\ oil}{Mass\ of\ seed\ sample} \times 100\%$$

The extraction efficiency was calculated based on the oil yield result obtained by Soxhlet extraction as this is the standard analytical method for the determination of oil content. The Soxhlet oil yield % was considered to be 100 % of the oil content in the seed. The extraction efficiencies of the other extraction methods were calculated in relation to the Soxhlet extraction using the equation(Nwabuebo, 2017):

$$Extraction\ effeciency = \frac{\%\ oil\ yield\ for\ other\ extraction\ method}{\%\ oil\ yield\ for\ soxhlet\ extraction} \times 100\%$$

4.3 Determination of physico-chemical characteristics

4.3.1 Oil preparation

Once room temperature, clear sediment-free oil samples were shaken up by hand used directly.

4.3.2 Colour and state of oils

The colour and state of the oils were determined by visual observation at 25 ℃ (Onyeike and Acheru, 2002).

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4.3.3 Determination of the acid value

The acid value (AV) was determined according to the AOAC Official Method 940.28 (Latimer, 2019). In a 100 mL conical flask, 1 g of oil was added, followed by 7 mL of alcohol previously neutralized by adding phenolphthalein solution and enough 0.10 M sodium hydroxide (NaOH) to produce a faint permanent pink. The mixture was vigorously stirred while being titrated with standardised 0.10 M NaOH until a permanent faint pink appeared and persisted for more than 1 min.

The AV was then calculated from the following equations (Wrolstad *et al.*, 2005; Cheikhyoussef, 2018):

$$\text{FFA as \% oleic acid} = \frac{\text{mL NaOH x normality of NaOH} \times 28.2}{\text{sample weight (g)}}$$

$$\text{Acid value (mg KOH / g oil)} = \text{\% FFA (as oleic acid)} \times 1.99$$

4.3.4 Determination of the saponification value and average molecular weight

The saponification value (SV) (mg KOH/g of oil) was determined according to the Association of Official Analytical Chemists (AOAC) Official Method: 920.160. Alcoholic potassium hydroxide (KOH) solution was prepared by adding crushed 20 g KOH and 22.5 g calcium oxide (CaO) to 500 mL alcohol. The mixture was shaken for 25 min and inverted several times during the day. The next morning the mixture was filtered and stored in a clean, dry, glass-stoppered bottle (Latimer, 2019).

Determination: 1 g of oil was weighed into a round bottom flask, 10 mL of alcoholic KOH was pipetted into the flask to dissolve the oil. The mixture was then refluxed for 30 min to completely saponify the oil. Once cool the saponified mixture was titrated with standardised 0.5 M hydrochloric acid

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(HCl) using a phenolphthalein indicator. The same procedure was followed for the blank omitting the oil.

Calculations:

$$\text{Saponification value (mg KOH /g of oil)} = \frac{(\text{V2} - \text{V1}) \times 56.1\text{N}}{\text{Sample weight (g)}}$$

Where N = Normality of HCl, V1 = Volume of HCl used in the sample titration and V2 = Volume of HCl used in the blank titration (Dangarembizi et al., 2015).

The average molecular weight (AMW) was calculated from the saponification value according to the following equation (Yang *et al.*, 2013; Cheikhyoussef, 2018):

Average molecular weight = (3*56.1*1000)/saponification value of oil

4.3.5 Determination of the ester value

Ester value (EV) was calculated from the difference between the determined saponification value (SV) and the acid value (AV) of the oil (Cheikhyoussef, 2018).

EV = SV – AV

4.3.6 Determination of the iodine value

The Iodine value (IV) (mg KOH/g of oil) was determined according to the Hanus Method AOAC Official Method: 920.158. Determination: Weighed 0.100 g into a 100 mL conical flask and dissolved it in 4 mL of chloroform ($CHCI_3$). Next, 10 mL of prepared Hanus diiodine (I_2) solution was pipetted into the flask and left to stand for 30 minutes in the dark. Thereafter 4 mL

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of 15% KI solution was added as well as 40 mL deionised (DI) water. The mixture was titrated with standardised 0.05 M sodium thiosulfate ($Na_2S_2O_3$) solution using a starch indicator. A blank run was performed using no oil.

The number of mL 0.05M $Na_2S_2O_3$ required by blank (B) minus mL used in sample determination (S) gives $Na_2S_2O_3$ equivalent of I_2 absorbed by the oil. Calculate % by weight of I_2 absorbed (I_2 number, Hanus method) :

$$\mathrm{I_2\ number} = \frac{(\mathrm{B} - \mathrm{S}) \times \mathrm{M} \times 12.69}{\mathrm{g\ test\ portion}}$$

Where M = molarity of $Na_2S_2O_3$ solution, B = Titer value of blank and S = Titer value of the sample.

4.3.7 Determination of the peroxide value

The peroxide value (PV) (milliequivalent peroxide/kg oil) was determined according to the AOAC Official Method: 965.33 (Latimer, 2019) in diffuse daylight. The acetic acid-chloroform solution was prepared by mixing three volumes acetic acid (CH_3COOH) with two volumes $CHCl_3$. Saturated potassium iodide (KI) solution was prepared by dissolving excess KI in distilled water until excess solid remained at the bottom of the amber bottle which was covered in foil. A 1% starch solution was prepared by dissolving 0.5 g soluble starch powder in 50 mL boiling water.

Determination: 1 g of oil was weighed into a 100 mL conical flask. 6 mL CH_3COOH - $CHCl_3$ was added to dissolve the oil. Next, 100 µL saturated KI solution was added, the mixture was swirled and left to stand for 1 minute before 6 mL of distilled water was added. The mixture was slowly titrated with standardised 0.01M $Na_2S_2O_3$, with vigorous shaking until pale yellow. 100 µL of 1% starch solution was then added, and titration

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continued until the blue colour just disappeared. The same procedure was followed for the blank omitting the oil. Calculations were as follows:

$$\text{Peroxide value (meq/ kg of oil)} = \frac{(\text{S} - \text{B}) \text{ x M x } 1000}{\text{Sample weight (g)}}$$

Where S= mL $Na_2S_2O_3$ for sample, B= mL $Na_2S_2O_3$ for blank, and M = molarity $Na_2S_2O_3$ solution.

4.3.8 Determination of density and specific gravity

The bulk density of seed oils was determined using a 1000 μL micropipette at room temperature calibrated using deionised water. Three successive additions of 500 μL of distilled water and samples were accurately weighed in a pre-weighed Eppendorf tube. Specific gravity was then calculated as the mass of seed oil divided by the mass of DI water at the same temperature (Gbogouri *et al.*, 2011). Calculations were as follows (Taylor, 1967; Paquot, 1979; Latimer, 2019):

Density=(W1-W0) / Volume of oil

Specific gravity = (W1-W0) / (W2-W0)

Where W_1 = weight of tube with oil, W_0 = weight of the empty tube, and W_2 = weight of the tube with water.

4.3.9 Determination of the refractive index

The refractive indices of the oil samples were determined according to AOAC Official Method 921.08 (Latimer, 2019) using an Abbe refractometer at 20 °C. Readings were standardised using DI water at 20 °C.

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4.4 Determination of thermal properties

The thermal properties of selected oils were studied using a differential scanning calorimeter (DSC). Differential scanning calorimetry data were collected using a Mettler Toledo DSC 3 with aluminium pans under nitrogen gas (flow rate = 10 mL/min). Exothermic events were shown as peaks. Samples were heated and cooled to determine melting points, recrystallization as well as any additional phase transitions. The temperature and energy calibrations were performed using pure indium (purity 99.99%, Melting point 156.6 °C, the heat of fusion: 28.45 J g^{-1}) and pure zinc (purity 99.99%, Melting point 479.5°C, the heat of fusion: 107.5 J g^{-1}). Samples were heated to 80 °C from 25 °C and held as an isotherm for 5 minutes before being cooled to -80 and held at that temperature for 5 min. Finally, the sample was heated back to 25 °C. The heating/cooling rate was done at 5 °C/min. Figure 21 below illustrates the DSC instrument used in this study.



Figure 21: Illustration of the DSC instrument used in this study

4.5 Fourier Transform-Infrared Spectroscopy analysis

Fourier transform infrared spectroscopy (FT-IR) analysis was used to expose the functional groups present in the twenty-three oil samples

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(Prakash Maran *et al*. 2015). The FTIR spectra of extracted oil samples were recorded using a Bruker Tensor 27 FT-IR spectrophotometer. The FT-IR spectrum was reported in % transmittance. The wavenumber region for the analysis was 4000-400 cm^{-1}. Figure 22 below illustrates the FT-IR spectrophotometer used in this study



Figure 22: Illustration of the FT-IR spectrophotometer set-up

4.6 Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR and ^{13}C NMR spectroscopic analysis were performed once per selected sample using the Bruker Avance 500 MHz spectrometer (Germany) to determine acyl composition of selected seed oils using deuterated chloroform ($CDCl_3$) solvent at 25℃. Data were recorded as "chemical shifts (δ)" reported in ppm (Cheikhyoussef, 2018). Figure 23 below illustrates the NMR instrument used in this study.

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Figure 23: Illustration of the NMR set-up

4.7 Determination of fatty acid content

A stock solution of standard fatty acid methyl esters (FAMEs standard mix, 37 components) was prepared with high - performance liquid chromatography (HPLC) grade hexane and used as a reference for identification and quantification of the fatty acid content in the various oils. The twenty-three oil samples were prepared as follows: about 50 mg of oil was dissolved in 5 mL hexane in a centrifuge tube followed by adding 0.5 mL potassium hydroxide methanol solution (2 M). The tube was capped and vigorously shaken (Labcon 150 revolutions per minute (RPM)) for 15 min and then centrifuged (Hettich zentrifugen ROTOFIX 32 A 4000 RPM) for 15 min. A portion of the top hexane layer containing the FAME's was removed into a gas chromatography (GC) vial and briefly stored at -20 °C

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until analysis (Yang *et al.*, 2013; Cheikhyoussef, 2018). Instrumentation details were as follows gas chromatography electron capture detector (GC-ECD) Agilent Technologies, (7890A) with a BPX5 capillary column (29.616 x 0.25 mm ID, 0.25 m film thickness) and Chemstation software was used for analysis. An injection volume of 1 µL was used, oven temperature programme started with an initial target temperature of $50\,°C$ for 1 min., increased to 150 $°C$ at 15 $°C$/min and lastly to 250 $°C$ at 5 $°C$/min for 5 min. with a solvent delay of 1.7 min. The carrier gas used was helium at a flow rate of 1.4 mL/min with an injector temperature maintained at 250 $°C$ and operated in a splitless mode. The transfer line temperature was maintained at 285 °C. Figure 24 below illustrates the gas chromatography – mass spectrometry (GC-MS) instrument used in this study.



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Figure 24: Illustration of the GC-MS set-up

4.8 Effect of sunlight exposure

Samples of selected oils were placed in clear glass vials and left on a laboratory benchtop exposed to full natural sunlight. Physical observations; namely physical state, colour and odour; were made at the start of the study and changes were noted accordingly over various time intervals. Figure 25 below is an illustration of the various samples at time zero (T0) of the sunlight exposure study.

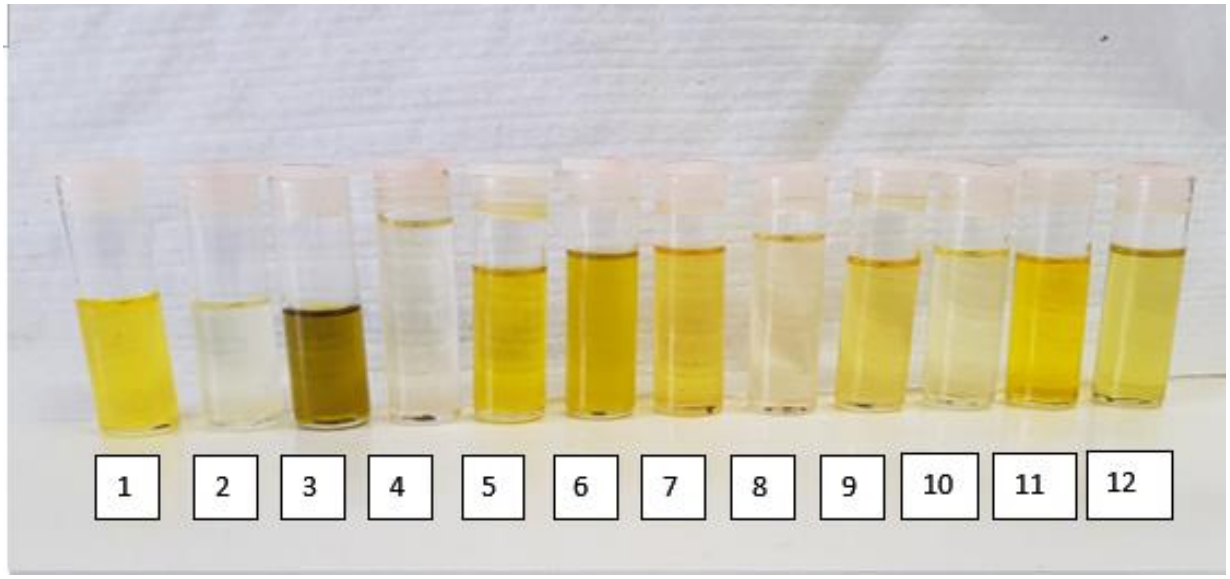

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Figure 25: Illustration of the selected oils at the start of the sunlight exposure study (14/12/2020). Where 1= Jojoba seed oil. 2= Grapeseed oil, 3= Avocado oil, 4= Sunflower oil, 5= Sunflower oil, 6= Lefakong Farming cold-press MO oil, 7= Bright mountains MO oil, 8= Marula Seed Oil, 9= Mongongo Seed oil, 10= Kalahari Melon Seed Oil, 11= Baobab Seed Oil, and 12= Hemp seed oil

4.9 Determination of radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The radical scavenging activity of each oil was determined by monitoring the decrease in absorbance at 517 nm using an Agilent Technologies Cary Series ultraviolet – visible (UV-Vis) Spectrophotometer. A 5 mL freshly prepared 0.01 mM ethanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was added to 5 mL of a 5 mg/mL extracted oil sample. The sample was mixed thoroughly and left to stand for thirty minutes in the dark to allow the radical-antioxidant reaction to occur. Thereafter, the absorbance was measured at 517 nm against a blank in triplicate. A synthetic antioxidant, ascorbic acid was also prepared in the same manner as the oil sample and used as a positive control or reference standard for this assay (Brand-Williams, Cuvelier and Berset, 1995; Delfan-Hosseini *et al.*, 2017; Nwabuebo, 2017). A further investigation was done on a selected oil exposed to different storage conditions to see its impact on radical scavenging activity.

4.10 Statistical methods and analysis

Experiments were carried out in triplicate unless otherwise stated, and means were compared with an analysis of variance (ANOVA) on Microsoft excel. Extracted MO oil data were further analysed with the Tukey test using excel. A probability value at $P<0.05$ was considered a statistically

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significant difference. A covariance matrix of results was conducted for quantitative parameters.

4.11 Trial formulation application of cosmetic products

Cosmetic bases purchased from Nautica Organics; namely a cream, lotion, and castile soap, were modified with 1% of a MO and Kalahari melon seed oil bend (1:1). The control samples (unmodified sample) and modified samples were given to a few friends and family members with different skin types in neutral packaging with no indication of the differences between the samples. They were then asked to use the two samples in the same category on equal but opposite parts of the body and give basic feedback on skin feel and the overall impression of which one they would purchase (Appendix E). This simple application was done to physically feel the effect of seed oils added to cosmetic formulations to get an understanding of consumer preference.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 *Moringa oleifera* oil extraction yield and efficiency percentages

Amongst the extraction techniques, Soxhlet extraction of *Moringa oleifera* (MO) gave the highest % yield of 32.50 ± 1.04 % in comparison to the other techniques. According to de Oliveira and co-workers, this means that Soxhlet extraction is the most economically viable technique. This was due to the use of heat which increases solubility and diffusivity which improves mass transfer (Bhutada et al., 2016). This was also due to the high purity of the solvent during each Soxhlet extraction reflux causing a high concentration gradient between seed sample and extraction solvent favouring oil solubility (de Oliveira *et al.*, 2014). A common difficulty found with Soxhlet, Ultrasound-assisted and Shaker method extraction was

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residual solvent. The oil produced from these methods had a soft nutty smell with a slight solvent smell as opposed to the cold-pressed oil which purely had a nutty smell. According to the ANOVA single factor test, the % yield, as well as efficiency % results, are significantly different from each other (p-value =3.92 $x10^{-3}$). For both % yield and % extraction efficiency results obtained for Soxhlet extraction was not significantly different ($p<0.05$) to those obtained for shaker method extraction, however they were significantly different ($p<0.05$) to the results obtained for Cold-press and Ultrasound-assisted extraction as indicated in Table 8 below.

For Soxhlet extraction, average literature values (Table 7) were 32.87 ± 10.11% and 100.00 ± 1.00% for % yield and % extraction efficiency respectively whereas experimentally they were found to be 32.50 ± 1.04 % and 100 % respectively which was very similar. Ultrasound-assisted extraction gave a % yield and % extraction efficiency lower than the average literature values. Cold-press and shaker method extraction of MO oil however gave higher experimental values than those in literature. Cold-press extraction gave experimental values of 28.77 % and 88.52 % for % yield and % extraction efficiency respectively whereas in literature averages of 26.3 % and 74.5% respectively were obtained (Tsaknis *et al*., 1998). Shaker method extraction gave experimental values of 30.47 ± 0.67 % and 93.76 % for % yield and % extraction efficiency respectively whereas in literature Nwabuebo obtained 27.19 ± 0.91% and 77.01 ± 0.55 % respectively (Nwabuebo, 2017).

Discrepancies between experimental values and literature values may be caused by several factors such as differences in experimental techniques due to differences in apparatus and equipment available as well differences in seed source. Seeds from different regions have different oil content due to factors such as water availability, soil conditions, nutrients, seasonal temperatures, and as well as the maturity of the trees (Anwar and Bhanger, 2003).

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Table 8: MO oil extraction yield and extraction efficiency percentages

Extraction method	% Yield	%Extraction efficiency
Cold-press	28.77[b]	88.52[b]
Soxhlet	32.50 ± 1.04[a]	100[a]
Ultrasound	28.55 ± 0.14[b]	87.83[b]
Shaker	30.47 ± 0.67[a,b]	93.76[a,b]

Values are mean ± SD of MO oils from different extraction methods analyzed in triplicate except for the cold-press method. Mean values in the same column followed by different superscript letters are significantly different ($p<0.05$) for the oil yield and extraction efficiency %, respectively.

5.2 Physico-chemical characteristics of the various oils

In Table 9 below; physico-chemical characteristics of the twenty-three various oils studied are presented; namely, the extracted four *Moringa oleifera* (MO) oils, nine commercially available MO oils, Kalahari melon, Marula, Baobab, Mongongo, Hemp, Olive, Sunflower, Jojoba, Grapeseed and Avocado oil.

5.2.1.1 Acid value

The chemical property acid value (AV) is a relative measure of an oil's rancidity at the time of analysis and therefore determines the quality of the oil. AV reflects the total acidity of the oil sample; therefore a lower value indicates less degradation and therefore higher quality of the oil and vice versa. Many factors contribute to this parameter such as seed source, extraction method, water content which results in hydrolytic degradation, storage conditions (sunlight, temperature etc.) etc.(Nwabuebo, 2017). Extraction conditions namely temperature and organic solvent negatively affect the natural antioxidants content in oils which reduces their ability to scavenge the free fatty acids present thereby leading to a lower quality of oil (Kaul *et al*., 2009). Oils with higher AVs would be better suited to anhydrous cosmetic formulations as the presence of water may accelerate their degradation and poorly affect the quality of the final cosmetic product over time.

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Amongst the extracted MO oils results obtained at the time of analysis, Shaker extracted MO AV is significantly different to Soxhlet, Ultrasound-assisted and Cold-pressed MO oil. The value obtained for Shaker extraction, 1.41 ± 0.15 mg KOH/g oil, was by far the highest out of the extracted MO oils however lower than the result obtained by Nwabuebo which was 3.31 ± 0.06 mg KOH/g oil (Nwabuebo, 2017). Many of the commercially purchased *Moringa oleifera* (MO) oils had very high AV results in comparison to the MO oil samples extracted for this study, namely Moyo moringa SA- Moringa Behen oil for skin, Leaf- Moringa seed oil, Moringa 5000 Pure Moringa Ben oil, Bright mountain - Moringa Seed Oil, and Karibaa - Moriveda moringa basic oil. This was expected since the duration from oil extraction to AV analysis was shorter for the extracted MO oil samples compared to the commercially available MO oils. In other words, the longer storage period undergone by these commercially available MO oils was most likely the reason for the higher AV results (Oomah *et al.*, 2002).

5.2.2 Saponification value and Average molecular weight

The chemical property saponification value (SV) is a measure of the chain length of fatty acids present in the oil and is used to calculate an average molecular weight (AMW). SV and AMW are inversely proportional such that higher SVs, which indicate shorter fatty acid chains, produce lower AMWs and vice versa. Low AMW may also indicate the formation of lower molecular weight oxidation products such as aldehydes and carboxylic acids (Chindo *et al.*, 2010). SV would be determined by the oil's inherent fatty acid content and may therefore be affected by seed source, which is influenced by growing conditions, geographical location etc.

It is known that oils with higher SV and therefore lower AMW are better suited for soap making due to their chain length (Ilesanmi *et al.*, 2021). The specific SV range optimal for soap making is 188 to 199 mg KOH/ 1g

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oil (Francis and Tahir, 2016). Therefore from the oils studied Plush Organics Moringa and Hemp seed oil having SV of 189.23 ± 0.95 mg KOH /1g oil and 195,05 ± 0.56 mg KOH /1g oil respectively would be most suitable for application in soap making. Amongst the extracted MO oils, all of which were significantly different from each other, Lefakong farming cold-pressed MO had the highest SV and therefore lowest AMW of 176.83 ± 0.59 mg KOH /1g oil and 951.78 ± 3.16 g/mol respectively. These values are most similar to Marula, Grapeseed and Olive oil. The results differ significantly from Özcan and co-worker's findings of 181.3 ± 3.67 mg KOH /1g oil and 928.30 g/mol respectively however they are more comparable to the findings by Tsaknis and co-workers of 179.80 mg KOH /1g oil and 936.04 g/mol respectively for cold-pressed MO oil.

5.2.3 Ester value

The chemical property ester value (EV) is the amount of mg KOH required to saponify esters contained in one gram of fat or oil. The greater the number, the higher the ester content of the oil sample (Istiningrum *et al.*, 2017). It is also a measure of the amount of saponifiable glyceride that is present in the oil (Dileesh *et al.*, 2013). Since this parameter is calculated using acid value (AV) and saponification value (SV), variables affecting these parameters will influence the EV result.

The simulated EV result obtained by Özcan and co-workers for cold-pressed *Moringa oleifera* (MO) oil is 181.11 ± 3.67 mg KOH/g oil. This is higher than the value obtained for Lefakong farming cold-pressed MO oil of 175.97 ± 0.58 mg KOH/g oil this is due to the differences in AV and SV as beforementioned.

5.2.4 Iodine value

The chemical property iodine value (IV) is an indication of the degree of unsaturation within the oils. The higher the IV, the more unsaturated the

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fatty acids are in the oil making the oil more susceptible to oxidation and free radical production which overall makes the oil less stable compared to oils with lower IVs (Yang *et al.*, 2013). Since IV depends on the saturation of the fatty acids present in the oil, this parameter may vary with seed source, which is influenced by growing conditions, geographical location etc. It is also affected by age, storage conditions as well as exposure to heat and therefore the extraction method used (Nkafamiya *et al.*, 2007; Alajtal *et al.*, 2018).

Oils with IV's in the range 130- 200 g/100g are classified as drying oils; those with IV's between 90-130 g/100g are semi-drying oils whereas those with IV's below 90 g/100g are considered as non-drying oils (Lambert J, 1979). Oils with IVs between 100- 150 g/100g are said to be useful to soap making due to their oxygen-absorbing properties (Anhwange *et al.*, 2010). According to this classification and the obtained results *Moringa oleifera* (MO), Marula and Olive oil are non-drying. Baobab, Avocado and Jojoba oils are semi-drying. Kalahari melon, Mongongo, Hemp, Grapeseed and Sunflower oil are drying and fall in the range most useful for soap making.

The general IV range for MO oil (65-88 g/100g) was comparable only to Marula seed oil. Amongst the extracted MO oils there was notable variability as only Soxhlet extracted MO and Ultrasound-assisted MO are not significantly different. From the solvent extracted techniques (Soxhlet, Ultrasound-assisted and Shaker method) there was an increase in IV with an increase in temperature used during the extraction. Amongst the extracted MO oils, Lefakong farming cold-pressed MO oil had the highest IV value of 74.99 ± 0.82 g/100g which is higher than the value reported by Özcan and co-workers (67.53 ± 1.37 g/100g). This discrepancy may be caused by the variables affecting this parameter beforementioned.

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5.2.5 Peroxide value

Peroxide value (PV) is a chemical characteristic used to determine the extent of oxidative deterioration of the oil at the time of analysis. It gives a quantitative measure of the products of primary oxidation in the oil. Therefore, a higher PV indicates more extensive deterioration of the oil at the time of analysis. The rate of oxidation is affected by the age of the oil, quality of seed source as well as exposure to temperature, light, moisture, metals and oxygen (Nwabuebo, 2017).

Amongst the extracted *Moringa oleifera* (MO) samples Lefakong farming cold-press MO oil was significantly higher than the other samples most likely caused by the age of the oil in comparison to the other three methods samples. From the solvent extracted techniques (Soxhlet, Ultrasound-assisted and Shaker method) the PV value for Soxhlet (1,75 ± 0,01 mequiv/kg) was higher than Ultrasound-assisted extraction (0,97 ± 0,18 mequiv/kg) and Hexane shaker method extraction (1,54 ± 0,33 mequiv/kg). Although both Ultrasound-assisted extraction and Hexane shaker method extraction do not use high heat for the extraction process, there is a higher PV for the Shaker method sample. This was most likely caused by the longer extraction time used for the shaker method as well as the exposure to sunlight in the shaker instrument.

In general, the commercially available MO oils that were purchased had a higher PV (1.80-63.68 mequiv/kg) as opposed to the extracted MO samples (0.97-6.42 mequiv/kg). The reason for this would most likely be the difference in age of the oils as well as possible poor storage conditions before purchase causing further deterioration of these oils.

5.2.6 Density and specific gravity

Density and specific gravity are physical properties that give information on how heavy the oil is. These characteristics affect the sensoriality

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properties of the oil on its own and in cosmetic products, it also affects industrial processing in terms of moving the oils through pipelines and the force needed for mixing etc.(Alomair *et al.*, 2016). Density and specific gravity are affected by temperature such that density decreases with an increase in temperature (Dinesha *et al.*, 2018). Since density is directly proportional to mass, any sediment in the oil would give a false-positive result and likewise, density is inversely proportional to volume, therefore, any residual solvent may give a false-negative result.

The lowest density and specific gravity values belonged to Jojoba seed whereas the highest was obtained by Mongongo seed oil. Amongst the extracted *Moringa oleifera* (MO) there is no significant difference between the different extraction techniques. This indicated that different extraction temperatures did not affect the density and specific gravity which was in agreement with the findings of Adejumo and co-workers. Özcan and co-workers only reported a density value for cold-pressed MO of 0.819 ± 0.013 g/ml which is lower than the density value of Lefakong farming cold-pressed MO oil of 0.84 ± 0.20 g/ml which was most likely due to the sediment that was present in this sample.

5.2.7 Refractive index

The physical property refractive index (RI) is a dimensionless number that describes how radiation such as light, propagates through a medium. For oils, RI is characteristic and therefore indicates the purity of the oil as well as the rancidity development in oil (Sarkar *et al.*, 2015). The RI of oils is related to the degree of saturation as well as free fatty acid content, oxidation, and heat treatment (Ethiopia, 2018). An increase in the degree of saturation increases iodine values and therefore increases the refractive index of the seed oil (Mitei *et al.*, 2008).

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Amongst the significantly different extracted *Moringa oleifera* (MO) RI values, the shaker method extracted MO oil had the lowest RI value of 1.46 ± 0.00. This may be due to a higher amount of residual solvent being present in the batch analysed as hexane has a RI of 1.375. RI results in Table 9 below follow the same trends as mentioned for iodine value above such that the same oil samples fall in the categories of drying, semi-drying and non-drying which as beforementioned affects the usefulness of the oil in soap making.

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Table 9: Physico-chemical characteristics of the various oils

Sample	**Acid value (mg KOH/g oil)**	**Saponification value (mg KOH /1g oil)**	**Average molecular weight (g/mol)**	**Ester value (mg KOH/g oil)**	**Iodine value (g/100g)**	**Peroxide value (meq/kg oil)**	**Density (g/ml)**	**Specific gravity**	**Refractive index**
Soxhlet extracted MO oil	0.66 ± 0.08[a]	166.00 ± 0.28[a]	1013.84 ± 1.72[a]	165.40 ± 0.28	70.31 ± 0.89[a]	1.75 ± 0.01[a]	0.86 ± 0.06[a]	0.86 ± 0.06	1.47 ± 0.00[a]
Shaker extracted MO oil	1.41 ± 0.15[b]	160.48 ± 0.45[b]	1048.76 ± 2.93[b]	159.06 ± 0.42	65.93 ± 0.80[b]	1.55 ± 0.33[a]	0.84 ± 0.02[a]	0.84 ± 0.02	1.46 ± 0.00[b]
Ultrasound extracted MO oil	0.74 ± 0.06[a]	170.60 ± 0.55[c]	986.53 ± 3.20[c]	169.86 ± 0.55	69.27 ± 0.31[a]	0.97 ± 0.18[a]	0.89 ± 0.06[a]	0.89 ± 0.06	1.47 ± 0.00[c]
Lefakong Cold-pressed MO oil	0.85 ± 0.06[a]	176.83 ± 0.59[d]	951.78 ± 3.16[d]	175.97 ± 0.58	74.99 ± 0.82[c]	6.42 ± 0.62[b]	0.84 ± 0.20[a]	0.85 ± 0.20	1.47 ± 0.00[d]
Moyo moringa SA- Moringa Behen oil for skin	8.30 ± 0.23	180.29 ± 0.87	933.49 ± 4.50	172.51 ± 0.84	74.95 ± 0.89	63.68 ± 0.23	0.86 ± 0.06	0.87 ± 0.06	1.47 ± 0.00
Leaf- Moringa seed oil	2.57 ± 0.03	177.70 ± 0.42	947.09 ± 2.23	175.13 ± 0.42	75.61 ± 0.16	26.26 ± 0.52	0.84 ± 0.02	0.84 ± 0.02	1.47 ± 0.00
Essentially Natural Moringa oil	0.67 ± 0.05	180.32 ± 0.67	933.37 ± 3.48	179.64 ± 0.67	89.55 ± 0.80	44.96 ± 0.89	0.86 ± 0.03	0.86 ± 0.03	1.47 ± 0.00
Moringa 5000 Pure Moringa Ben oil	2.25 ± 0.13	175.92 ± 0.89	956.73 ± 4.81	173.67 ± 0.88	73.44 ± 0.58	21.85 ± 0.16	0.85 ± 0.02	0.85 ± 0.02	1.47 ± 0.00
Bright mountain - Moringa Seed Oil	6.86 ± 0.42	179.83 ± 0.84	935.91 ± 4.36	172.97 ± 0.73	76.18 ± 0.47	1.80 ± 0.21	0.83 ± 0.02	0.84 ± 0.02	1.47 ± 0.00
Karibaa - Moriveda moringa basic oil	1.57 ± 0.21	173.59 ± 0.99	969.55 ± 5.57	172.02 ± 0.97	77.59 ± 0.68	14.53 ± 0.21	0.86 ± 0.01	0.86 ± 0.01	1.47 ± 0.00
Plush Organics Moringa	0.48 ± 0.04	189.23 ± 0.95	889.39 ± 4.47	188.76 ± 0.95	88.49 ± 0.69	14.41 ± 0.16	0.85 ± 0.01	0.85 ± 0.01	1.47 ± 0.00

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Tree of life Pure organic moringa oil	0.91 ± 0.02	171.39 ± 0.60	982.00 ± 3.42	170.47 ± 0.59	76.31 ± 0.30	21.07 ± 0.56	0.84 ± 0.03	0.85 ± 0.03	1.47 ± 0.00
Moringa world- MO oil	1.14 ± 0.12	180.40 ± 0.42	932.93 ± 2.15	179.25 ± 0.40	72.98 ± 0.81	9.28 ± 0.58	0.87 ± 0.02	0.87 ± 0.02	1.47 ± 0.00
Baobab Seed Oil	0.40 ± 0.01	183.75 ± 0.99	915.95 ± 4.94	183.35 ± 0.99	91.30 ± 0.98	6.67 ± 0.13	0.83 ± 0.01	0.83 ± 0.01	1.47 ± 0.00
Kalahari Melon Seed Oil	0.46 ± 0.05	204.25 ± 0.42	823.97 ± 1.69	203.79 ± 0.42	131.90 ± 0.76	5.73 ± 0.06	0.88 ± 0.01	0.89 ± 0.01	1.48 ± 0.00
Marula Seed Oil	2.95 ± 0.13	176.16 ± 0.73	955.37 ± 3.95	173.21 ± 0.72	79.48 ± 0.36	1.17 ± 0.27	0.90 ± 0.05	0.90 ± 0.05	1.47 ± 0.00
Mongongo Seed oil	0.67 ± 0.06	202.28 ± 0.33	832.00 ± 1.38	201.61 ± 0.33	133.70 ± 0.26	25.91 ± 0.44	0.91 ± 0.05	0.91 ± 0.05	1.48 ± 0.00
Hemp seed oil	0.43 ± 0.01	195.05 ± 0.56	862.85 ± 2.49	194.62 ± 0.56	147.66 ± 0.97	12.54 ± 0.53	0.87 ± 0.02	0.88 ± 0.02	1.48 ± 0.00
Avocado oil	2.16 ± 0.09	181.33 ± 0.90	928.17 ± 4.61	179.17 ± 0.90	90.92 ± 0.65	12.42 ± 0.79	0.88 ± 0.01	0.88 ± 0.01	1.47 ± 0.00
Grapeseed oil	0.17 ± 0.02	176.07 ± 0.46	955.88 ± 2.48	175.90 ± 0.46	142.86 ± 0.73	9.07 ± 0.16	0.88 ± 0.01	0.88 ± 0.01	1.48 ± 0.00
Jojoba seed oil	0.58 ± 0.01	90.03 ± 0.58	1869.45 ± 12.07	89.45 ± 0.58	90.87 ± 0.24	0.97 ± 0.08	0.81 ± 0.01	0.81 ± 0.01	1.47 ± 0.00
Sunflower oil	0.30 ± 0.03	183.49 ± 0.98	917.25 ± 4.91	183.19 ± 0.98	130.67 ± 0.52	13.23 ± 0.08	0.87 ± 0.01	0.87 ± 0.01	1.48 ± 0.00
Olive oil	0.85 ± 0.03	173.22 ± 0.17	971.62 ± 0.97	172.36 ± 0.17	89.62 ± 0.86	14.61 ± 0.46	0.87 ± 0.01	0.87 ± 0.01	1.47 ± 0.00
p-value (ANOVA)	1×10^{-51}	3×10^{-65}	4×10^{-73}	N/A	5×10^{-69}	2×10^{-68}	0.89	N/A	2×10^{-81}

Data are shown as means with ± SD of three replicates. The p-value between sample groups determined using the ANOVA single factor test are presented where samples values within each parameter are significantly different if $p<0.05$ except for EV and SG as they are calculated using averages and therefore have no replicates. Extracted MO oil means with different letters in the same row are significantly different ($p<0.05$) as determined with Tukey's test for EV and SG as they are calculated using averages and therefore have no replicates.

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5.3 Thermal properties of selected oils

Figure 26 below shows the DSC cooling profiles of Cold-pressed MO, Soxhlet extracted MO, Baobab and Kalahari melon seed oil. This information correlates to the summary in Table 10. Figure 27 below shows the DSC heating profiles of Cold-pressed MO, Soxhlet extracted MO, Baobab and Kalahari melon seed oil. This information correlated to the summary in Table 11.

The enthalpy values indicated whether the phase change experienced by the oil took place by absorbing energy in other words endothermically or by releasing energy in other words exothermically (Gbogouri *et al.*, 2011). For the cooling profiles (Figure 26) there were two exothermic peaks, which indicated crystallisation, for cold-pressed MO, Kalahari melon and Soxhlet extracted MO oil whereas only one exothermic peak for baobab oil. For the heating profiles (Figure 27) there were four endothermic peaks for all the oils which indicated melting of the crystalline fraction.

Gbogouri and co-workers obtained similar DSC results for Kalahari melon seed oil of two exothermic peaks for the cooling profile and four endothermic peaks for the heating profile. The cooling profiles exothermic peaks occurred at -10.2$\pm$0.2 $°C$ and -39.5 $\pm$ 0.5 $°C$, and the heating profiles endothermic peaks occurred at -36.5$\pm$1.5 $°C$, -18.1$\pm$0.3 $°C$, -8.8$\pm$0.1 $°C$, and -1.6$\pm$0.1 $°C$. In this study, the Kalahari melon seed oil cooling profile had exothermic peaks that occurred at -6.96 $°C$ and -29.43 $°C$, and the heating profile had endothermic peaks occurred at -46.03 $°C$, -31.32 $°C$, -16.05 $°C$, and -12.28 $°C$.

Abdulkarim and co-workers, as well as Zhong and co-workers previously analysed Soxhlet extracted *Moringa oleifera* seed oil, amongst other oils, using DSC. Abdulkarim and co-workers reported three endothermic peaks in the heating profile at -24.95 ℃, -12.6 ℃ and 14.7 ℃. Whereas Zhong

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and co-workers reported three endothermic peaks in the heating profile at −17.8 °C, -0.35 ℃ and 23.1℃, as well as two exothermic peaks in the cooling profile at 4 ℃ and −37 ℃. In this study, DSC results for Soxhlet extracted MO oil displayed four endothermic peaks in the heating profile at −20.43 °C, -12.96 °C, 5.71℃ and 17.10℃ and two exothermic peaks in the cooling profile at 6.34℃ and −35.32 °C.

The multiple transition temperatures are said to have been caused by the oil sample's unique triacylglycerol (TAG) composition as well as the melting-recrystallization of the original TAG crystallites and their subsequent melting known as the polymorphism phenomenon (Tan and Che Man, 2000; Gbogouri *et al.*, 2011). Therefore, when heated, the lowest temperature transitions are caused by the least stable polymorphs melting. The transitions that occur at higher temperatures are then caused by the remaining TAG rearranging and recrystallizing into more stable polymorphs. From both sets of data, there are clear differences between the different types of oils namely moringa, baobab and Kalahari melon seed oil. However, there were slight differences between the two moringa oil samples that were extracted in different ways namely by Soxhlet extraction and cold-pressing. This is in agreement with conclusions made by Mohammadpour and co-workers who studied Moringa peregrina oil extracted with different techniques namely Ultrasound-assisted extraction and Soxhlet extraction.

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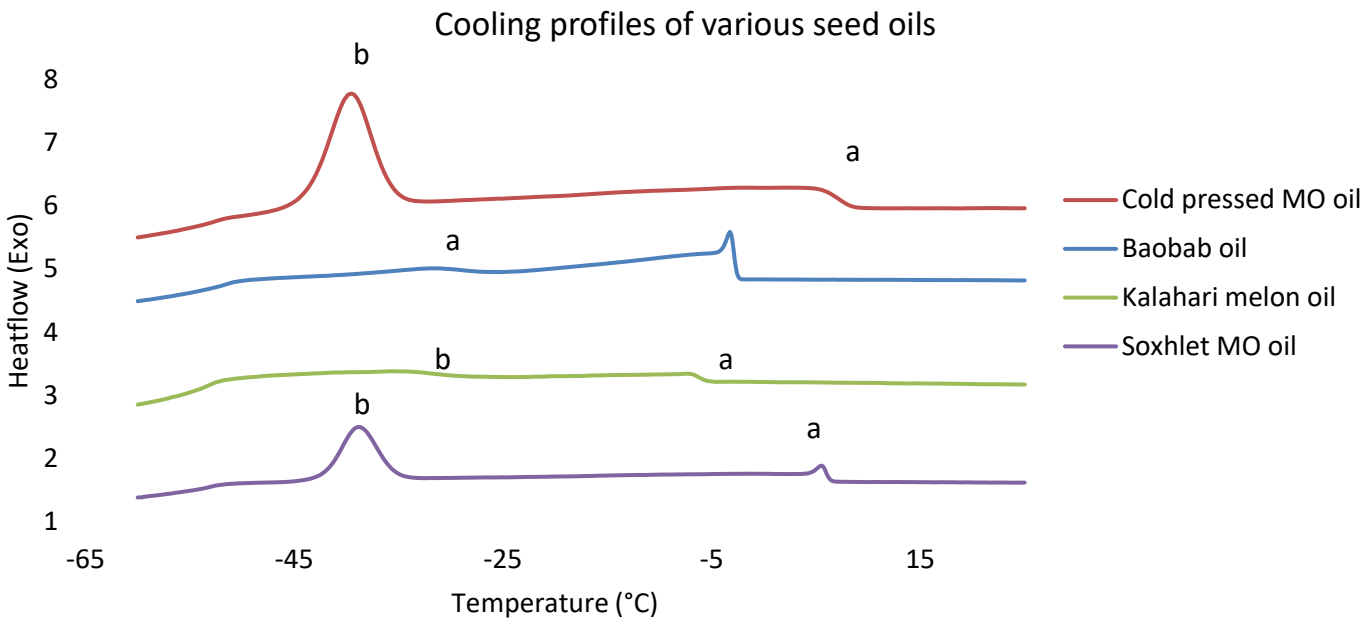

Figure 26: DSC cooling profile of various seed oils

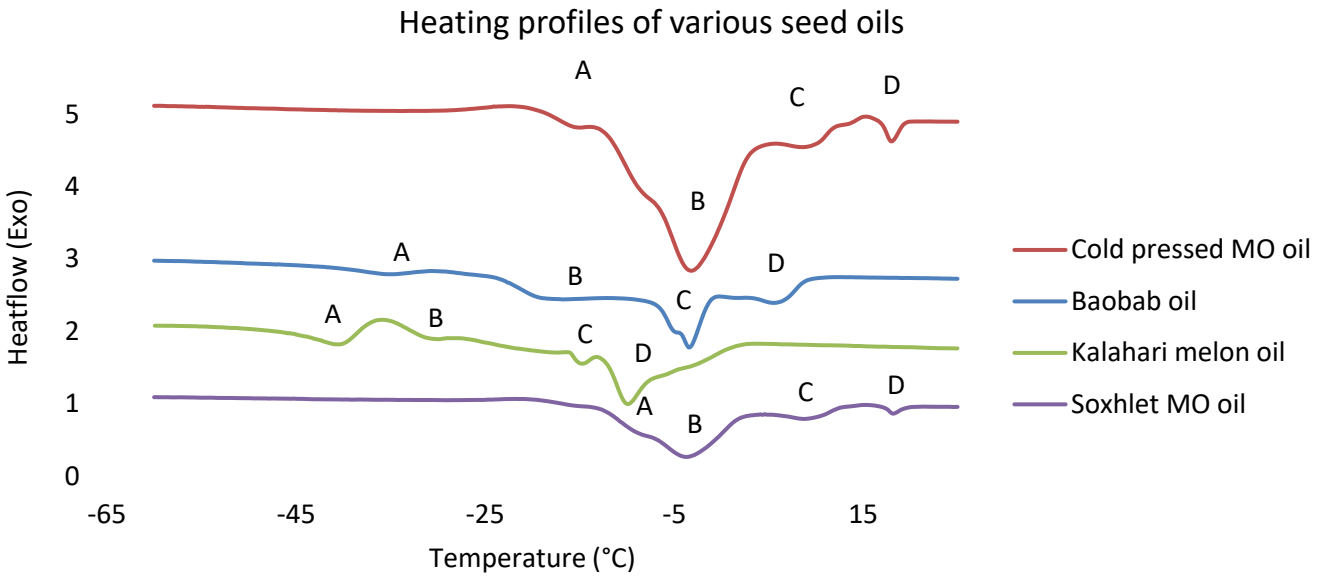

Figure 27: DSC heating profile of various seed oils

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Table 10: Transition temperatures during cooling processes of various seed oils

Sample	Transition	Onset temperature (°C)	Enthalpy ∆H (J/g)
Cold-pressed MO oil	a	8.58	N/A
	b	-35.51	30.34
Baobab oil	a	-26.30	8.26
Kalahari melon oil	a	-6.96	N/A
	b	-29.43	14.81
Soxhlet extracted MO oil	a	6.34	2.21
	b	-35.32	30.62

Table 11: Transition temperatures during heating processes of various seed oils

Sample	Transition	Onset temperature (°C)	Enthalpy ∆H (J/g)
Cold-pressed MO oil	A	-18.35	-0.62
	B	-9.38	-47.69
	C	6.13	-1.88
	D	16.79	-1.55
Baobab oil	A	-40.35	-1.23
	B	-23.14	-3.74
	C	-6.83	-7.68
	D	3.22	-1.07
Kalahari melon oil	A	-46.03	-8.72
	B	-31.32	-0.17
	C	-16.05	-1.38
	D	-12.28	-28.41
Soxhlet extracted MO oil	A	-20.43	-49.66
	B	-12.96	
	C	5.71	-4.06
	D	17.10	-1.56

5.4 Nuclear Magnetic Resonance (NMR) spectroscopy of selected oils

The assignments of the main resonances in the ^{13}C and ^{1}H NMR spectra were assigned according to Cheikhyoussef and Popescu and co-workers and displayed in Table 12 and 13 below. Relative integral percentages presented in Table 14 were used to calculate the relative saturates fatty acid (SAFA) content as described by Popescu and co-workers as well as

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Manina and co-workers. Individual spectra are presented in Appendix B (Figure 37-44).

From the ^{13}C NMR data in Table 12, four major signal groupings were evident at 14.27-34.36 ppm for the aliphatic carbons, 62.24-69.01 ppm for the glycerol backbone, 129.81-130.36 ppm for the unsaturated carbons and 172.98-173.45 ppm for the carbonyls (Cheikhyoussef, 2018). ^{1}H NMR resonance groups signals were integrated, which reflects the number of equivalent nuclei in the environment. The percentage of each group was then determined from the total signal (Mannina *et al*., 2003). From Table 14, the largest contribution signal (51.8-62.75%) to the total organic signal obtained for the selected oil samples was for the 1.25-1.35 ppm signal corresponding to methylene protons $(CH_2)_n$.The lowest contribution signal (0.90-0.97%) to the total organic signal obtained for the selected oil samples was for the 5.24-5.28 ppm signal corresponding to glycerol (triacylglycerols).

From the values in Table 14, the relative level of saturation of the oils could be estimated in the form of % Saturated Fatty Acid (SAFA) and Iodine value (IV). A high content of linolenic acid would decrease %SAFA and increase the IV, whereas a high content of oleic acid would increase %SAFA and decrease IV (Popescu *et al.*, 2015). From the %SAFA results obtained for the selected oils saturation was in the order of Kalahari melon seed oil, Lefakong farming cold-pressed MO oil, Soxhlet extracted MO oil and the highest being Baobab seed oil. However, according to the relative IV degree of saturation was in the order of Kalahari melon seed oil, Baobab seed oil, Lefakong farming cold-pressed MO oil, and the highest being Soxhlet extracted MO oil. This discrepancy is due to the low accuracy of this estimation using relative percentages.

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Table 12: Chemical shifts and assignments of main resonances in ppm (δ) of the ^{13}C NMR spectra of selected seed oils

Assignment	Lefakong farming cold-pressed MO oil	Soxhlet extracted MO oil	Kalahari melon seed oil	Baobab seed oil
Solvent ($CDCl_3$)	77.16	77.16	77.16	77.16
C18 (ω1)	14.27	14.27	14.23-14.27	14.23-14.27
C17 (ω2)	22.84	22.84	22.73-22.84	22.73-22.84
C3	24.99	nd	24.98	24.99
C11, C14	25.01-25.03	25.00-25.04	25.02-25.77	25.01-25.77
C8, C11	27.32-27.37	27.33-27.38	27.32-27.37	27.32-27.37
$(CH_2)n$	29.14-29.92	29.15-29.92	29.19-29.92	29.20-29.92
C16 (ω3)	31.94	31.94	31.67	31.68
C2, sn-1,3	32.06-32.08	32.06-32.08	32.06-32.08	32.06-32.08
C2, sn-2	34.17-34.34	34.18-34.36	34.17-34.33	34.20
CH_2O-, sn-1,3	62.24	62.24	62.24	62.24
CHO-, sn-2	69.01	69.01	69.01	69.01
C10, C12, C13	nd	nd	128.01-128.21	128.02-128.21
C9, C10	129.81-129.97	129.82-129.97	129.84	129.82-129.84
C9, C13	130.06-130.16	130.07-130.16	130.11-130.35	130.11-130.36
C1, sn-2	172.99	nd	172.98	172.98
C1, sn-1,3	173.42-173.44	173.42-173.45	173.39-173.44	173.40-173.44

nd, no data

Table 13: Chemical shifts and assignments of main resonances in ppm (δ) of the 1H NMR spectra of selected seed oils

Assignment	Lefakong farming cold-pressed MO oil	Soxhlet extracted MO oil	Kalahari melon seed oil	Baobab seed oil
Solvent ($CDCl_3$)	7.26	7.26	7.26	7.26
—CH_2—CH_2—CH_2—C**H**$_3$	0.86-0.89	0.86-0.89	0.87-0.90	0.86-0.90
(C**H**$_2$)$_n$	1.25-1.34	1.25-1.34	1.25-1.35	1.25-1.35
—C**H**$_2$—CH_2—COOH	1.57-1.63	1.59-1.63	1.60	1.60-1.62
—C**H**$_2$—CH=CH—	1.99-2.02	1.98-2.20	2.02-2.05	2.00-2.05

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—C**H**$_2$—COOH	2.29-2.33	2.29-2.33	2.29-2.33	2.29-2.38
—CH=CH—C**H**$_2$—CH=CH—	nd	nd	2.75-2.78	2.75-2.78
—C**H**$_2$—OCO—R	4.12-4.16	4.12-4.6	4.12-4.6	4.12-4.6
—C**H**$_2$—O—COR	4.27-4.31	4.27-4.31	4.27-4.31	4.27-4.31
C**H**—OCO—R	5.25-5.27	5.24-5.28	5.25-5.27	5.24-5.27
–C**H**=C**H**–	5.30-5.37	5.30-5.37	5.31-5.39	5.30-5.39

nd, no data

Table 14: Integrals of the ^{1}H NMR main resonance groups for the selected oil samples (% from the total area of the signals)

Assignment	**Lefakong farming cold-pressed MO oil**	**Soxhlet extracted MO oil**	**Kalahari melon seed oil**	**Baobab seed oil**
—CH$_2$—CH$_2$—CH$_2$—C**H**$_3$	8.10 %	8.29 %	8.59 %	8.78 %
(C**H**$_2$)$_n$	62.75 %	62.53 %	51.80 %	59.24 %
—C**H**$_2$—CH$_2$—COOH	6.19 %	6.27 %	7.04 %	6.92 %
—C**H**$_2$—CH=CH—	8.56 %	8.59 %	9.59 %	7.36 %
—C**H**$_2$—COOH	5.45 %	5.47 %	5.87 %	5.92 %
—CH=CH—C**H**$_2$—CH=CH—	nd	nd	3.75 %	1.72 %
—C**H**$_2$—OCO—R	1.85 %	1.78 %	1.99 %	1.98 %
—C**H**$_2$—O—COR	1.80 %	1.75 %	1.97 %	1.95 %
C**H**—OCO—R	0.93 %	0.90 %	0.97 %	0.92 %
–C**H**=C**H**–	4.36 %	4.41 %	8.42 %	5.21 %
SAFA	21%	22%	16%	37%
Relative IV	69.49	68.54	126.36	76.44

nd, no data

5.5 Fourier Transform-Infrared Spectroscopy analysis of the various oils

Distinct functional groups are seen across all the oil samples (Appendix C Figure 45-67) characteristic to plant oils presented in Table 15 below. These include a weak broad O-H stretching at 3200-2700 cm^{-1}, strong alkane C-H stretch at 3000-2840 cm^{-1}, a strong C=O stretch at 1750-1735 cm^{-1}, a medium alkane C-H bending at 1465 cm^{-1}, a strong ester C-O

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stretching at 1210-1163 cm^{-1} and a strong alkene C=C bending at 730-665 cm^{-1}. These findings confirm the presence of the ester and carboxylic acid functional groups expected for plant oils. Slight variations in wavenumber and intensities are due to the different fatty acid compositions in the various oils. In Figure 28 below, the FT-IR spectra of the four extracted MO oils are overlapped. They are almost identical except for the weak peak for Lefakong farming cold-pressed MO at around 1096.24 cm^{-1} which may be an impurity. This indicates that the extraction method used has virtually no effect on the molecular fingerprint, in other words, the functional groups, present in the final oil product.

Thirugnanasambandham who investigated Ultrasound-assisted extracted MO using FT-IR analysis reported similar results of an ester functional group (CO stretch) at 1776 cm^{-1}, an ether linkage at 1168 cm^{-1}, broad OH stretch peak at 3420 cm^{-1} and peaks of ortho-substituents at 753 cm^{-1}. An alcohol or phenol OH stretch at 3510–3100 cm^{-1}, CH stretching at 2925 cm^{-1} and 2860 cm^{-1}, an ester functional group in the region 1770-1668 cm^{-1} and a carboxylic acid signal in the region 3000-2700 cm^{-1}(Thirugnanasambandham, 2018).

Table 15: Common FT-IR signals for the various seed oils (Appendix C Figure 45-67)

Wavenumber range cm^{-1}	Functional group	Mode of vibration	Intensity
3200-2700	O-H	Stretching	Weak
3000-2840	C-H	Stretching	Strong
1750-1735	C=O	Stretching	Strong
1465	C-H	Bending	Medium
1210-1163	C-O	Stretching	Strong
730-665	C=C	Bending	Strong

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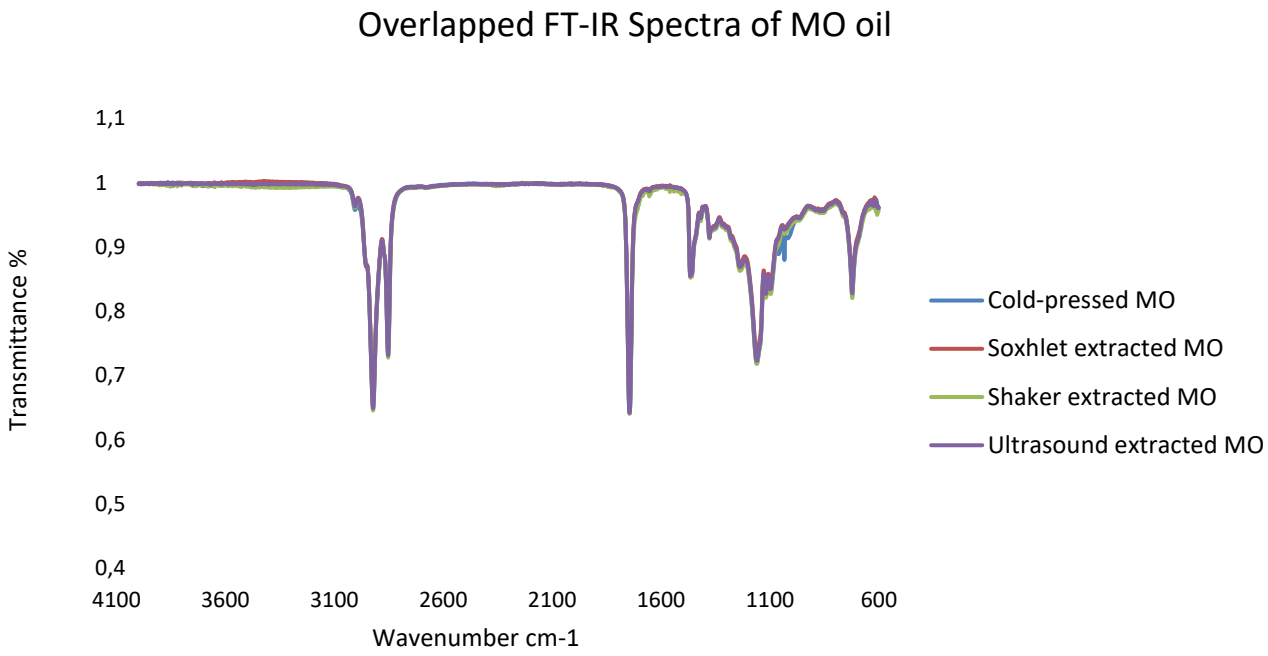

Figure 28: Overlapped FT-IR Spectra of extracted MO oil

5.6 The relative fatty acid content of the various oils

Analysis of the Supelco 37 FAMES (Fatty Acid Methyl Esters) standard using the described Gas Chromatograph-Mass Spectroscopy (GC-MS) methodology (4.7), resulted in all standards being eluted and visible in a similar order to that found in literature (Danish and Nizami, 2019). Reference Table 19 ad Figure 68 were used in processing the oil samples results. There were however difficulties in peak identification for the oil samples due to matrix effects resulting in a wider retention time search range per analyte to be used. As a result, analyte of similar elution times became problematic to differentiate and be correctly picked up by the automatic processing function. Hence, in general, larger standard deviations resulted for fatty acids in the range of 17.00-22.00 minutes.

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In Table 19 below, a sample of relative fatty acid content of various oils are presented, any results below 0.01% were regarded as not detected (nd). The relative percentage of each fatty acid was determined out of the total fatty acid content which was used to quantify the total Saturated Fatty Acid % (SAFA %) and The Total Unsaturated Fatty Acid % (TUFA %). Figure 29 is a sample chromatogram of Shaker extracted *Moringa oleifera* (MO) oil. The remaining relative fatty acid content, GC-MS chromatograms and major mass spectra are presented in Appendix D below.

Recovery calculations were not possible in this analysis as the standard was already in a derivatised form and could not be used to spike samples undergoing derivatisation before analysis, hence 100% recovery is assumed across all results. In Table 16 results for three oils; namely Mongongo seed oil, Plush Organics Moringa oil, and Olive oil; of sample sizes n=3 and n=9 are displayed to show the close similarity in results hence the remaining oils were analysed as n=3.

Amongst the extracted *Moringa oleifera* (MO) oils there were a few notable differences between the individual fatty acids detected and their relative percentages but a similarity in their saturation trends. Discrepancies may be caused by contamination of the sample or the instruments column. The highest relative fatty acid % (24.46-57.95%) across the extracted MO oils was cis-9-Oleic acid methyl ester (Oleic acid) and the second highest being Methyl palmitate (Palmitic acid) (14.40-25.56%). This order is in agreement with results presented by Özcan and co-workers for Soxhlet and Cold-pressed MO oil. However, the third highest fatty acid % reported by Özcan and co-workers was for Methyl docosanoate (Behenic acid) however, results obtained in this study indicate Methyl Arachidate (Arachidonic acid) was the third highest fatty acid present (8.97-14.03%).

Discrepancies in the percentage values of fatty acids in comparison to literature values may be contributed to by different ranges of analyte of

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interest. Such that many researchers focussed only on major fatty acids such as oleic, linoleic, stearic, palmitic etc. whereas in this study fatty acid % was calculated out of the total 37 fatty acid range. The relative fatty acid percentages present in seed oils may also depend on seed source, extraction method and storage conditions (Nwabuebo, 2017). Soxhlet extracted MO oil had high SAFA% and PUFA% which suggests that these triglycerides require high temperature and a solvent for their extraction which is in agreement with findings by Nwabuebo (Nwabuebo, 2017).

GC-MS analysis of oils fatty acid content gives insight into which fatty acids are present and absent within the oil which affects the benefits in cosmetic application. The major fatty acids important to cosmetic application are palmitic acid, linoleic acid, and oleic acid for their benefits beforementioned (2.1). The level of saturation in the oil affects the state of the oil at different temperatures as well as the oils long term stability as susceptibility to oxidative degradation is affected by functional groups present required for degradation reactions to take place. A high percentage of oleic acid in oil is said to make the oil desirable in terms of nutrition and high stability cooking and frying oil (Bhutada *et al.*, 2016). This correlates with the results obtained as the highest oleic acid content was obtained for Olive oil which is used for such applications. The highest relative percentage of Palmitic acid belonged to Grapeseed oil and the highest relative percentage of Linoleic acid belonged to Kalahari melon seed oil. This would indicate that Grapeseed oil and Kalahari melon seed oil are very valuable to skin formulations focussed on protecting the skin barrier from dying and damage.

As beforementioned, and presented in Table 9, Iodine value (IV) is related to the degree of unsaturation such that the higher the IV the more unsaturated the oil. This parameter as well as the relative IV estimates calculated from ^{1}H NMR data (5.4) correlates well with the GC-MS findings. The IV obtained for the extracted MO oils (65.93-74.99 g/100g)

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was lower than that obtained for Baobab seed oil (91.30 g/100g) and Kalahari melon seed oil (131.90 g/100g). Relative IV from ^{1}H NMR data for Lefakong farming cold-pressed MO oil and Soxhlet extracted MO oil (68.54-69.49 g/100g) was lower than that obtained for Baobab seed oil (76.44 g/100g) and Kalahari melon seed oil (126.36 g/100g). The TUFA% results for the extracted MO oils (33.93-64.33%) is lower than the TUFA% obtained for Baobab seed oil (65.61%) and Kalahari melon seed oil (72.24%). Overall, this indicated that Kalahari melon seed oil was the most unsaturated followed by Baobab seed oil and MO oil was the least unsaturated from this sample group.

The highest relative fatty acid % obtained for Mongongo seed oil was Methyl Linoleate (Linoleic acid) which is in agreement with the results presented in literature (Cheikhyoussef, 2018). The highest relative fatty acid % obtained for Baobab seed oil was cis-9-Oleic acid methyl ester (Oleic acid) which is in agreement with the results presented in literature (Muthai *et al.*, 2019; Zimba *et al.*, 2005). The highest relative fatty acid % obtained for Marula seed oil was cis-9-Oleic acid methyl ester (Oleic acid) which is in agreement with the results presented in literature (Mariod, Matthaus and Eichner, 2004; Nwabuebo, 2017; Cheikhyoussef, 2018). The highest relative fatty acid % obtained for Kalahari melon seed oil was Methyl Linoleate (Linoleic acid) which is in agreement with the results presented in literature (Cheikhyoussef, 2018).

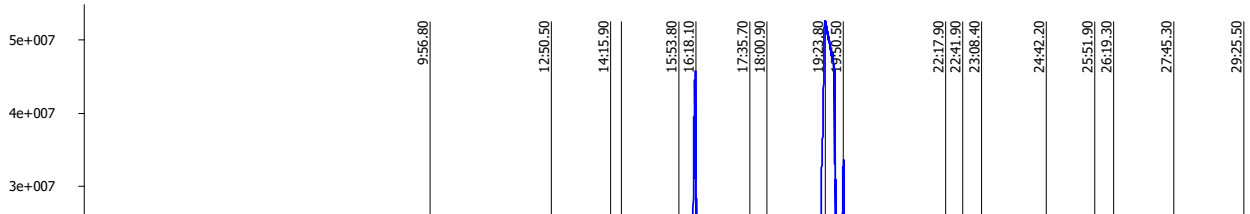

Figure 29: GC-MS chromatogram of Shaker extracted Moringa seed oil

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Table 16: Relative fatty acid composition of the various oils

Fatty acid	Lefakong Cold-pressed MO oil	Soxhlet extracted MO oil	Ultrasound extracted MO oil	Shaker extracted MO oil	Mongongo seed oil (n=9)	Mongongo seed oil (n=3)	Olive oil (n=9)	Olive oil (n=3)	Plush Organics Moringa oil (n=9)	Plush Organics Moringa oil (n=3)
Methyl laurate	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	nd	nd	nd	nd	nd	nd
Methyl tetradecanoate	0.49 ± 0.09	0.29 ± 0.08	0.28 ± 0.1	0.20 ± 0.05	0.07 ± 0.03	0.06 ± 0.01	0.02 ± 0.02	0.01 ± 0.02	0.02 ± 0.02	nd
Methyl pentadecanoate	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	nd	0.01 ± 0.01	0.01 ± 0.01
Methyl Palmitoleate	6.98 ± 1.71	4.65 ± 1.52	4.09 ± 0.61	3.33 ± 0.64	0.11 ± 0.05	0.12 ± 0.03	2.25 ± 1.02	2.07 ± 0.44	1.45 ± 0.42	1.14 ± 0.13
Methyl palmitate	25.26 ± 4.73	14.40 ± 10.65	20.55 ± 5.71	14.90 ± 2.83	15.27 ± 0.26	23.10 ± 6.29	9.81 ± 8.90	6.81 ± 2.12	18.92 ± 15.50	13.36 ± 13.43
cis-10-Heptadecenoic acid methyl ester	0.21 ±0.05	0.13 ± 0.03	0.10 ± 0.02	0.08 ± 0.02	0.05 ± 0.03	0.04 ± 0.01	0.36 ± 0.19	0.31 ± 0.02	0.35 ± 0.08	0.28 ± 0.05
Methyl heptadecanoate	0.39 ± 0.08	0.25 ± 0.05	0.21 ± 0.03	0.18 ± 0.03	0.18 ± 0.08	0.16 ± 0.05	0.31 ± 0.16	0.28 ± 0.04	0.27 ± 0.08	0.23 ± 0.03
Linolelaidic acid methyl ester	nd	nd	nd	nd	0.86 ± 1.71	1.13 ± 1.96	nd	nd	5.36 ± 6.80	5.93 ± 5.34
trans-9-Elaidic acid methyl ester	nd	6.32 ± 10.95	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Linolenate	nd	nd	nd	nd	nd	nd	12.20 ± 10.06	10.80 ± 9.57	14.78 ± 12.72	19.54 ± 3.20
Methyl Linoleate	nd	nd	nd	nd	27.05 ± 12.54	24.54 ± 4.78	nd	nd	nd	nd

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cis-9-Oleic acid methyl ester	35.63 ± 12.13	24.46 ± 24.78	40.66 ± 21.85	57.95 ± 11.42	8.01 ± 8.38	6.83 ± 5.92	62.80 ± 12.27	67.04 ± 12.06	47.36 ± 21.84	50.27 ± 7.17
Methyl octadecanoate	nd	21.58 ± 14.83	10.68 ± 4.55	5.07 ± 4.39	8.01 ± 5.48	5.97 ± 5.17	10.40 ± 3.33	10.86 ± 2.12	10.02 ± 2.63	8.02 ± 0.86
Methyl cis-5.8.11.14-Eicosatetraenoic	nd	nd	nd	nd	20.87 ± 4.82	19.46 ± 4.93	nd	nd	nd	nd
Methyl cis-5.8.11.14.17-Eicosapentaenoate	nd	nd	nd	nd	18.54 ± 8.12	18.45 ± 4.79	nd	nd	nd	nd
cis-8.11.14-Eicosatrienoic acid methyl ester	nd	nd	nd	nd	0.04 ± 0.08	nd	nd	nd	nd	nd
cis-11.14-Eicosadienoic acid methyl ester	nd	nd	nd	nd	0.37 ± 0.08	0.35 ± 0.10	nd	nd	0.59 ± 0.14	0.50 ± 0.04
Methyl cis-11-eicosenoate	5.31 ± 1.09	1.84 ± 3.18	4.41 ± 1.52	2.93 ± 0.54	0.45 ± 0.12	0.41 ± 0.11	0.64 ± 0.23	0.63 ± 0.13	nd	nd
cis-11.14.17-Eicosatrienoic acid methyl ester	nd	2.76 ± 2.50	nd	nd	nd	nd	nd	nd	nd	nd
Methyl heneicosanoate	10.50 ± 2.12	8.84 ± 1.73	8.60 ± 2.98	5.88 ± 1.13	0.40 ± 0.09	0.39 ± 0.09	0.98 ± 0.38	0.98 ± 0.22	0.79 ± 0.22	0.62 ± 0.03
All cis-4.7.10.13.16.19-Docosahexaenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

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cis-13-16-Docosadienoic acid methyl ester (22:2)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Erucate	0.08 ± 0.01	0.09 ± 0.03	0.09 ± 0.02	0.04 ± 0.03	nd	nd	nd	nd	nd	nd
Methyl Arachidate	14.03 ± 2.02	13.41 ± 2.61	9.71 ± 7.39	8.97 ± 1.76	0.03 ± 0.02	0.04 ± 0.01	0.18 ± 0.08	0.17 ± 0.02	0.07 ± 0.02	0.07 ± 0.02
Methyl docosanoate	0.06 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Methyl tricosanoate	0.04 ± 0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Nervonate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl lignocerate	0.95 ± 0.06	0.87 ± 0.34	0.54 ± 0.10	0.41 ± 0.07	0.04 ± 0.02	0.03 ± 0.00	0.03 ± 0.04	0.03 ± 0.04	0.01 ± 0.01	0.01 ± 0.02
SAFA %	51.78	59.74	50.65	35.67	24.02	29.77	21.76	19.16	30.12	22.34
MUFA %	48.22	31.17	49.35	64.32	8.62	7.40	66.05	70.04	49.16	51.70
PUFA %	5.31	2.76	0.09	nd	66.87	62.80	12.20	10.80	15.36	20.04
TFA %	nd	6.32	nd	nd	0.86	1.13	nd	nd	5.36	5.93
TUFA %	53.53	33.93	49.44	64.33	75.49	70.20	78.25	80.84	64.52	71.74

nd, not detected. SAFA = Saturated Fatty Acids, TFA = Trans Fatty Acids, MUFA = Monounsaturated Fatty Acids, PUFA = Polyunsaturated Fatty Acids, and TUFA = Total Unsaturated Fatty Acids. Data are shown as means with ± SD of n replicates.

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5.7 The effect of sunlight exposure on selected oils

It is known that exposure to higher temperatures, oxygen and light are the main external factors that contribute to the autoxidation of fatty acids (Nwabuebo, 2017). This experiment was intended to roughly indicate the selected oil's oxidative stability. Poor oxidative stability results in low-quality oil with a shorter shelf life. Poor oxidative stability results in rancidity which is indicated by an unpleasant odour and/or taste caused by the aldehydes and ketones formed during the degradation process (Nwabuebo, 2017).

There were clear trends in the decrease in the quality of these oils as they deteriorated, which can be seen in Figure 30 below. In terms of the property colour, the oils either remained the same colour or lost their pigment and became dull or completely transparent. In terms of the state of oils, all but one oil remained a homogeneous liquid, that oil being the cold-pressed MO oil from Lefakong farming. Over time this oil formed a white precipitate causing the oil sample to appear murky, this may have been sediment caused by poor filtration or the formation of a by-product caused by the sunlight exposure. The odours of the oils were by far been the most affected by this accelerated deterioration study. None of the oils had their original earthy, nutty and/or herbal smells by the end of this experiment. The odours intensities fluctuated and had changed to rancid, acidic and/or fishy smells which was a clear sign of deterioration of the sample.

Given that peroxide value (PV) (Table 9) is a quantitative measure of oxidative stability, there is a clear correlation between the PV results and the sunlight exposure results presented in Table 17 below. From the selected oils, Jojoba oil had the lowest PV of 0,97 ± 0,08 mequiv/kg whereas Mongongo oil had the highest with a value of 25,91 ± 0,44 mequiv/kg. From Table 17 below we see very little changes over the 2

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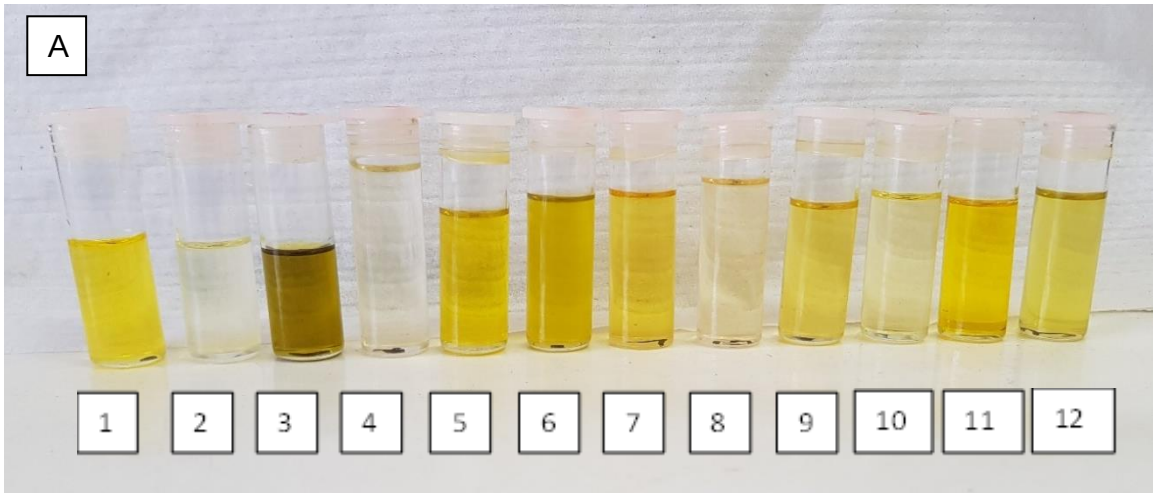
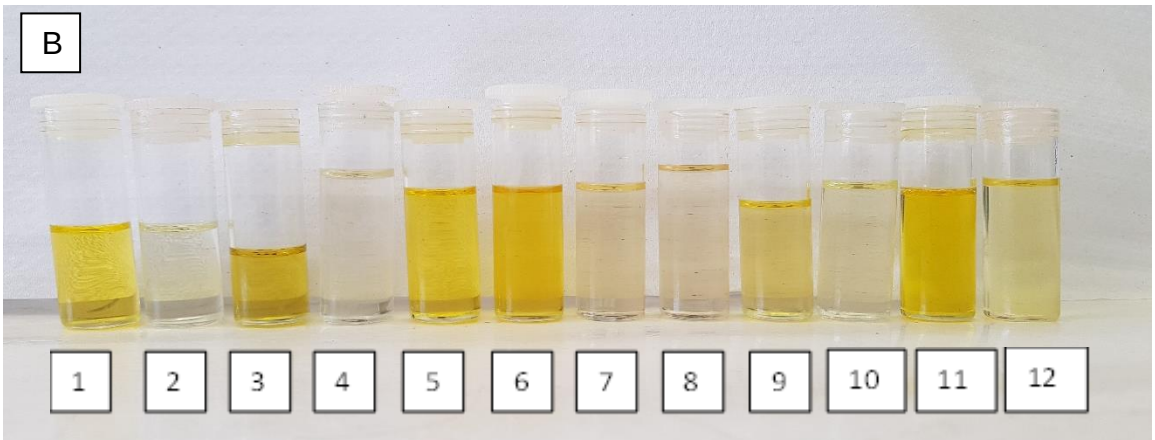
months for Jojoba oil whereas the Mongongo oil showed signs of rancidity after just 1 month indicating poorer oxidative stability in comparison to jojoba oil.

For application in cosmetics, oil's colour and odour are very important as it affects the quality and integrity of the final product over time. If oils susceptible to degradation are to be used in a formulation, additional active ingredient, namely antioxidants and chelating agents are necessary to protect the product's integrity by preventing decomposition of the natural oil phase.





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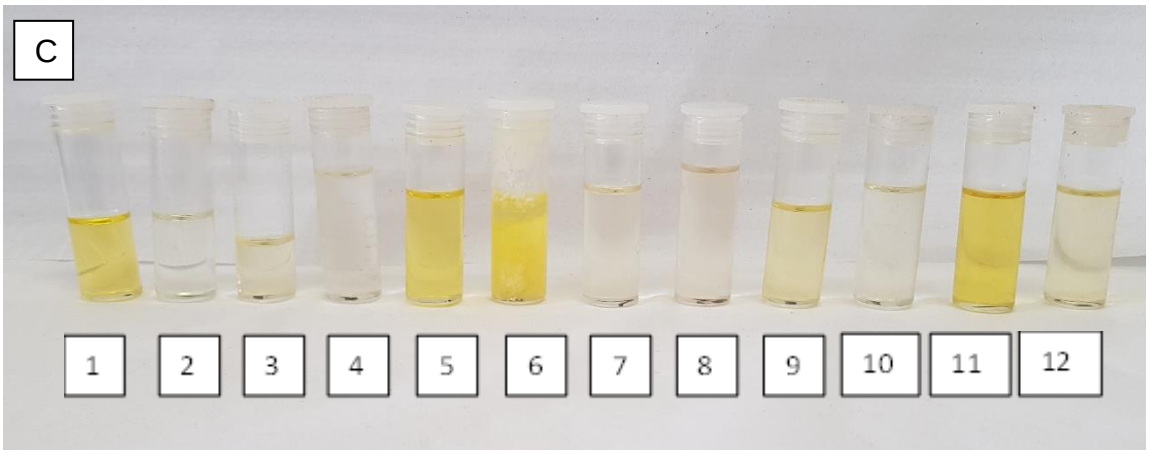

Figure 30: Images of the selected oils during the sunlight exposure study A= 14/12/2020, B= 14/05/2021 and C=14/08/2021. Oils were positioned as follows: 1= Jojoba seed oil. 2= Grapeseed oil, 3= Avocado oil, 4= Sunflower oil, 5= Sunflower oil, 6= Lefakong Farming cold-press MO oil, 7= Bright mountains MO oil, 8= Marula Seed Oil, 9= Mongongo Seed oil, 10= Kalahari Melon Seed Oil, 11= Baobab Seed Oil, and 12= Hemp seed oil

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Table 17: Physical effect of sunlight exposure summary on various oils

Sample	Original conditions	Condition after 1 month	Condition after 2 months	Condition after 3 months	Condition after 4 months	Condition after 5 months	Condition after 6 months	Condition after 7 months	Condition after 8 months
Jojoba seed oil	Bright yellow Homogeneous liquid Earthy aroma	Bright Yellow, earthy aroma	Bright Yellow, Faint, almost no smell.	Bright Yellow, Mild acidic smell.	Bright yellow. Acidic and herbal smell.	Yellow. Acidic and herbal smell.	Yellow. Rancid and herbal smell.	Pale yellow. Faint rancid smell.	Pale yellow. Faint rancid smell.
Grapeseed oil	Very pale yellow Homogeneous liquid Earthy acidic smell	Very pale yellow. Acidic rancid smell.	Very pale yellow. Faint rancid smell.	Very pale yellow. Rancid smell.	Very pale yellow almost clear. Rancid smell.	Very pale yellow almost clear. Rancid smell.	Very pale yellow almost clear. Pungent acidic smell.	Clear. Faint rancid smell.	Clear. Strong rancid acidic smell.
Avocado oil	Dark olive green Homogeneous liquid Herbal nutty aroma	Dark olive green. Herbal acidic smell.	Slightly paler olive green. Herbal acidic smell.	Slightly paler olive green. Pungent herbal acidic smell.	Pale olive green. Pungent herbal acidic smell.	Pale yellow-green. Pungent acidic smell.	Dull yellow. Pungent acidic smell.	Dull faint yellow. Pungent acidic herbal smell. Precipitate.	Very faint yellow. Pungent acidic herbal smell. Murky precipitate.
Sunflower oil	Very pale yellow Homogeneous liquid Slightly earthy	Very pale yellow. Slightly earthy	Very pale yellow. Faint acidic smell.	Very pale yellow. Faint rancid smell	Very pale almost completely clear. Faint	Completely clear. Strong rancid smell.	Completely clear. Mild herbal rancid	Completely clear. Faint rancid smell.	Completely clear. Strong rancid smell.

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	aroma	aroma.			rancid smell		smell.		
Olive oil	Bright yellow Homogeneous liquid Pungent earthy/ acidic	Bright yellow. Pungent earthy/ acidic smell.	Bright yellow. Mild acidic smell.	Bright yellow. Pungent acidic smell.	Bright yellow. Pungent acidic smell.	Bright yellow. Mild herbal acidic smell.	Dull yellow. Pungent herbal acidic smell.	Dull yellow. Pungent herbal acidic smell.	Dull yellow. Pungent herbal acidic smell.
Lefakong Farming cold-press MO oil	Dull yellow Homogeneous liquid Nutty aroma	Dull yellow. Acidic rancid smell.	Formed a separate murky pale-yellow layer. Mild nutty and rancid smell.	Dull yellow. Medium rancid smell.	Murky pale yellow. Strong rancid smell.	Murky pale yellow. Strong rancid nutty smell.	Pale yellow with white solids. Strong rancid smell.	Pale yellow with white solids. Faint rancid smell.	Pale yellow with white solids. Faint rancid smell.
Bright mountains MO oil	Bright yellow Homogeneous liquid Nutty aroma	Bright yellow. Acidic rancid smell.	Dull medium yellow. Mild nutty, earthy, and rancid smell.	Dull medium yellow. Medium earthy rancid smell.	Very pale peach. Medium earthy rancid smell.	Very pale peach almost clear. Strong earthy rancid smell.	Very pale peach almost clear. Strong earthy rancid smell.	Clear. Strong earthy rancid smell.	Clear. Strong earthy rancid smell.
Marula Seed Oil	Very pale yellow/ peach Homogeneous liquid Nutty roasted aroma	Very pale yellow/ peach. Soft earthy smell.	Very pale yellow/ peach. Faint, almost no smell.	Very pale yellow/ peach. Faint acidic smell.	Very pale almost clear. No smell.	Very pale. Mild fishy smell.	Very pale almost clear. Mild fishy earthy smell.	Clear. No smell.	Clear. Faint earthy rancid smell.
Mongongo Seed oil	Dull medium yellow Homogeneous liquid	Dull medium yellow. Earthy and	Dull medium yellow. Mild	Dull medium yellow. Pungent	Pale yellow. Pungent	Pale yellow. Pungent	Pale yellow. Pungent	Very pale yellow. Pungent	Very pale yellow. Pungent

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	Earthy aroma	acidic.	rancid smell.	rancid smell.	rancid smell.	rancid smell.	rancid smell.	rancid smell.	rancid smell.
Kalahari Melon Seed Oil	Pale yellow Homogeneous liquid Earthy acidic smell	Pale yellow. Earthy acidic smell.	Pale yellow. Very faint earthy smell.	Pale yellow. Acidic rancid smell.	Pale yellow. Pungent rancid smell.	Very pale yellow. Pungent acidic rancid smell.	Very pale yellow. Pungent rancid smell.	Very pale yellow almost clear. Faint rancid smell.	Clear. Faint rancid smell.
Baobab Seed Oil	Bright yellow Homogeneous liquid Nutty roasted aroma	Bright yellow. Nutty and acidic smell.	Bright yellow. Mild earthy and rancid smell.	Bright yellow. Mild earthy and rancid smell.	Bright yellow. Acidic and herbal smell.	Bright yellow. Acidic and herbal smell.	Dull yellow. Rancid and herbal smell.	Dull yellow. Faint rancid and herbal smell.	Dull yellow. Faint rancid smell.
Hemp seed oil	Dull medium yellow Homogeneous liquid Herbal/ earthy aroma	Dull medium yellow. Earthy and acidic.	Dull medium yellow. Earthy and acidic.	Dull medium yellow. Earthy and acidic.	Pale yellow. Pungent earthy and acidic smell.	Very pale. Pungent earthy and acidic smell.	Very pale. Pungent earthy and acidic smell.	Dull faint yellow. Pungent earthy and acidic smell.	Very faint yellow. Pungent earthy and acidic smell.

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5.8 Radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical of the various oils

In Table 18 below, the % inhibition results of the various samples as well as the positive control, ascorbic acid, are presented. Overall, according to the ANOVA single factor test, the results are significantly different from each other (p-value =7,06 $x10^{-87}$). The higher the percentage inhibition, the higher the sample's radical scavenging activity towards the DPPH radical. In the chemical reaction that takes place, the solution changes from purple to light yellow depending on the extent of the radical scavenging ability of that sample at the time of analysis. The synthetic antioxidant, ascorbic acid, as expected exhibited high radical scavenging ability having a % inhibition of 88.31 ± 1.82%. From the extracted MO oils, all results were significantly different ($p<0.05$) according to Tukey's test. Ultrasound-assisted and shaker method extracted MO had the higher % inhibition values. This is most likely due to their low/no heat extraction techniques which prevent the natural antioxidant content from being destroyed. Lefakong Farming cold-pressed MO had a surprisingly low % inhibition considering the absence of heat from the extraction process. This however correlates with other parameters conducted on the extracted oils as Lefakong Farming cold-pressed MO oil had the highest peroxide value (PV) of 6.42 ± 0.62 mequiv/kg as well as the highest acid value (AV) of 0.85 ± 0.09 mg KOH/g oil. This may be due to the age of the oil and/or the storage conditions of the product before receiving it which may have caused deterioration of the natural antioxidant content. As a result oils with high antioxidant content such as Mongongo and Kalahari melon seed oil would be best suited in antioxidant rich cosmetic formulations.

The remaining commercially available MO oil had a wide range of % inhibition values from 5.46 ± 0.03% for the Leaf moringa seed oil to 16.48 ± 0.02% for Plush organics moringa oil. There could be several reasons for this observation such as varying extraction methods, storage

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conditions, age of the sample, seed source etc., which may have affected the natural antioxidant content of the oils. In literature, some researchers used similar methods to determine their oil/s of interests % inhibition. Bayir and co-workers reported % inhibition values of 56-65% for sunflower oil (Baydır and Aşçıoğlu, 2018). Nwabuebo reported 54.24 ± 0.53% for screw pressed marula oil and 51.21 ± 0.53%, 53.33 ± 0.53% 56.06 ± 0.53% for Soxhlet extracted, shaker extracted and screw pressed moringa oil respectively (Nwabuebo, 2017). Zhong and co. workers reported a 10% inhibition % for ultrasound-assisted extracted moringa oil which falls in the range of the experimental values obtained in this study (Zhong *et al.*, 2018).

The comparison made by Nwabuebo showed that moringa seed oil had an overall higher radical scavenging ability compared to marula seed oil given her experimental data. The experimental data from this study however contradict her findings as results for commercially purchased marula and moringa seed oil from the same supplier (Bright Mountains) showed that marula oil had a lower AV (2.95 ± 0.13 mg KOH/g oil) in comparison to moringa oil (6,86 ± 0,42 mg KOH/g oil). As well as the results for PV, marula oil had a lower value of 1,17 ± 0,27 mequiv/kg whereas moringa oil had a higher value of 1,80 ± 0,21 mequiv/kg. These two parameters confirm the results obtained for the radical scavenging activity experiment as marula oil had a higher % inhibition of 11.68 ± 0.13% whereas the moringa oil had a lower result of 10.77 ± 0.02%. Therefore, from the results obtained in this study, for marula seed and moringa seed oil from the same supplier, marula oil had an overall higher radical scavenging activity.

To further investigate if storage conditions affected the natural antioxidant content of oils one oil selected at random, namely hemp seed oil was used. Hemp seed oil in three different conditions was analysed for radical scavenging activity. One sample was taken from the originally purchased

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bottle that was only opened once to decant a sample and thereafter has been stored in the fridge. The second sample was from the decanted portion of this oil stored in the fridge in a glass amber bottle but was opened multiple times for previous analyses and had therefore been exposed to oxygen and room temperature conditions several times. The third sample was taken from the clear glass vial of this oil used in the sunlight exposure study for 6 months. This sample was exposed to oxygen when opened and was constantly in full sunlight and room temperature conditions. From this study, as expected, storage conditions have a significant impact on the oil's radical scavenging ability. The Hemp seed oil sample exposed to sunlight for 6 months had the lowest % inhibition value of 3.55 ± 0.03%, whereas the decanted hemp seed oil sample used for prior studies had an intermediate result of 14.22 ± 0.1% and as suspected the original hemp sample opened only once had the highest value of 30.53 ± 0.87% inhibition. This indicated that it had the highest antioxidant content amongst the 3 samples at the time of the study. Seeing that all 3 of these samples come from the same source the only possible difference between them was their storage conditions which could have caused these results hence proper storage of plant oils is necessary to preserve their natural antioxidant content.

Discrepancies for this parameter may be caused by several factors such as the age of oil at the time of analysis, storage conditions, experimental procedures, oil or seed source etc. which may have caused deterioration of the natural antioxidant content.

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Table 18: % Inhibition results for various oils

Sample	% Inhibition
Ascorbic acid positive control	88.31 ± 1.82
Soxhlet extracted MO oil	2.97 ± 0.06[a]
Ultrasound-assisted extracted MO oil	12.17 ± 0.27[b]
Hexane shaker method extracted MO oil	13.47 ± 0.03[c]
Lefakong Cold- pressed MO oil	4.25 ± 0.06[d]
Moyo moringa SA- Moringa Behen oil for skin	11.20 ± 0.04
Leaf- Moringa seed oil	5.46 ± 0.03
Essentially Natural Moringa oil	19.63 ± 0.14
Moringa 5000 Pure Moringa Ben oil	13.57 ± 0.02
Bright mountain - Moringa Seed Oil	10.77 ± 0.02
Karibaa - Moriveda moringa basic oil	13.78 ± 0.03
Plush Organics Moringa Oil	16.48 ± 0.02
Tree of life Pure organic moringa oil	9.53 ± 0.05
Sell- SA - Moringa world MO oil	9.67 ± 0.07
Baobab Seed Oil	30.21 ± 0.03
Kalahari Melon Seed Oil	39.06 ± 0.03
Marula Seed Oil	11.69 ± 0.13
Mongongo Seed oil	64.19 ± 0.02
Avocado oil	12.27 ± 0.02

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Grapeseed oil	14.97 ± 0.01
Jojoba seed oil	10.26 ± 0.25
Sunflower oil	23.90 ± 0.19
Olive oil	16.80 ± 0.05
Original hemp sample	30.53 ± 0.57
Decanted hemp sample	14.22 ± 0.1
Hemp sample exposed to sunlight for 6 months	3.55 ± 0.03

Data are shown as means with ± SD of three replicates. Extracted MO oil means with different letters in the same row are significantly different ($p<0.05$) as determined with Tukey's test.

5.9 Statistical analysis of quantitative parameters

Figure 31 below illustrates a heat map of the covariance matrix results determined for the quantitative parameters of all twenty-three oils studied namely the extracted four *Moringa oleifera* (MO) oils, nine commercially available MO oils, Kalahari melon, Marula, Baobab, Mongongo, Hemp, Olive, Sunflower, Jojoba, Grapeseed and Avocado oil. A strong positive correlation or directly proportional relationship between two parameters are indicated by values close to one and indicated by the lighter range of colours. Whereas a strong negative correlation or inversely proportional relationship is indicated by values close to negative one and are indicated by the darker range of colours. It is evident that there is a directly proportional relationship between saponification value (SV) and ester value (EV), this is expected as EV is calculated by SV minus acid value (AV). There is also a directly proportional relationship between density (D) and specific gravity (SG) as D is used to calculate SG. SG and D relate to the thickness of the oils and would therefore affect cosmetic properties such as the skin feel of the final product.

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A directly proportional relationship is seen between iodine value (IV) and refractive index (RI), this is due to their connection with the degree of saturation. Such that high iodine and specific gravity values indicate a higher presence of unsaturated compounds whereas low iodine and specific gravity values indicate a higher presence of saturated compounds (Craig, 1961). Therefore a positive correlation is also observed between radical scavenging ability (RSA) and RI, as the main antioxidants present in naturals are tocopherols which contain high degrees of unsaturation in their structures as shown in Figure 3. The degree of saturation affects the behaviour of oils in a cosmetic application as it affects the state of the oil at different temperatures, this was evident as the MO oil samples were solid at fridge temperature whereas hemp seed oil was in a liquid state at this temperature.

On the other hand, there is an inversely proportional relationship between SV and average molecular weight (AMW) as AMW is calculated by dividing a constant by SV hence the relationship. This leads to the inversely proportional relationship between AMW and EV as SV and EV are directly proportional due to the reasons before mentioned. Oils with higher SV and therefore lower AMW are better suited for soap making (Ilesanmi *et al.*, 2021) due to chain length. AMW would also affect the degree of penetration of the oils such that higher AMW oils would penetrate the skin and hair surface to a lesser degree and therefore form a thicker occlusive layer that affects properties such as lubrication and shine in final products.

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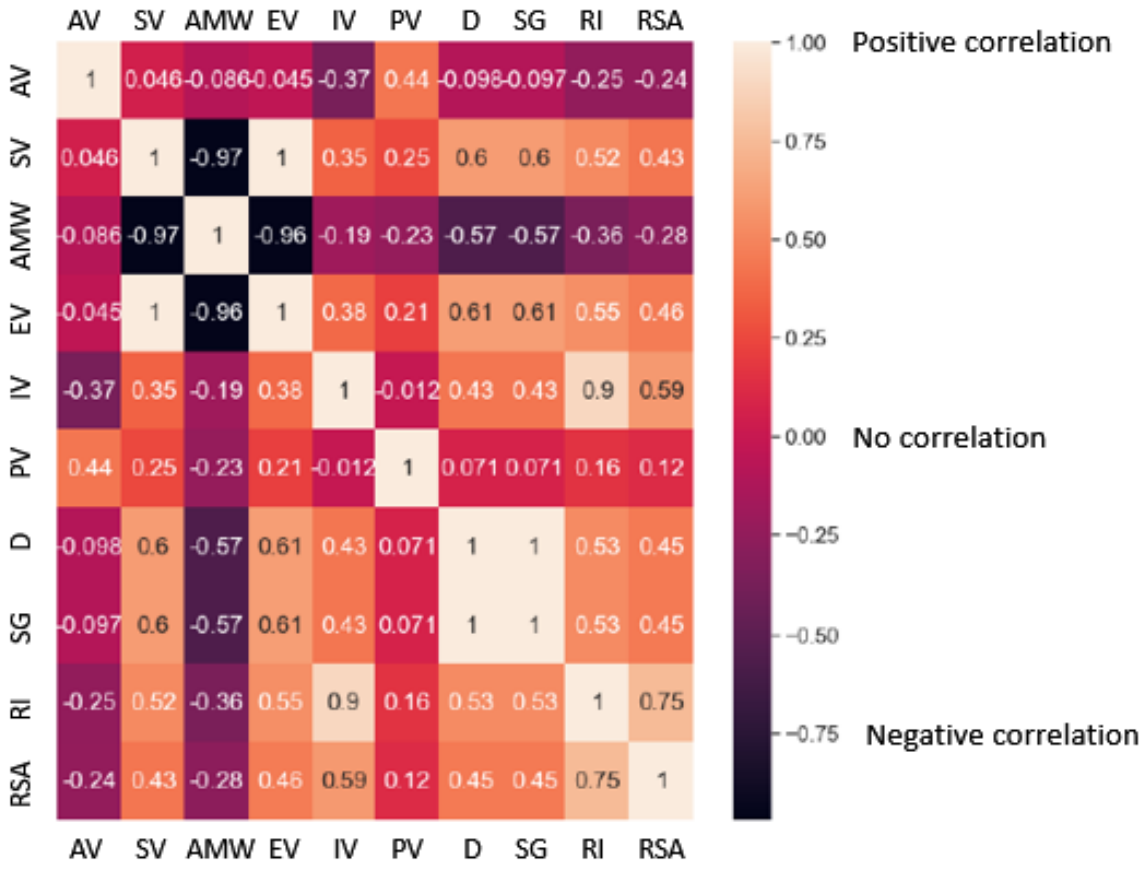

Figure 31: Covariance matrix of quantitative results. Where AV = Acid value, SV = Saponification value, AMW = Average molecular weight, EV = Ester value, IV = Iodine value, PV = Peroxide value, D = Density, SG = Specific gravity, RI = Refractive index, and RSA = Radical scavenging ability

5.10 Trial application of cosmetic products

A few friends and family members were given a pair of indistinguishable samples of each type of cosmetic product namely a liquid hand soap, lotion, and cream (Figure 23). One sample from the pair from each type of cosmetic product was modified with an additional 1% *Moringa oleifera* (MO) and Kalahari melon seed oil and the unmodified base that was unaltered was used as a control. Kalahari melon seed oil was chosen for

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its high antioxidant and linoleic fatty acid content and MO oil was chosen for its high oleic fatty acid content; both of these fatty acids are important in cosmetic formulations as previously mentioned (2.1). For the liquid hand soap, the overall experience was that the oil-enriched sample left the hands feeling less dry compared to the control after washing. For the lotion and cream, the modified sample gave a longer playtime to the products, in other words, was more spreadable compared to the control, it also left the skin slightly shinier and softer which most people favoured. The overall consensus was that the modified samples were preferred, and the majority would purchase the oil-enriched samples over the control. It is evident that even a small addition of seed oils to cosmetics provides a richness to products that most consumers prefer.



Figure 32: Illustration of cosmetic product samples

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CHAPTER 6: CONCLUSION

This study aimed to investigate the effect of extaction technique used on the characterisation and physico-chemical composition of *Moringa oleifera* (MO) seed oil. This was achieved through the extraction of MO seed oil using Cold-press, Soxhlet, Ultrasound-assisted and Shaker extraction techniques from the same seed source. In addition, characterisation and physico-chemical composition of commercial Moringa, Marula, Kalahari melon, Mongongo, Baobab and Hemp seed oils were determined alongside common commercial oils for comparison namely olive oil, sunflower seed oil, grapeseed oil, jojoba seed oil, and avocado oil.

From the extraction of MO oil, as expected, Soxhlet extraction produced the highest oil yields amongst the different oils, making it the most economically valuable. Many characterisation results were indifferent to the extraction method namely, density, thermal properties determined by Differential scanning calorimetry (DSC), Nuclear Magnetic Resonance (NMR) signals, Fourier transform infrared spectroscopy (FT-IR) results as well as fatty acid content. This indicated that the inherent structure and nature of the oil is relatively unaffected by the extraction type, as these are fingerprint type parameters. However, characteristics that affect the shelf life in other words stability and longevity of the oil sample, is largely affected by the extraction type. The parameters significantly affected by extraction type include acid value, saponification value, average molecular weight, ester value, iodine value, peroxide value, refractive index, as well as radical scavenging activity.

Through this research, the various oils studied are now comparable as variability in methodology is eliminated. For the extracted MO oils, variability of seed source is eliminated as they were all extracted from the same seed source, variability only existed in the storage time from extraction until analysis. In general, one of the major contributing factors to

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long term stability of oils was shown to be storage conditions as proven by the extended radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical evaluation of Hemp seed oil. This indicated a clear decrease in antioxidant content with increased sunlight, air and temperature exposure. Enhancement of cosmetic bases with Kalahari melon and MO oil proved to positively improve the performance of the products and had an overall preferred richness in texture and feel.

Results obtained through this research contributes to the database of knowledge available on underutilised African oils, which can be used to assess their economic importance and assist in the development of value-added products.

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CHAPTER 7: RECOMMENDATIONS

- Other oil extraction methods could be explored for moringa oil extraction with the same seed source, to determine the quality of those oils in comparison to the extraction methods used in this study, namely cold-pressing, Soxhlet, ultrasound and shaker-method extraction. To add, for example, steam distillation, microwave-assisted extraction, supercritical fluid extraction etc.
- Further studies on application-specific processing of the crude oils would be advantageous, such as for the food and biodiesel industries, for the further value-addition initiative of the crude oils. To date, the oils studied here are scarce and relatively highly priced in comparison to other common oils available commercially.
- Results produced in this study, in particular NMR data, could be used in the future in the classification of the oils to assess the quality of oils produced to detect cases of adulteration such as dilution of oils with cheaper carrier oils etc. (Popescu *et al.*, 2015).
- Further confirmation of the studied oils in the cosmetic application can be professionally tested for their synergistic effects on hair and skin.
- It is recommended to study the tocopherol and sterol content of the seed oils.

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APPENDICES

APPENDIX A: DSC thermal profiles

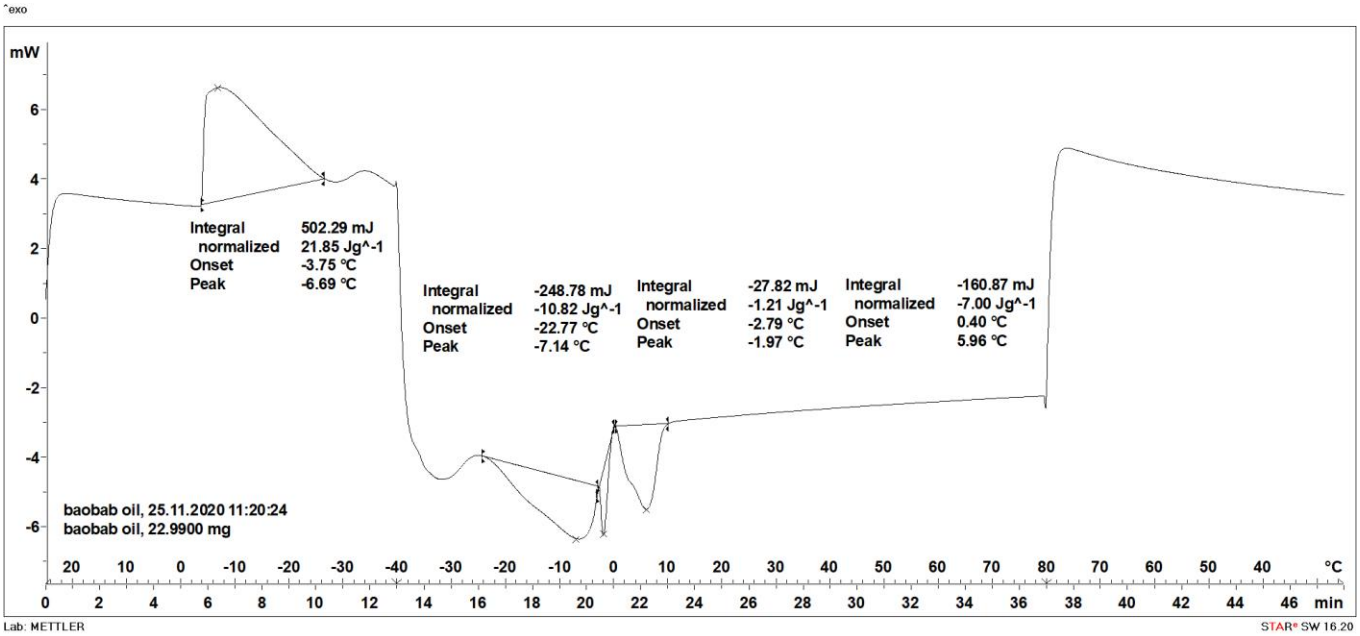

Figure 33: Complete DSC thermal profile of baobab oil

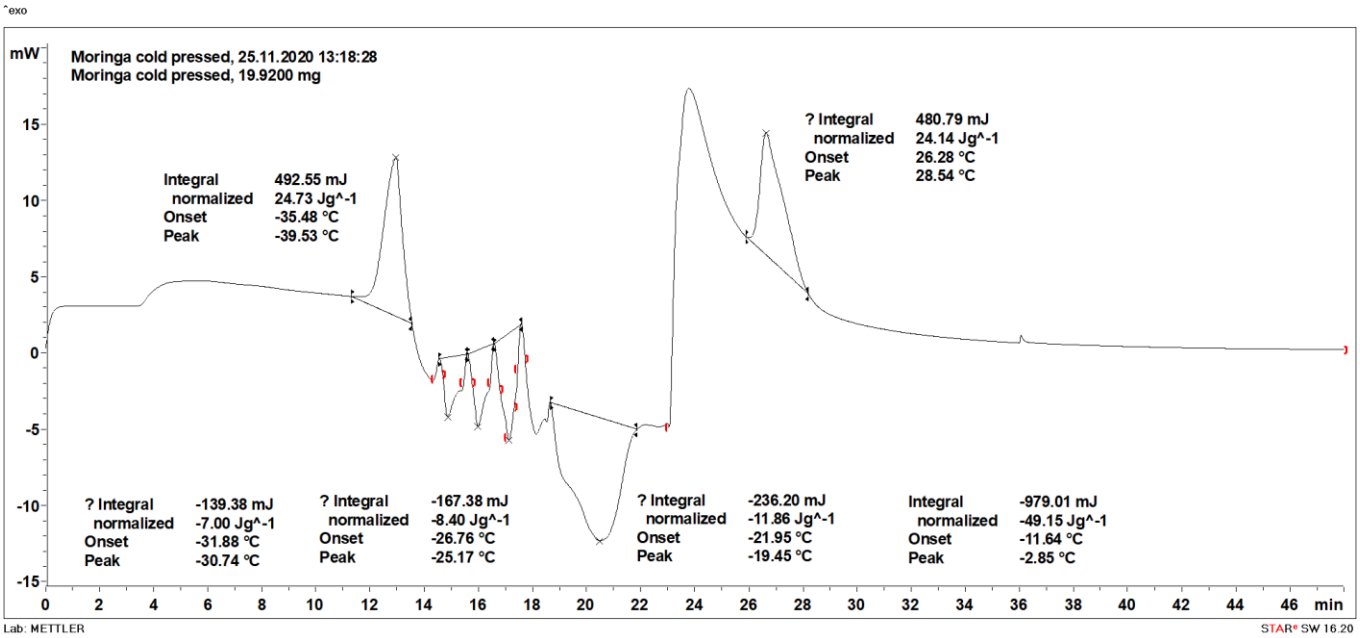

Figure 34: Complete DSC thermal profile of cold-pressed MO oil

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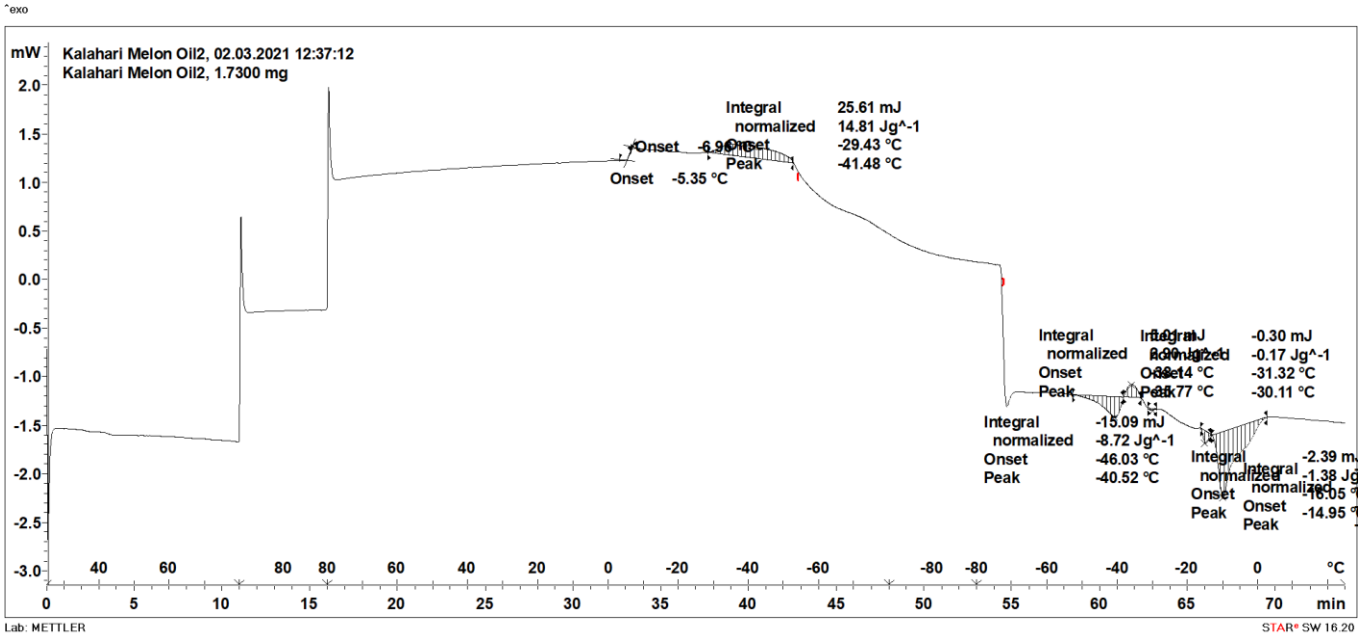

Figure 35: Complete thermal profile of Kalahari melon seed oil

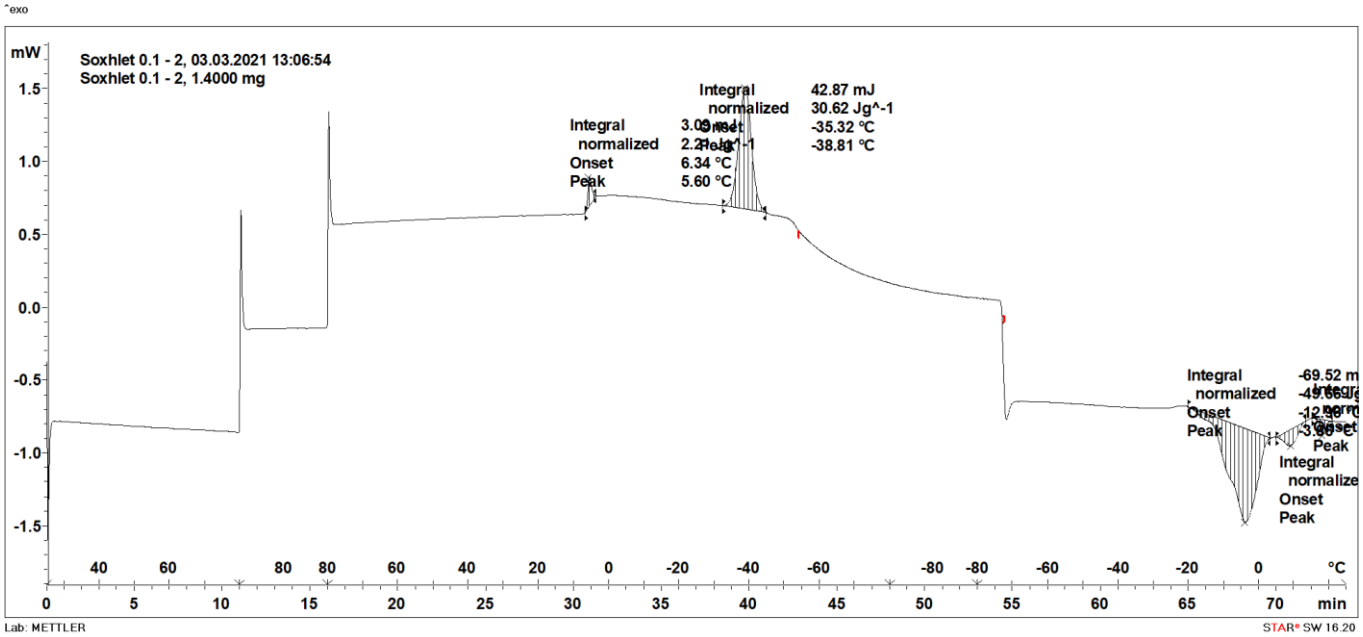

Figure 36: Complete thermal profile of Soxhlet extracted MO oil

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APPENDIX B: NMR Spectra

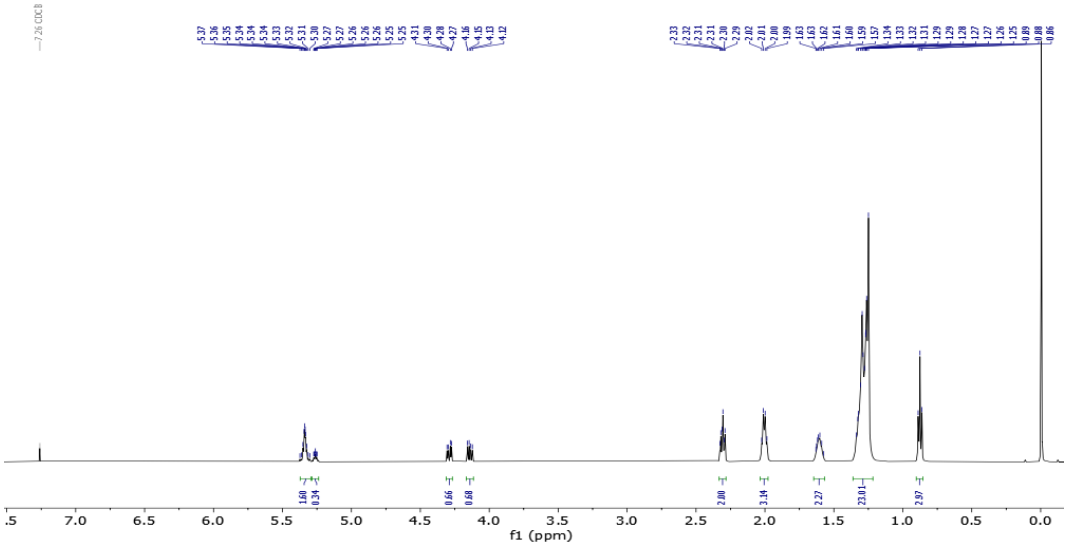

Figure 37: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of Lefakong farming cold-pressed MO seed oil

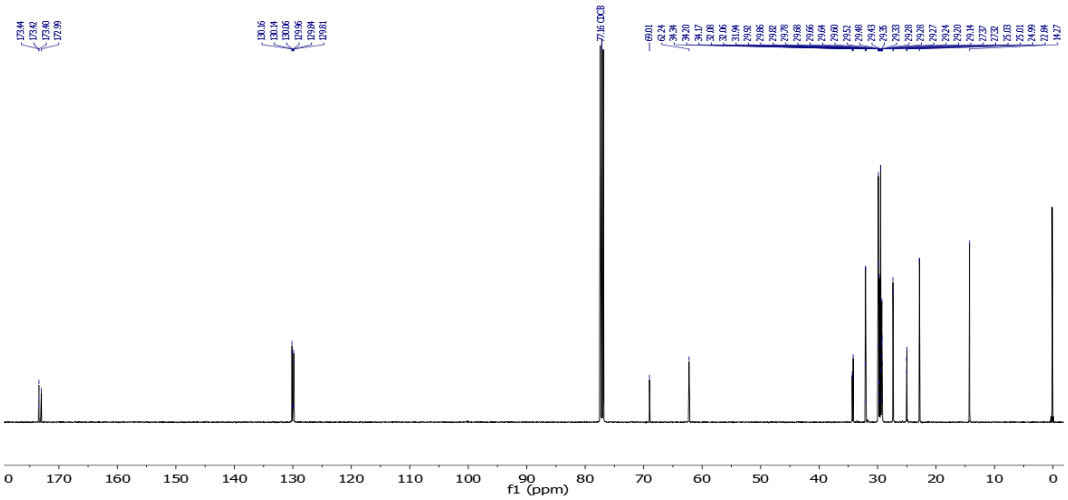

Figure 38: ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of Lefakong farming cold-pressed MO seed oil

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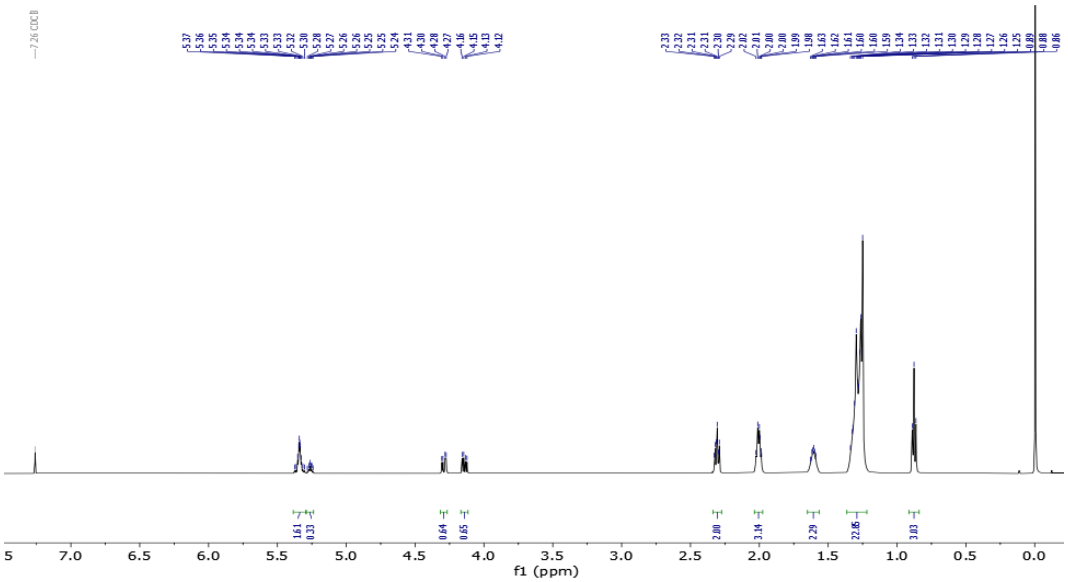

Figure 39: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of Soxhlet extracted MO seed oil

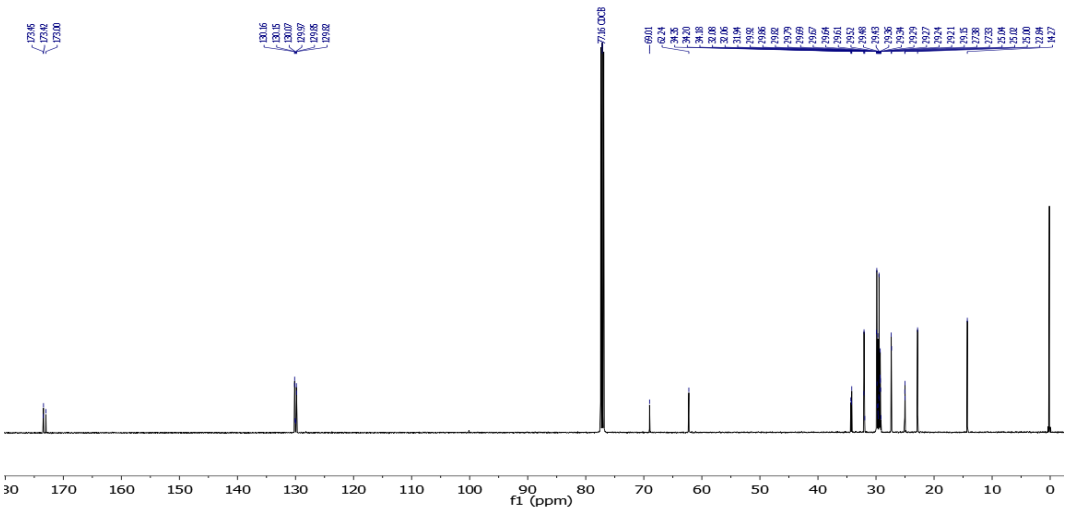

Figure 40: ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of Soxhlet extracted MO seed oil

C1 - Internal use

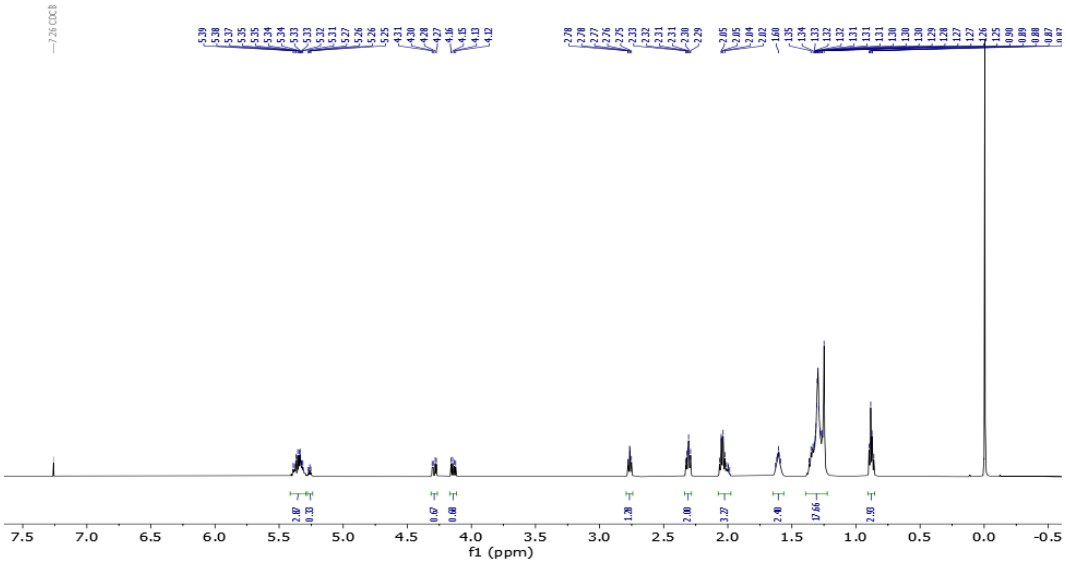

Figure 41: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of Kalahari melon seed oil

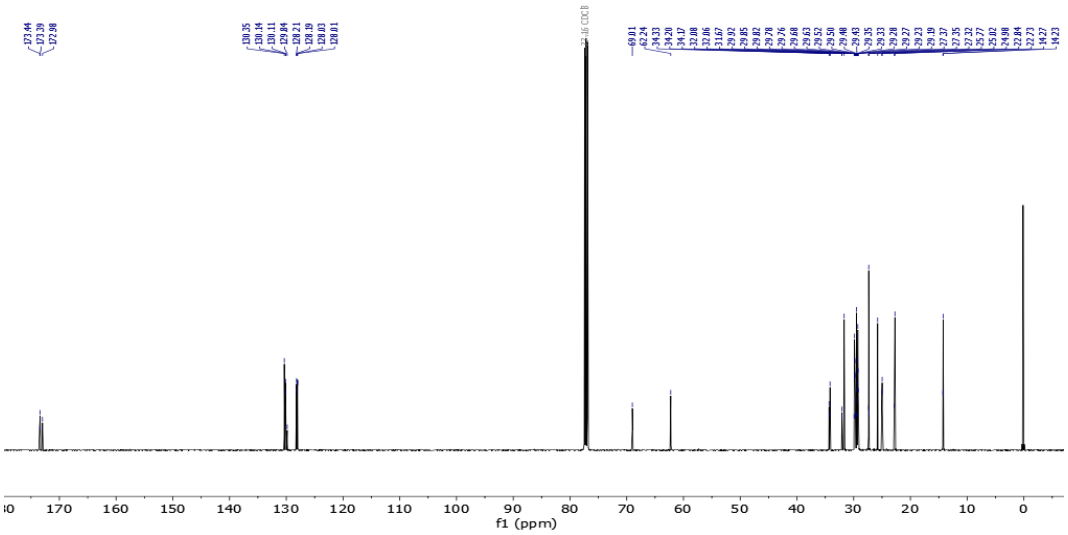

Figure 42: ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of Kalahari melon MO seed oil

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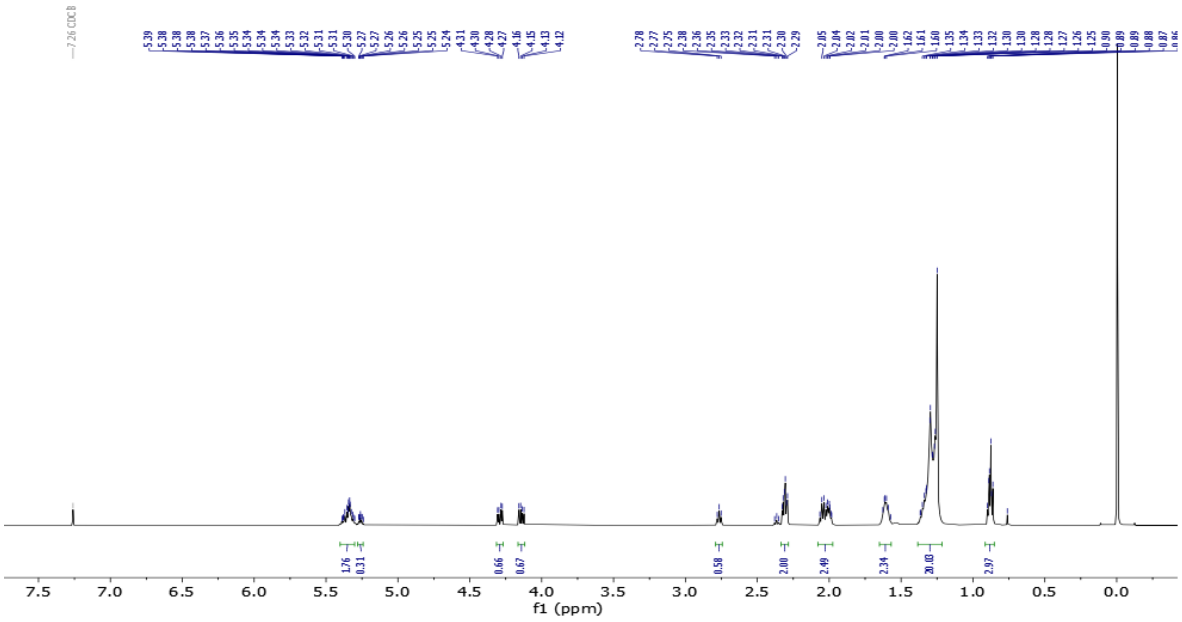

Figure 43: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum Baobab seed oil

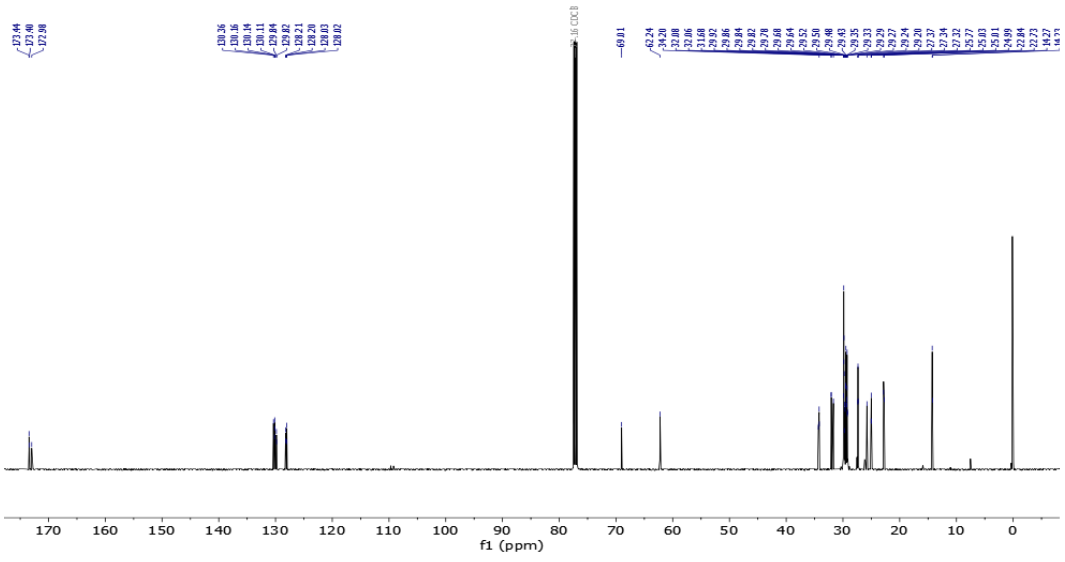

Figure 44: ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of Baobab seed oil

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APPENDIX C: FT- IR Spectra

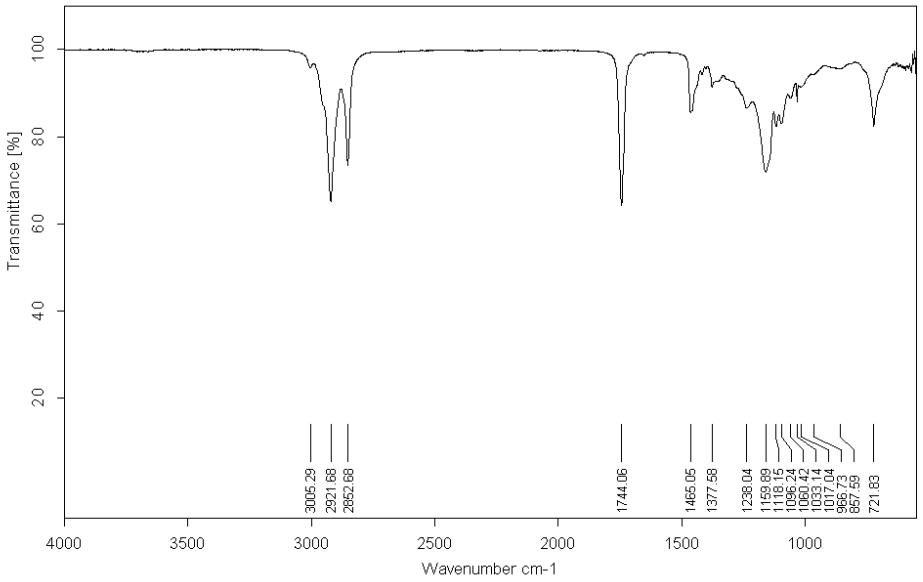

Figure 45: FT-IR spectra of Ultrasound-assisted extracted MO seed oil

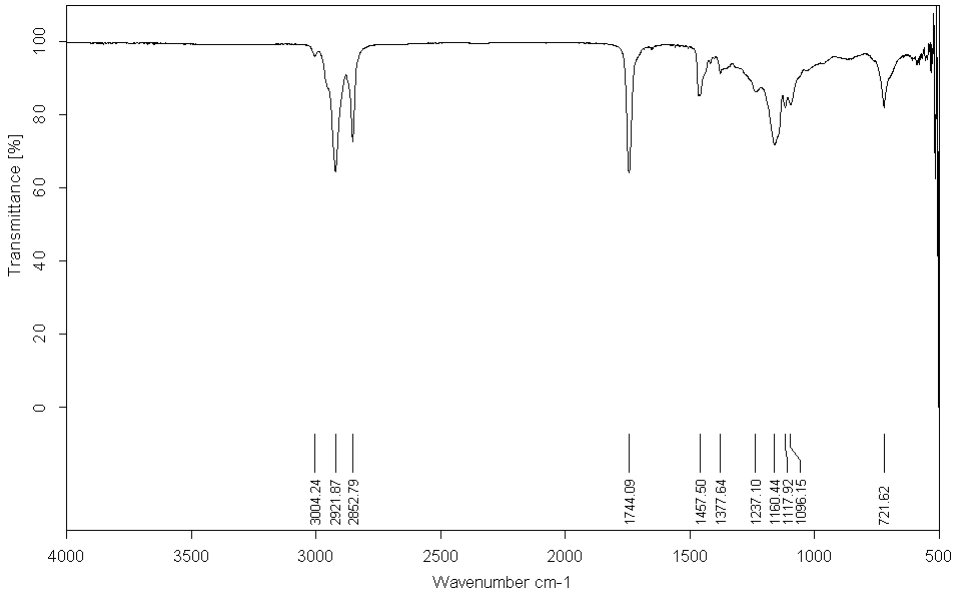

Figure 46: FT-IR spectra of Lefakong farming cold-press MO seed oil

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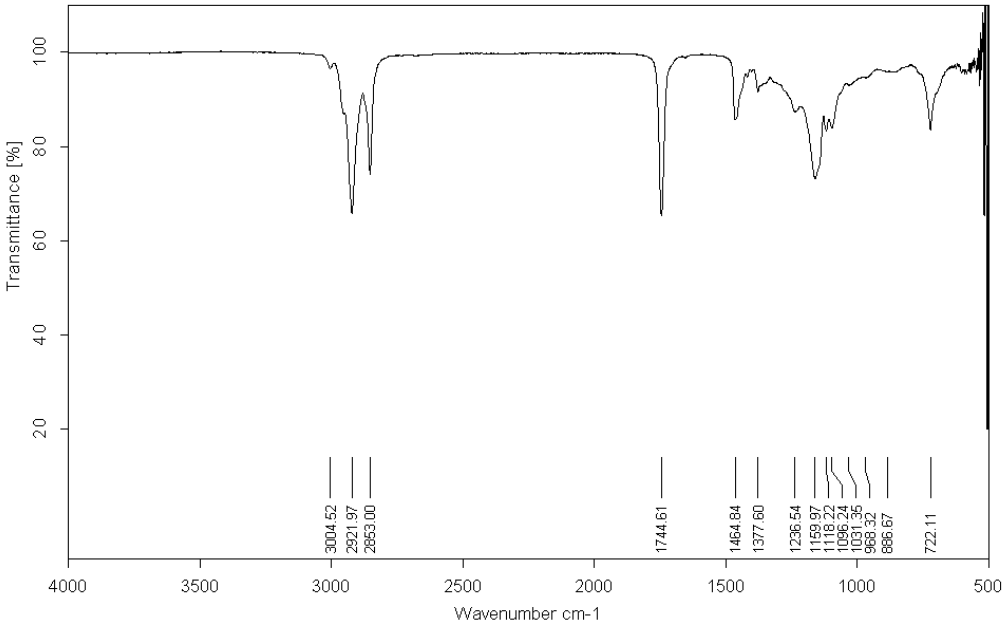

Figure 47: FT-IR spectra of Soxhlet extracted MO seed oil

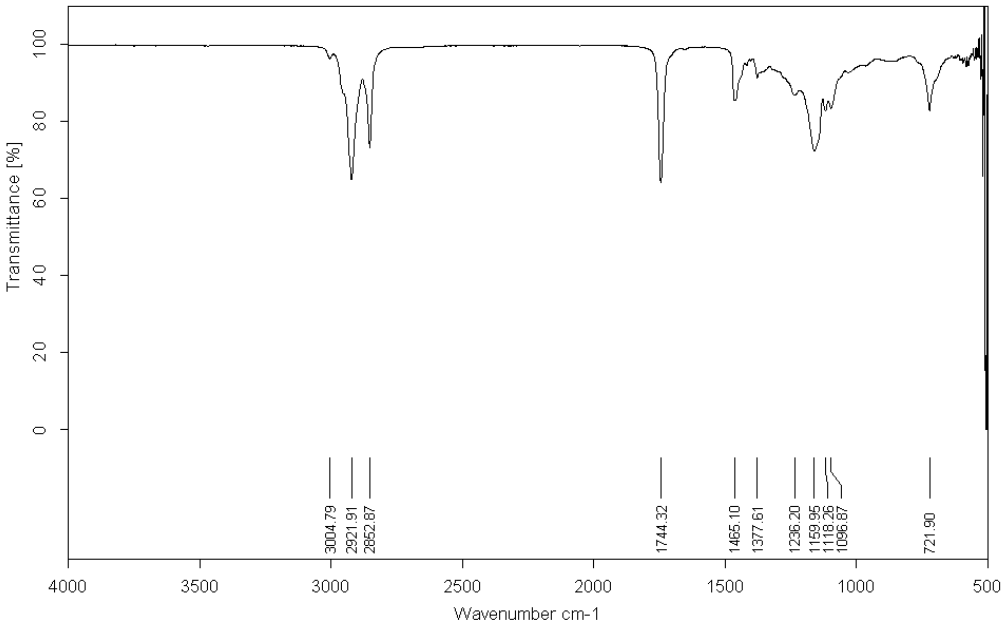

Figure 48: FT-IR spectra of Shaker method extracted MO seed oil

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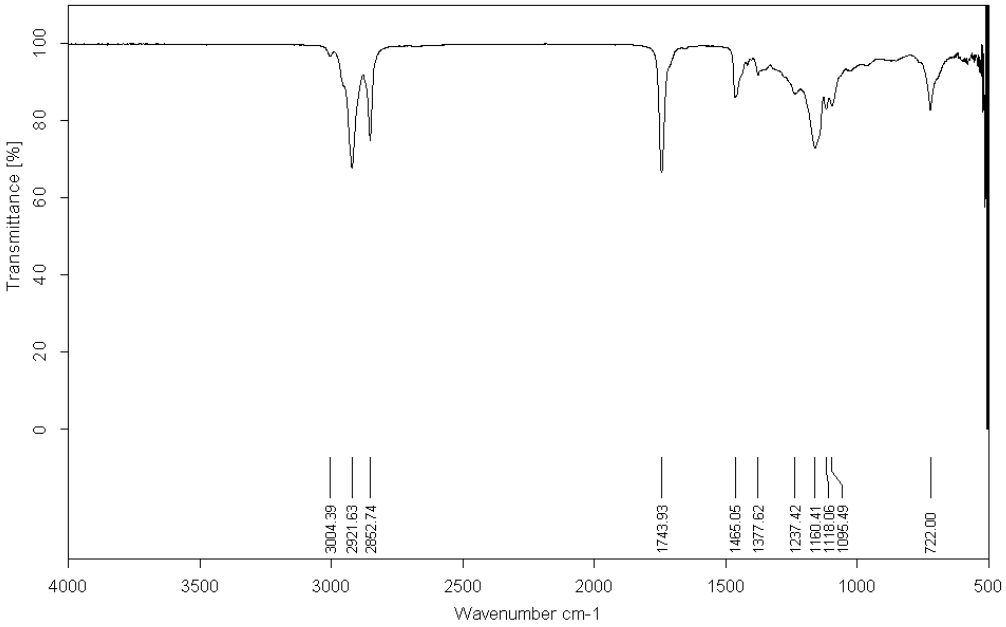

Figure 49: FT-IR spectra of Moyo moringa SA seed oil

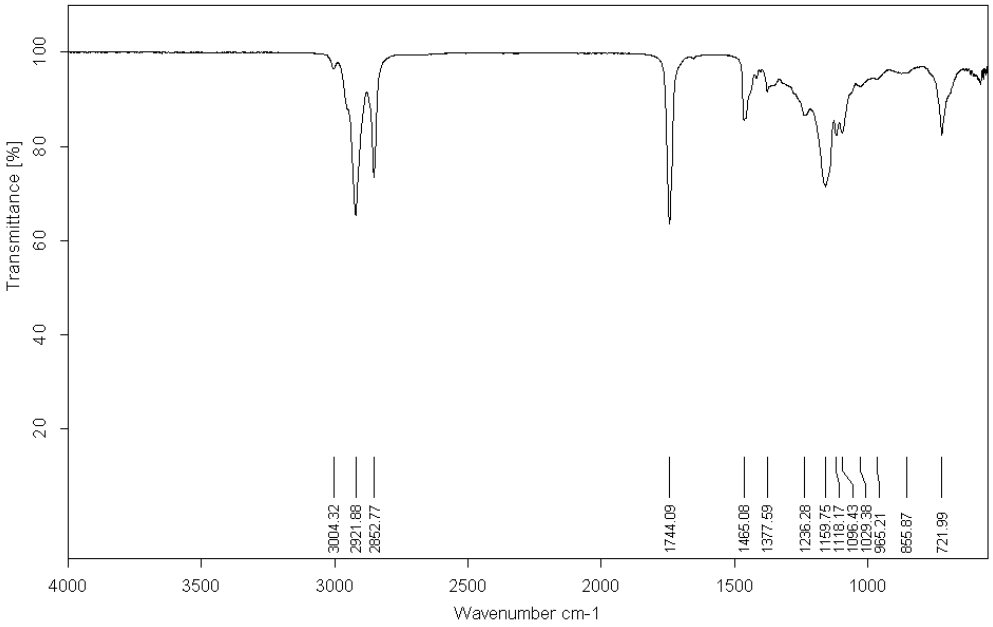

Figure 50: FT-IR spectra of Leaf- Moringa seed oil

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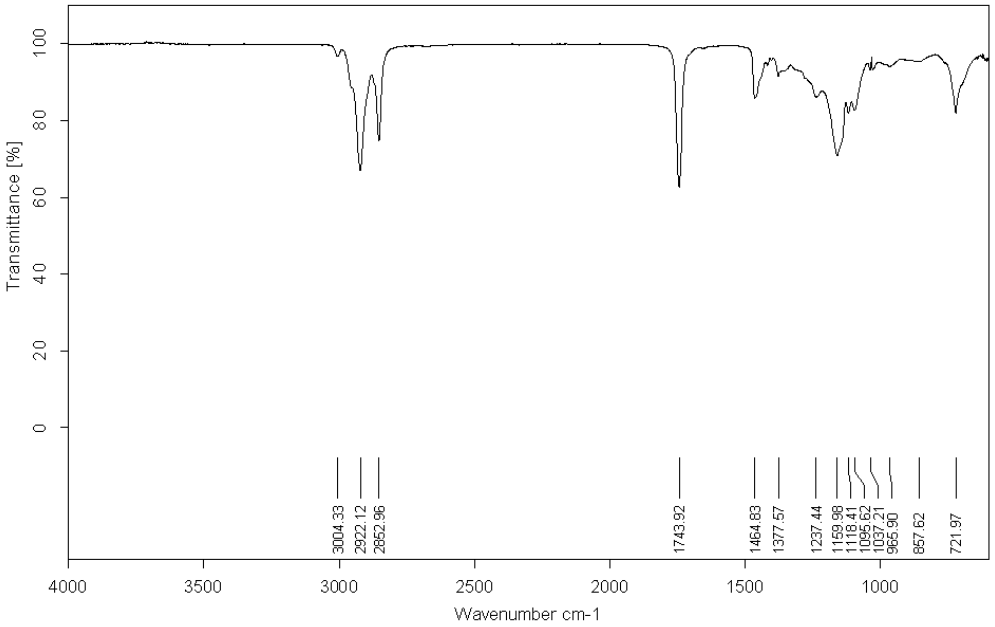

Figure 51: FT-IR spectra of Essentially Natural Moringa oil

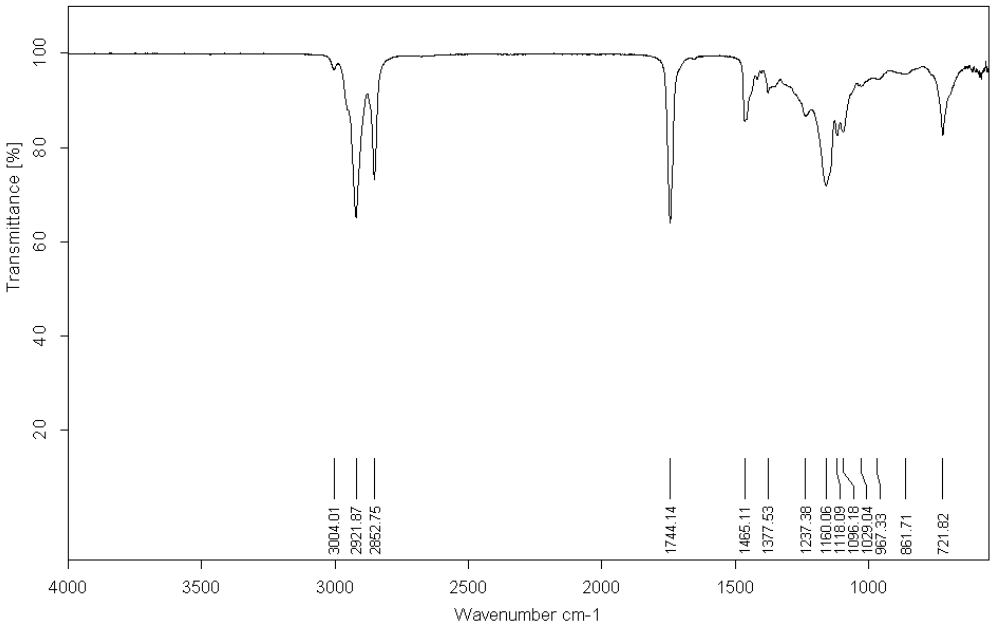

Figure 52: FT-IR spectra of Moringa 5000 Pure Moringa Ben oil

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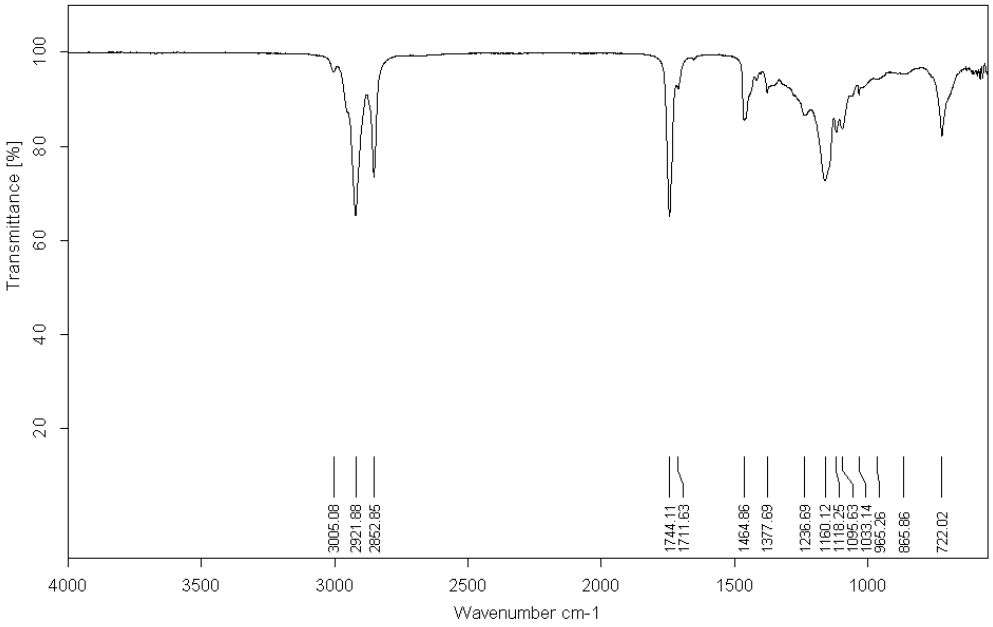

Figure 53: FT-IR spectra of Bright mountain - Moringa Seed Oil

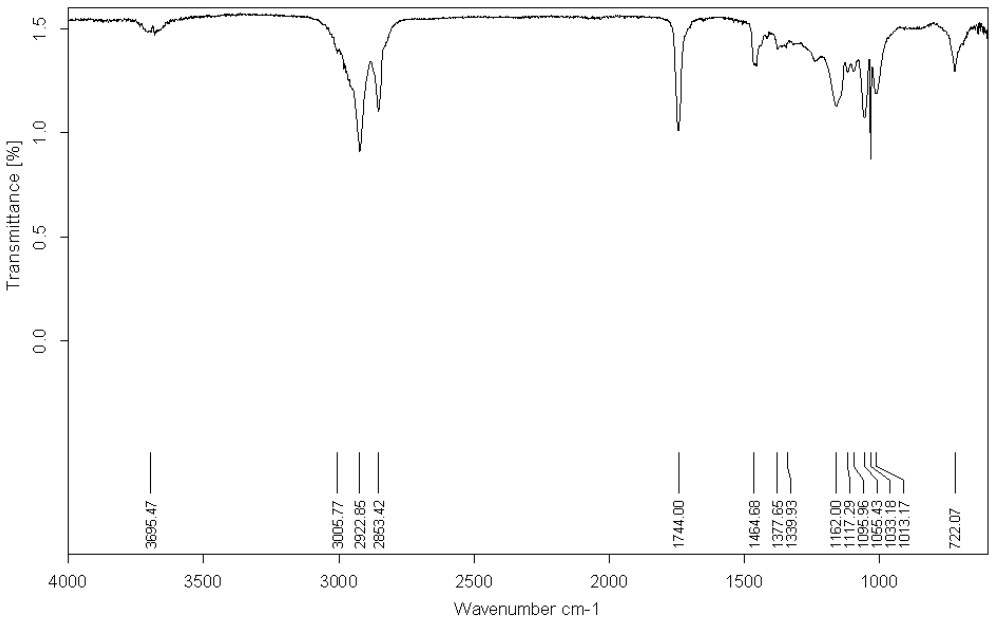

Figure 54: FT-IR spectra of Moriveda moringa basic oil

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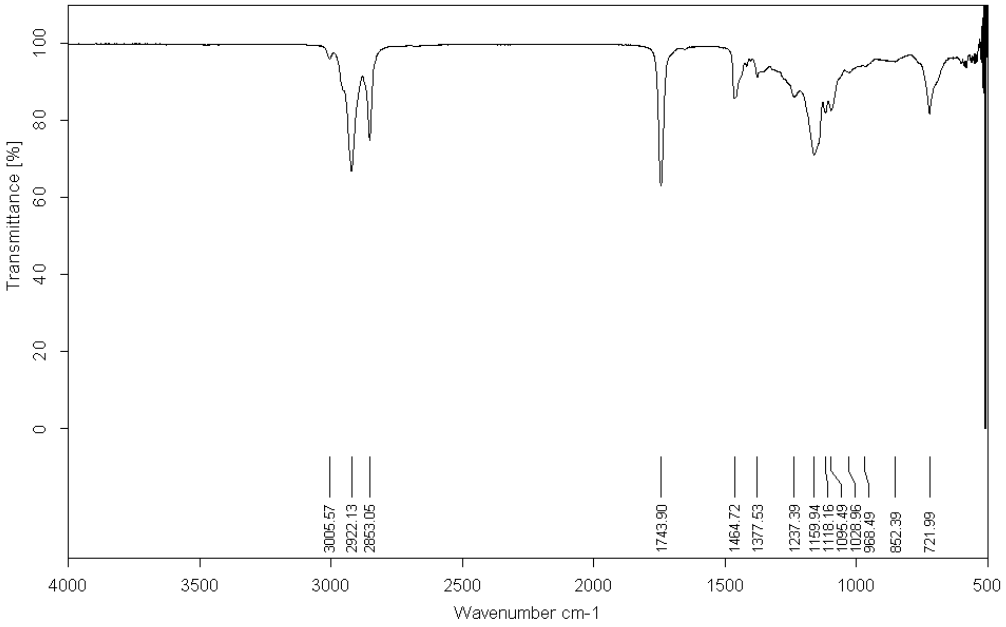

Figure 55: FT-IR spectra of Plush Organics Moringa Oil

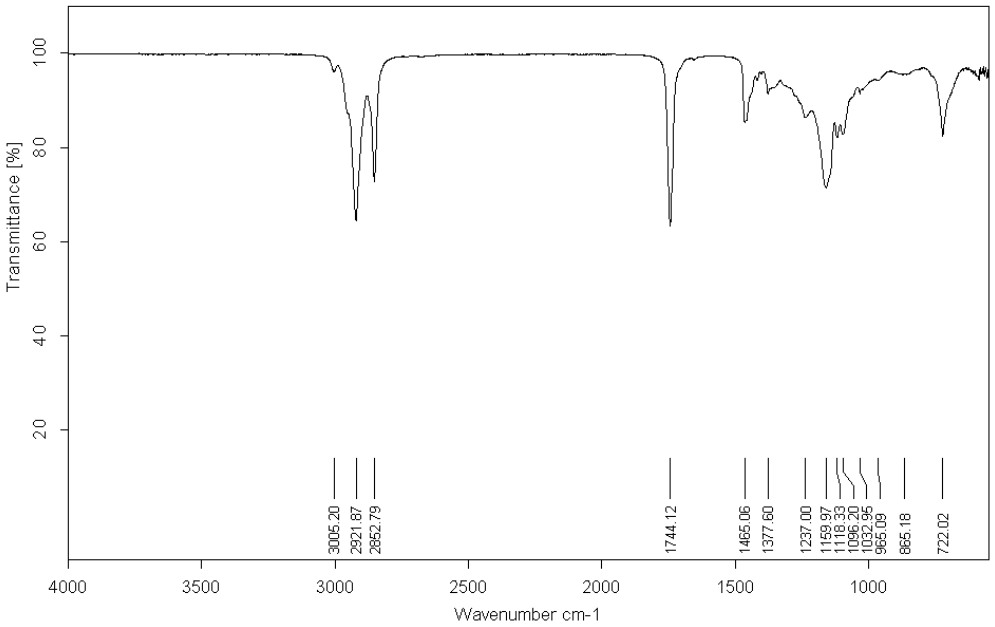

Figure 56: FT-IR spectra of Tree of life Pure organic moringa oil

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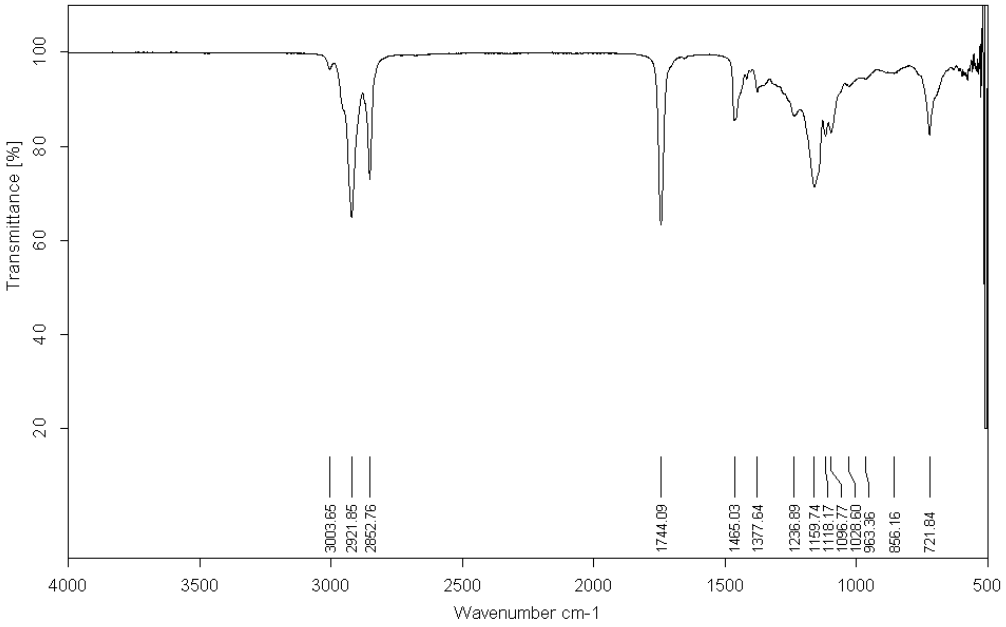

Figure 57: FT-IR spectra of Moringa world MO oil

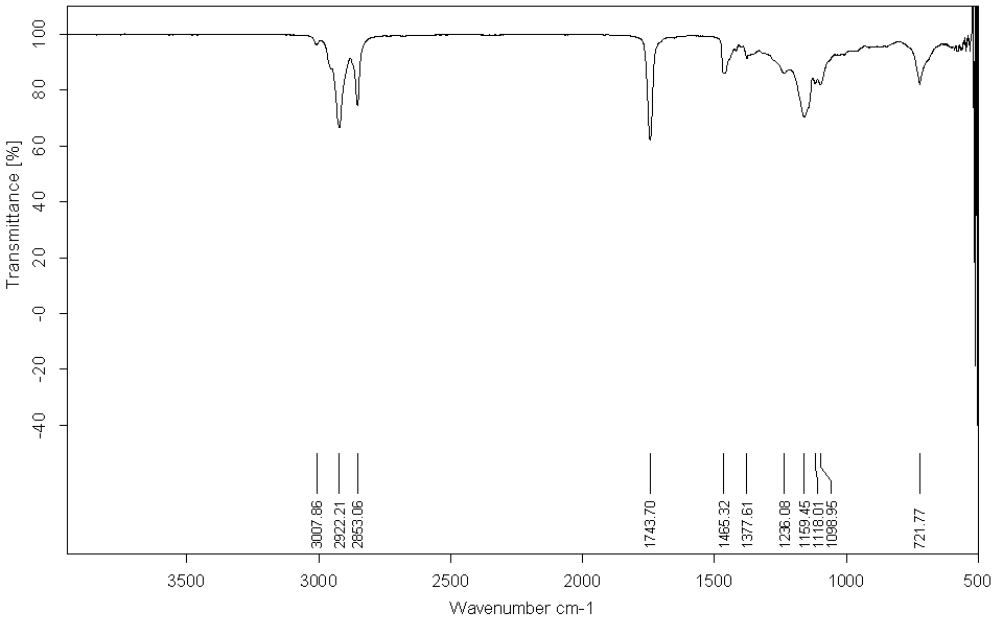

Figure 58: FT-IR spectra of Baobab seed oil

C1 - Internal use

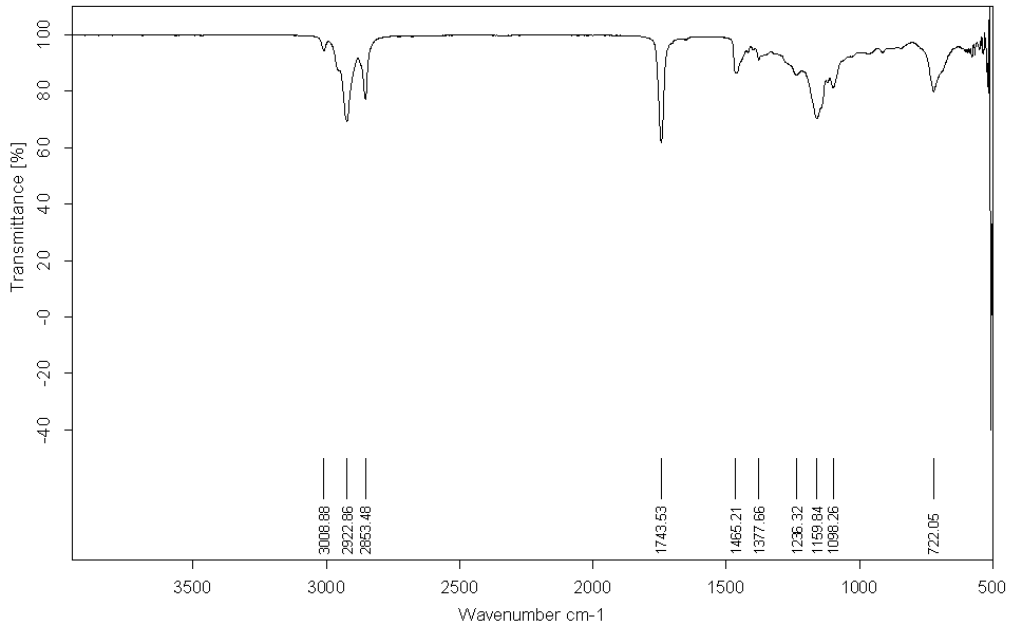

Figure 59: FT-IR spectra of Kalahari Melon seed oil

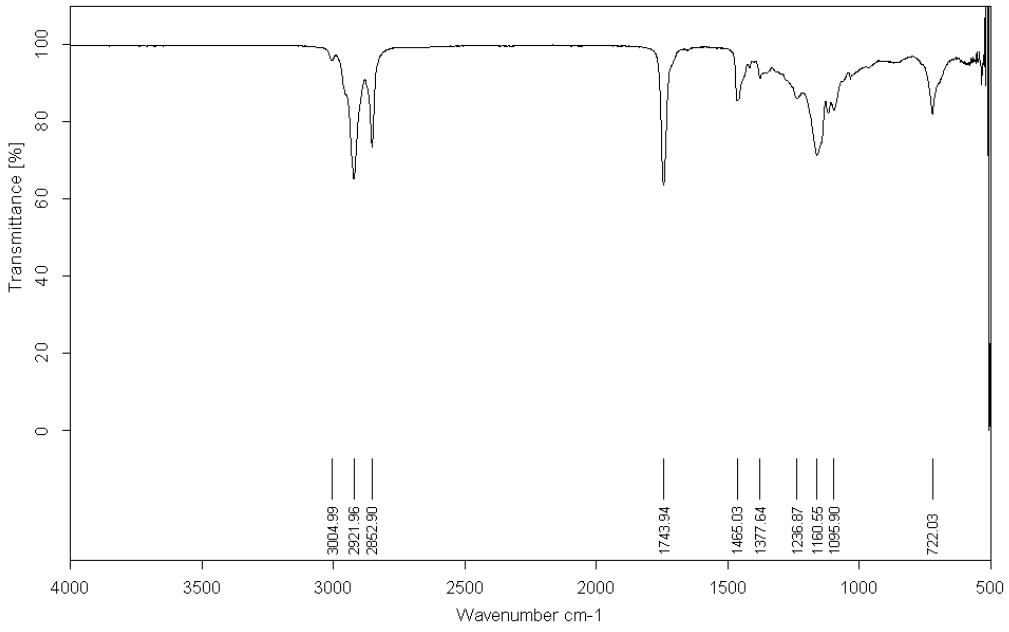

Figure 60: FT-IR spectra of Marula seed oil

C1 - Internal use

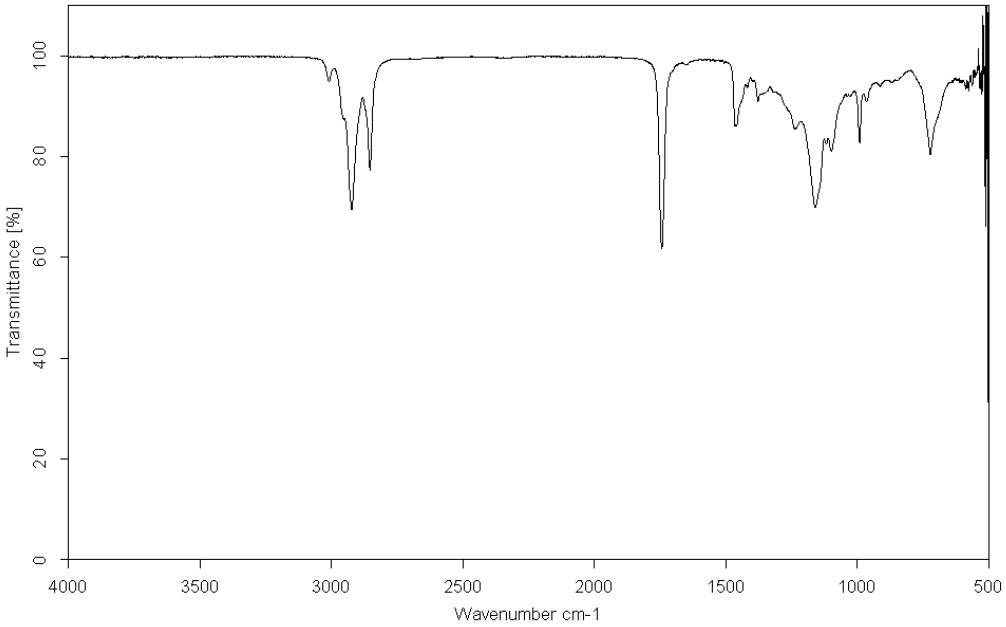

Figure 61: FT-IR spectra of Mongongo seed oil

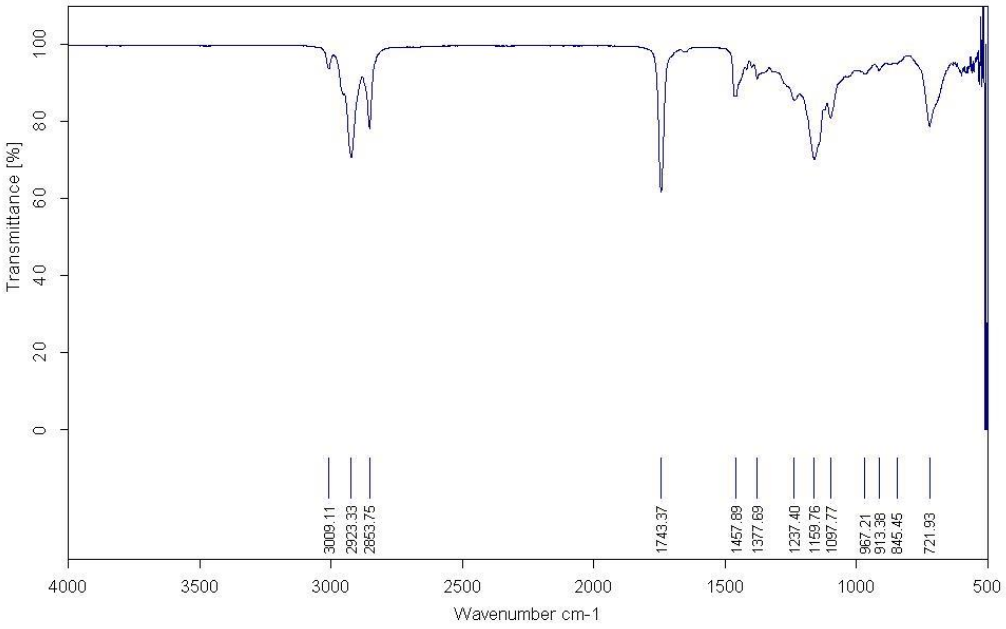

Figure 62: FT-IR spectra of Hemp seed oil

C1 - Internal use

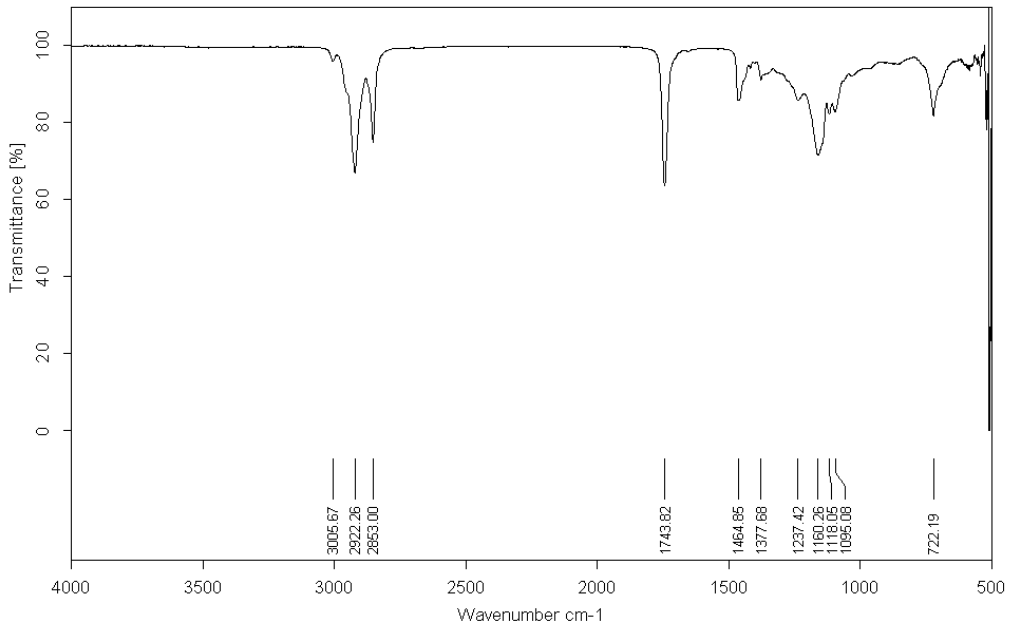

Figure 63: FT-IR spectra of Avocado seed oil

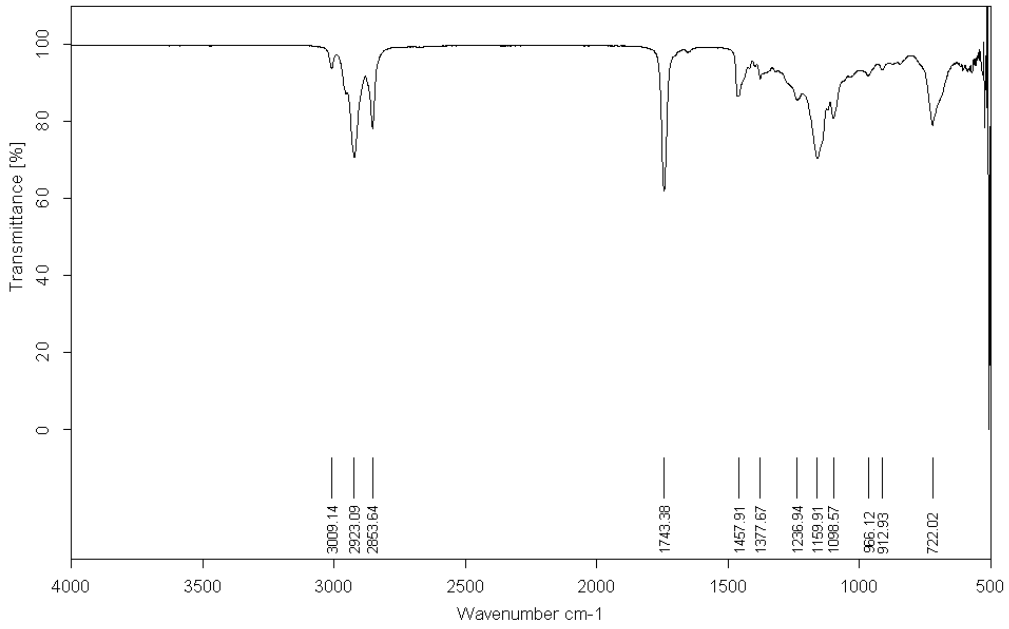

Figure 64: FT-IR spectra of Grapeseed oil

C1 - Internal use

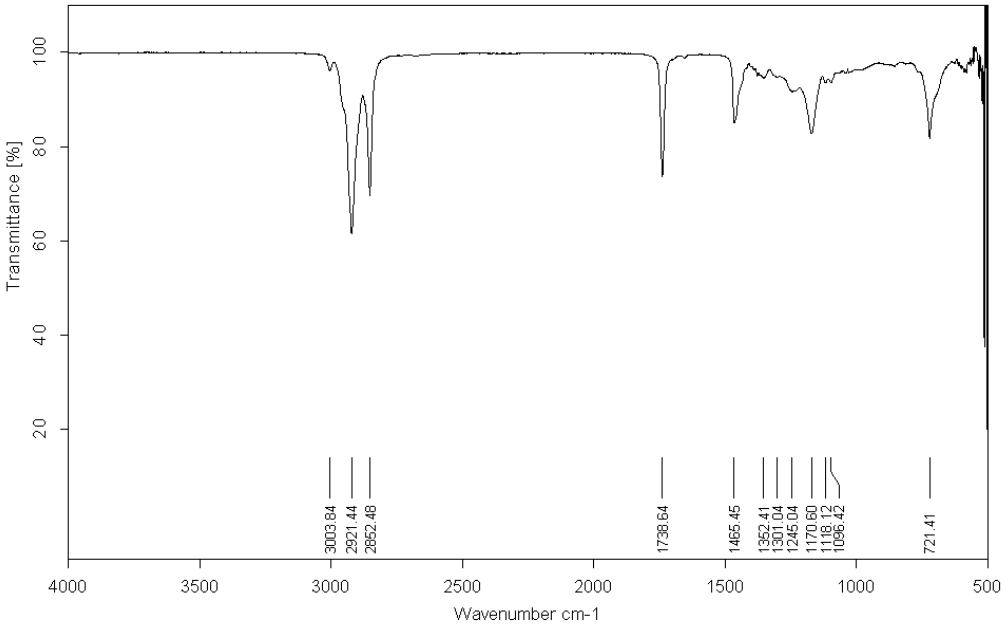

Figure 65: FT-IR spectra of Jojoba seed oil

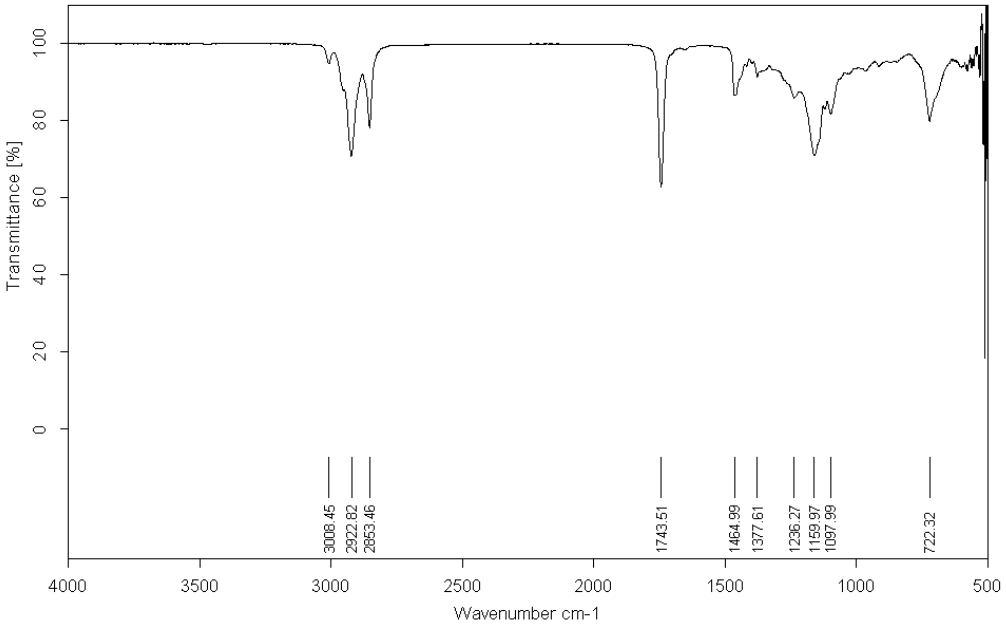

Figure 66: FT-IR spectra of Sunflower seed oil

C1 - Internal use

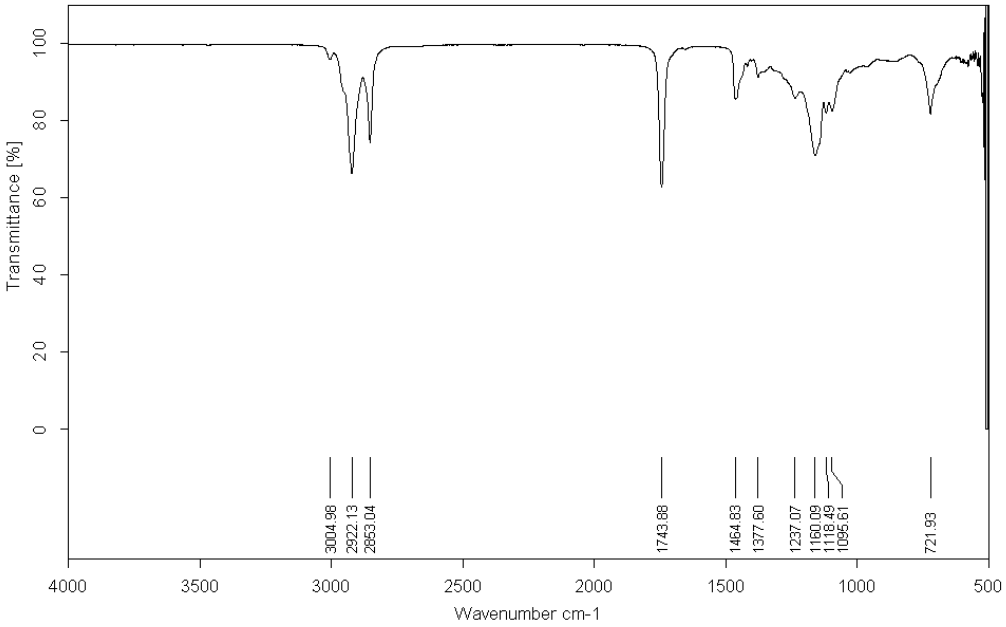

Figure 67: FT-IR spectra of Olive oil

C1 - Internal use

APPENDIX D: Gas chromatograms for fatty acid profile

Table 19: Supelco 37 component FAME Mix FAME analysis data used for quantitation & identification of the unknown peaks in the oil samples.

Elution number	Fatty acid name	Short name	Formula	Molecular weight	Fatty acid group	Retention time	Peak area	% Peak area
1	Methyl butyrate	C4:0	$C_5H_{10}O_2$	102,13	SAFA	01:45.5	33995304	0.37
2	Methyl hexanoate	C6:0	$C_7H_{14}O_2$	130.19	SAFA	03:38.1	357833716	3.93
3	Methyl octanoate	C8:0	$C_9H_{18}O_2$	158.24	SAFA	05:46.8	436416214	4.80
4	Methyl decanoate	C10:0	$C_{11}H_{22}O_2$	186.3	SAFA	07:43.2	603834101	6.64
5	Methyl undecanoate	C11:0	$C_{12}H_{24}O_2$	200.32	SAFA	08:43.7	267788121	2.94
6	Methyl laurate	C12:0	$C_{13}H_{26}O_2$	214.34	SAFA	09:53.8	568933997	6.26
7	Methyl tridecanoate	C13:0	$C_{14}H_{28}O_2$	228.37	SAFA	11:16.5	225475120	2.48
8	Myristoleic Acid Methyl Ester	C14:1	$C_{15}H_{28}O_2$	240.38	MUFA	12:38.2	140557619	1.55
9	Methyl tetradecanoate	C14:0	$C_{15}H_{30}O_2$	242.40	SAFA	12:49.1	549061088	6.04
10	cis-10-Pentadecenoic acid methyl ester	C15:1	$C_{16}H_{30}O_2$	254.41	MUFA	14:18.0	139710243	1.54
11	Methyl pentadecanoate	C15:0	$C_{16}H_{32}O_2$	256.42	SAFA	14:29.1	266309612	2.93
12	Methyl Palmitoleate	C16:1	$C_{17}H_{32}O_2$	268.40	MUFA	15:51.5	146133894	1.61
13	Methyl palmitate	C16:0	$C_{17}H_{34}O_2$	270.50	SAFA	16:13.6	820295541	9.02
14	cis-10-Heptadecenoic acid methyl ester	C17:1	$C_{18}H_{34}O_2$	282.50	MUFA	17:36.3	149315963	1.64
15	Methyl heptadecanoate	C17:0	$C_{18}H_{36}O_2$	284.50	SAFA	17:57.9	203516168	2.24
16	gamma-Linolenic acid methyl ester	C18:3	$C_{19}H_{32}O_2$	292.50	PUFA	18:52.0	105588174	1.16
17	Linolelaidic acid methyl ester	C18:2	$C_{19}H_{34}O_2$	294.50	TFA	19:09.4	70377637	0.77
18	trans-9-Elaidic acid methyl ester	C18:1	$C_{19}H_{36}O_2$	296.50	TFA	19:16.2	668087447	7.35
19	Methyl Linolenate	C18:3	$C_{19}H_{32}O_2$	292.50	PUFA	19:18.6	196680452	2.16
20	Methyl Linoleate	C18:2	$C_{19}H_{34}O_2$	294.50	PUFA	19:18.6	196680452	2.16
21	cis-9-Oleic acid methyl ester	C18:1	$C_{19}H_{36}O_2$	296.50	MUFA	19:22.0	375404916	4.13
22	Methyl octadecanoate	C18:0	$C_{19}H_{38}O_2$	298.50	SAFA	19:42.5	484913192	5.33
23	Methyl cis-5,8,11,14-Eicosatetraenoic	C20:4	$C_{21}H_{34}O_2$	318.49	PUFA	21:55.2	55050320	0.61
24	Methyl cis-5,8,11,14,17-Eicosapentaenoate	C20:5	$C_{21}H_{32}O_2$	316.48	PUFA	22:03.0	92375013	1.02

C1 - Internal use

25	cis-8,11,14-Eicosatrienoic acid methyl ester	C20:3	$C_{21}H_{36}O$	320.51	PUFA	22:14.0	103451752	1.14
26	cis-11,14-Eicosadienoic acid methyl ester	C20:2	$C_{21}H_{38}O_2$	322.50	PUFA	22:33.5	81084318	0.89
27	Methyl cis-11-eicosenoate	C20:1	$C_{21}H_{40}O_2$	324.50	MUFA	22:37.9	132493822	1.46
28	cis-11,14,17-Eicosatrienoic acid methyl ester	C20:3	$C_{21}H_{36}O$	320.51	PUFA	22:41.3	219009213	2.41
29	Methyl heneicosanoate	C21:0	$C_{22}H_{44}O_2$	340.00	SAFA	23:04.0	383469197	4.22
30	All cis-4,7,10,13,16,19-Docosahexaenoate	C22:6	$C_{23}H_{34}O_2$	342.51	PUFA	25:02.3	65131261	0.72
31	cis-13-16-Docosadienoic acid methyl ester (22:2)	C22:2	$C_{23}H_{42}O_2$	350.60	PUFA	25:46.4	41408205	0.46
32	Methyl Erucate	C22:1	$C_{23}H_{44}O_2$	352.60	MUFA	25:49.1	202421856	2.23
33	Methyl Arachidate	C20:0	$C_{21}H_{42}O_2$	326.60	SAFA	26:13.0	272159250	2.99
34	Methyl docosanoate	C22:0	$C_{23}H_{46}O_2$	354.60	SAFA	27:42.0	87817531	0.97
35	Methyl tricosanoate	C23:0	$C_{24}H_{48}O_2$	368.60	SAFA	27:42.0	87607511	0.96
36	Methyl Nervonate	C24:1	$C_{25}H_{48}O_2$	380.60	MUFA	28:56.0	62259343	0.68
37	Methyl lignocerate	C24:0	$C_{25}H_{50}O_2$	382.70	SAFA	29:22.4	200978834	2.21

SAFA = Saturated Fatty Acids, TFA = Trans Fatty Acids, MUFA = Monounsaturated Fatty Acids, and PUFA = Polyunsaturated Fatty Acids.

C1 - Internal use

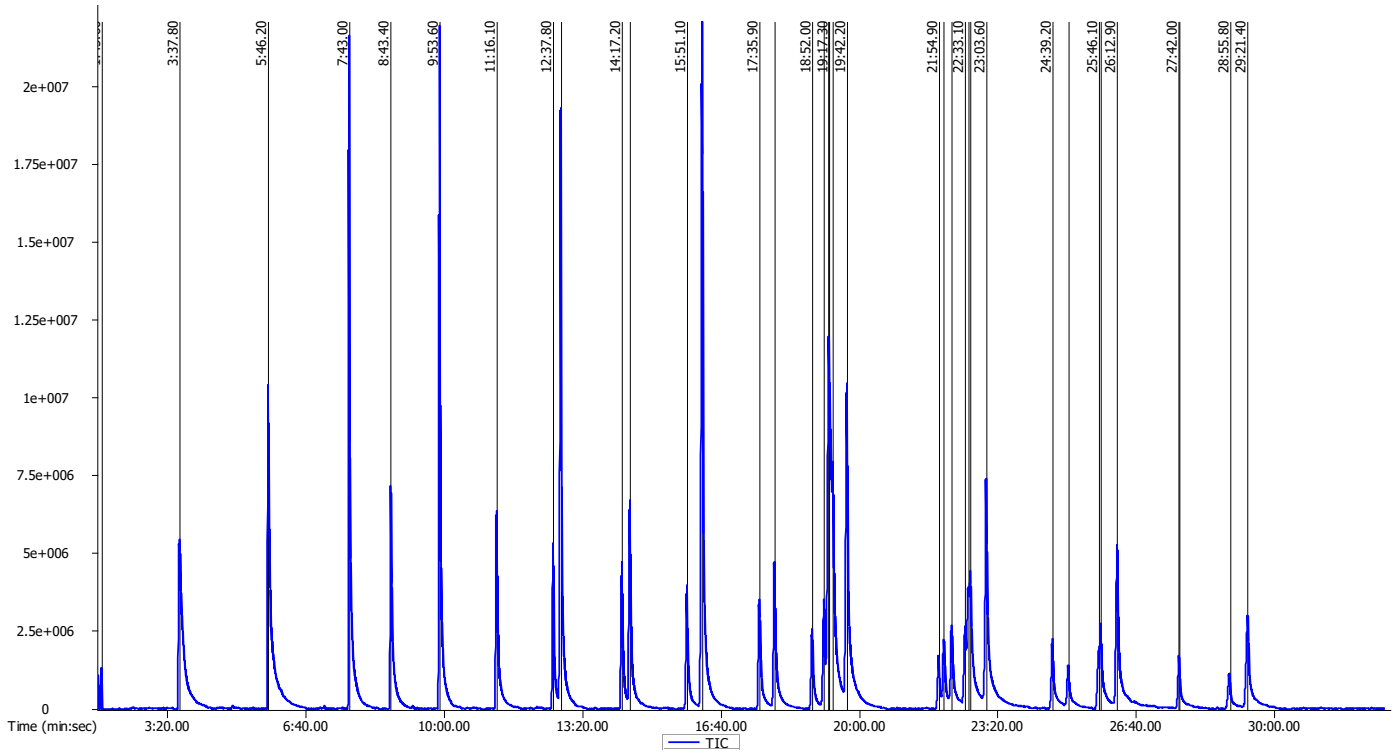

Figure 68: GC-MS chromatogram of Supelco 37 component FAME mix reference

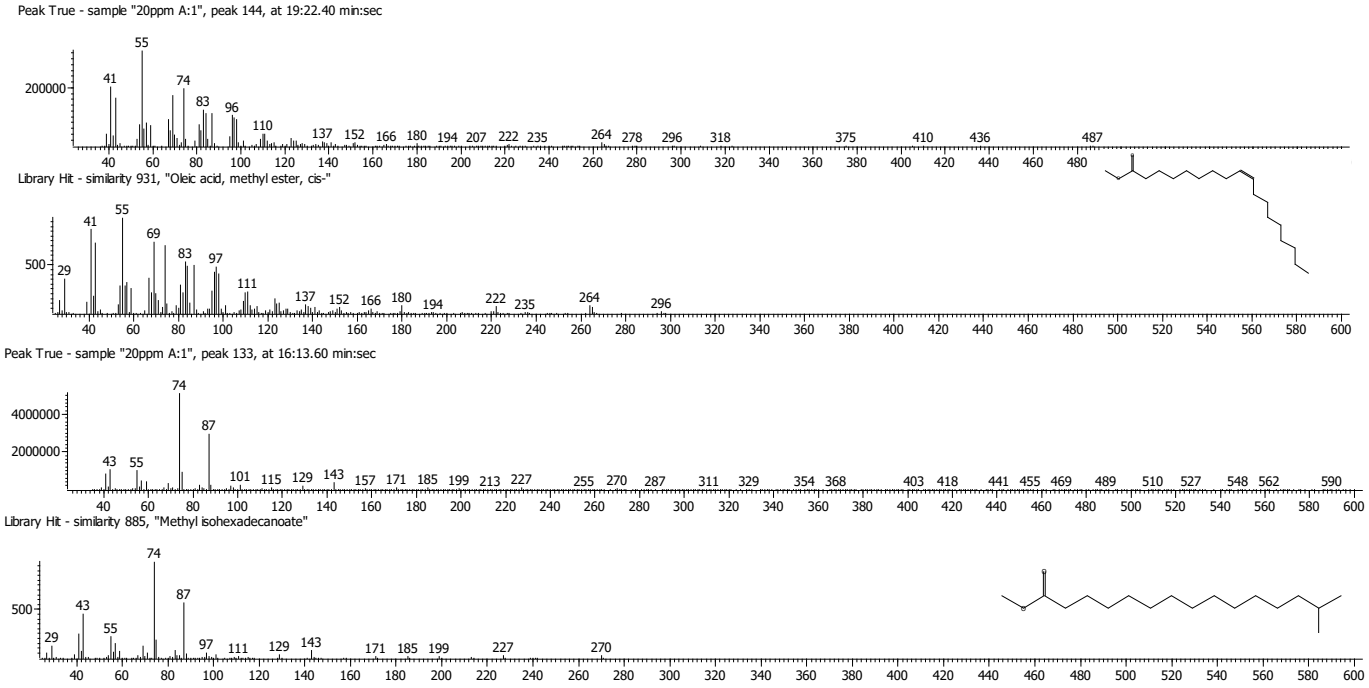

Figure 69: Mass spectra of major FAMES oleic acid and palmitic acid

C1 - Internal use

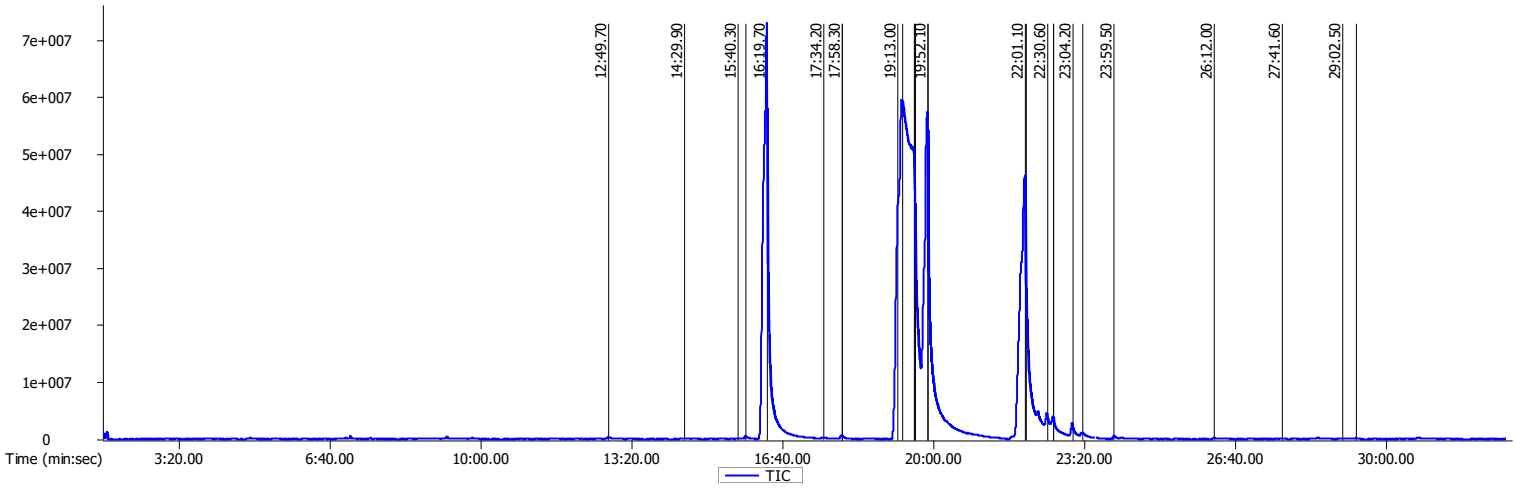

Figure 70: Gas chromatogram of Mongongo seed oil

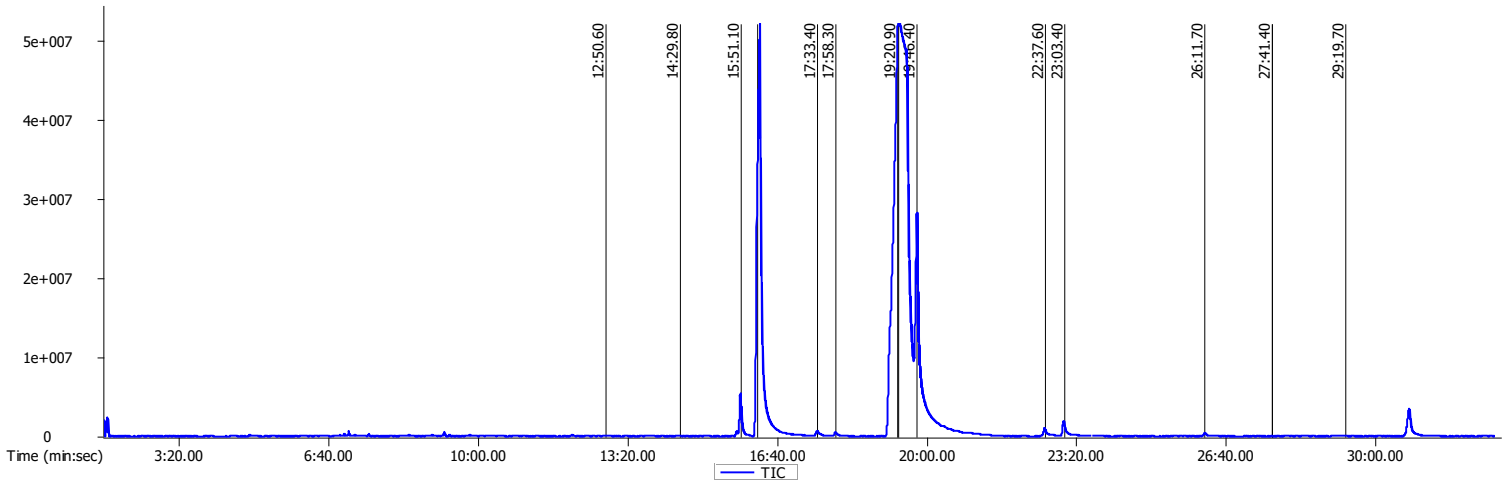

Figure 71: Gas chromatogram of Olive oil

C1 - Internal use

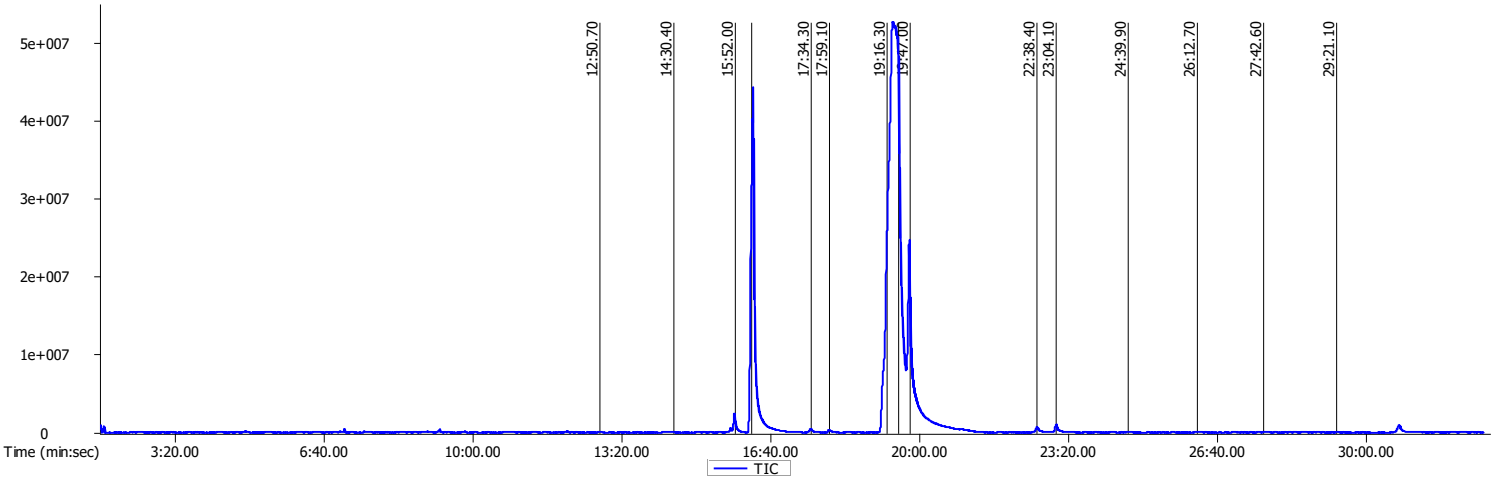

Figure 72: Gas chromatogram of Plush organics Moringa oil

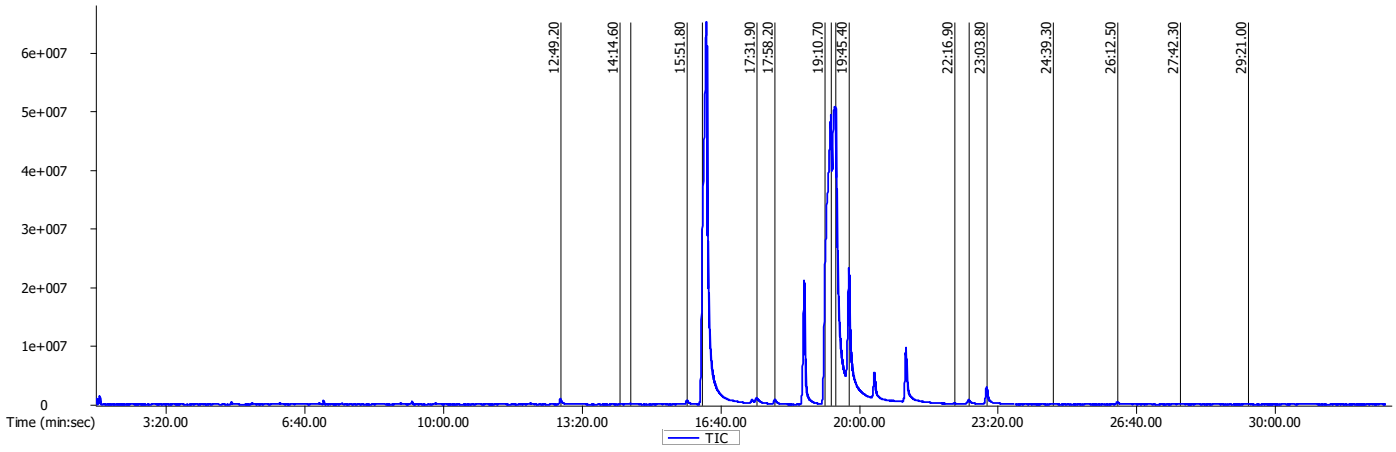

Figure 73: Gas chromatogram of Baobab seed oil

C1 - Internal use

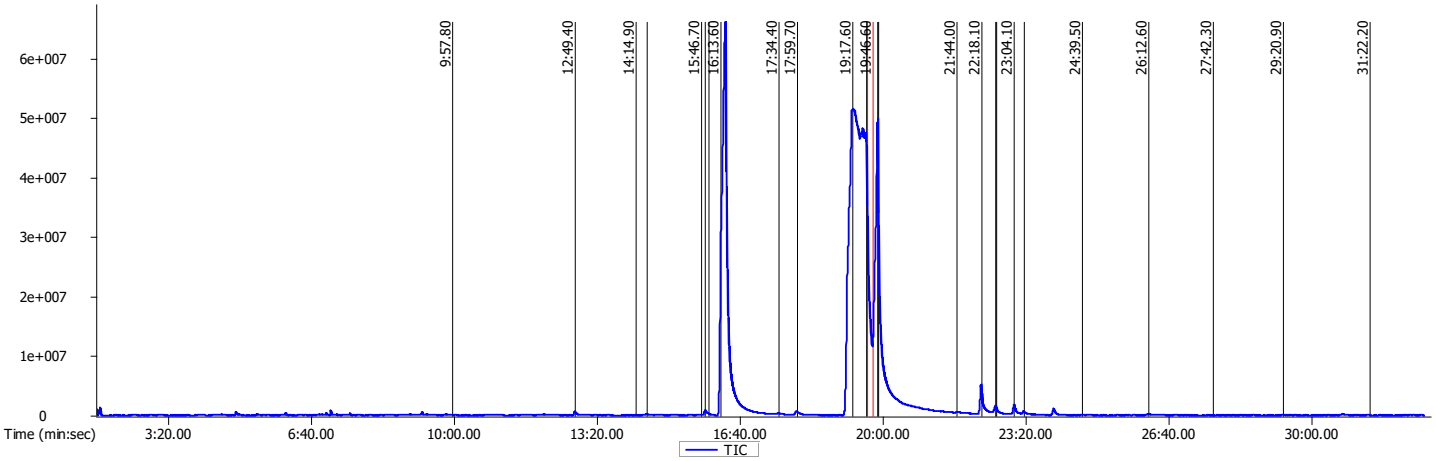

Figure 74: Gas chromatogram of Kalahari melon seed oil

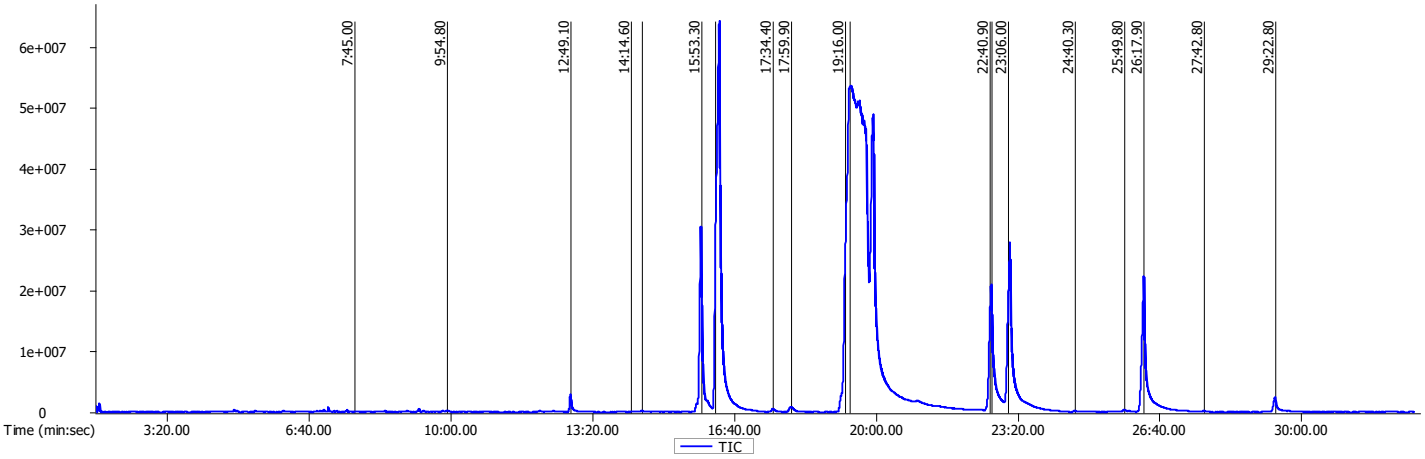

Figure 75: Gas chromatogram of Bright mountain Moringa se**ed oil**

C1 - Internal use

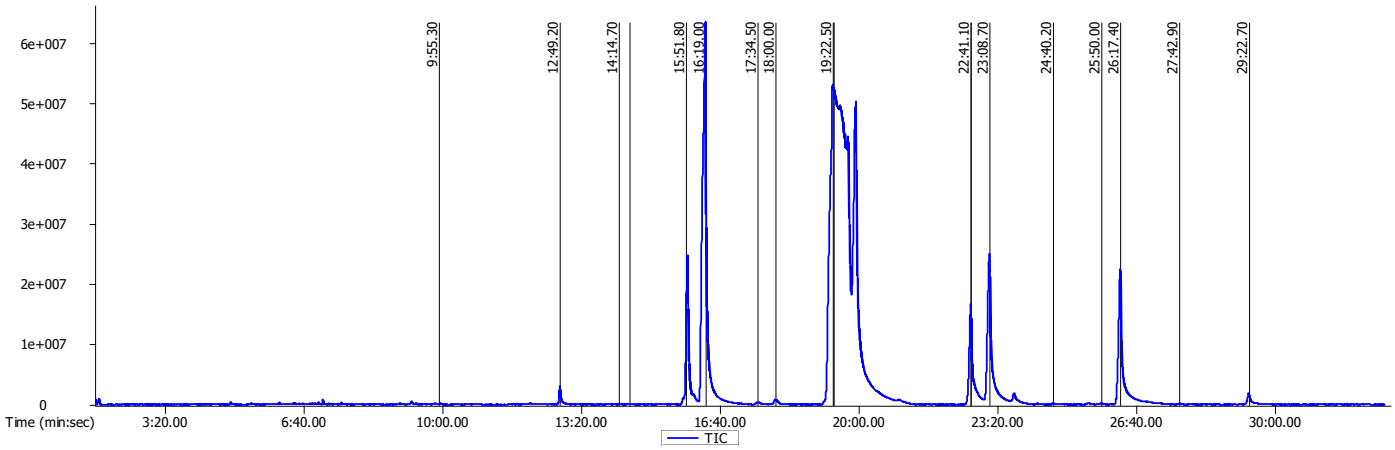

Figure 76: Gas chromatogram of Moyo moringa SA oil

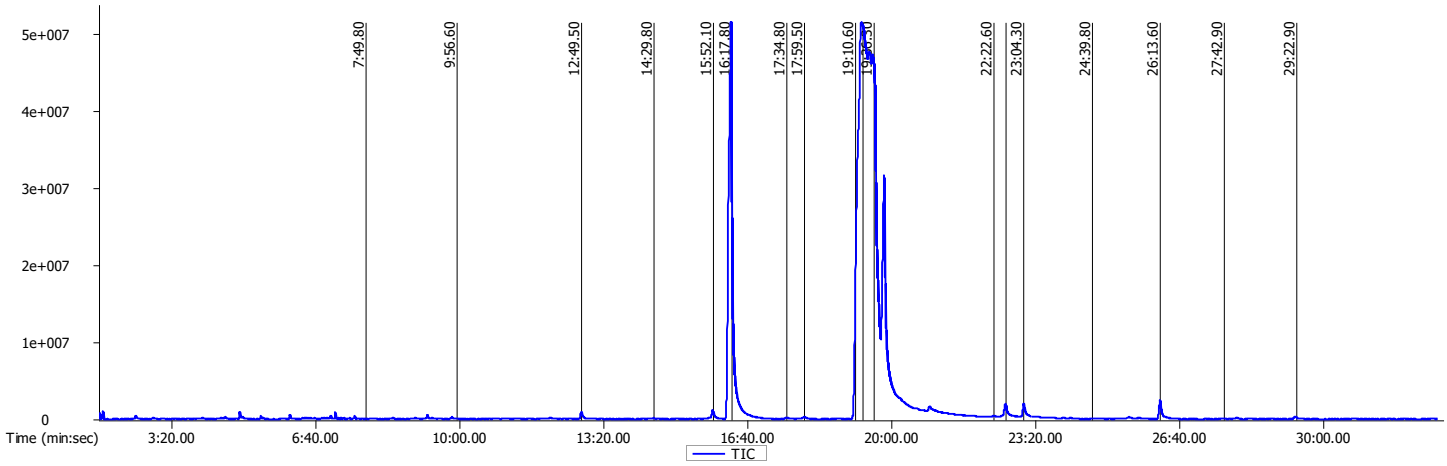

Figure 77: Gas chromatogram of Sunflower seed oil

C1 - Internal use

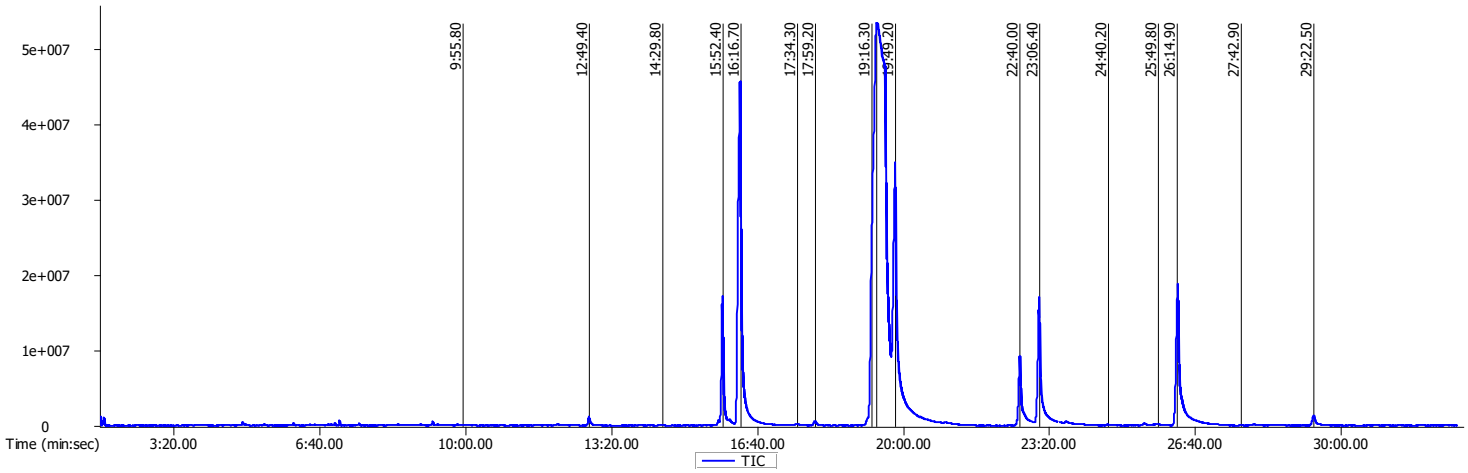

Figure 78: Gas chromatogram of Moringa world oil

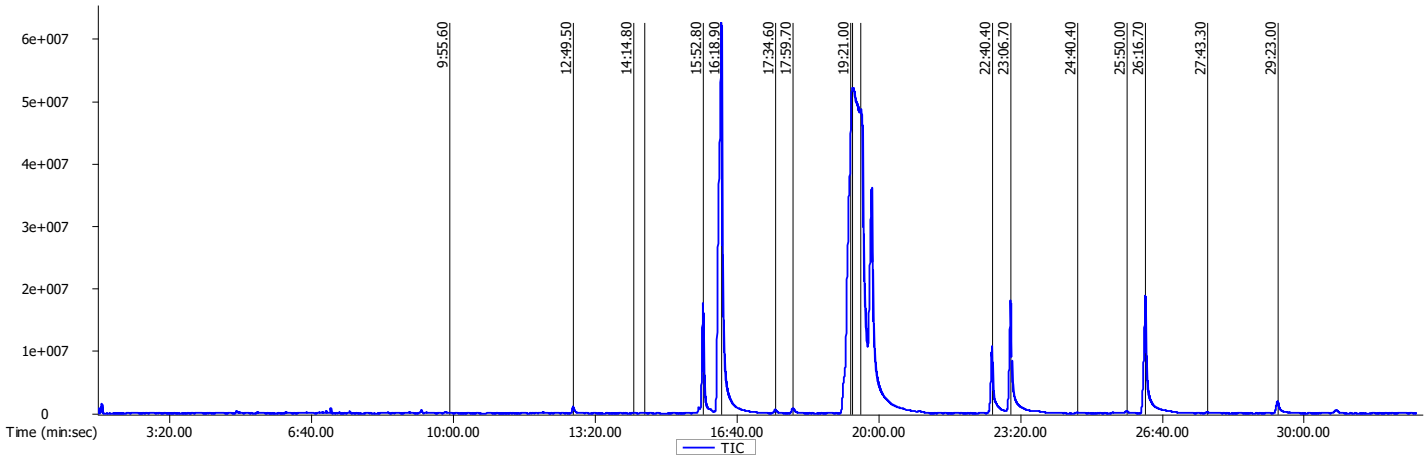

Figure 79: Gas chromatogram of Karibaa - Moriveda moringa basic oil

C1 - Internal use

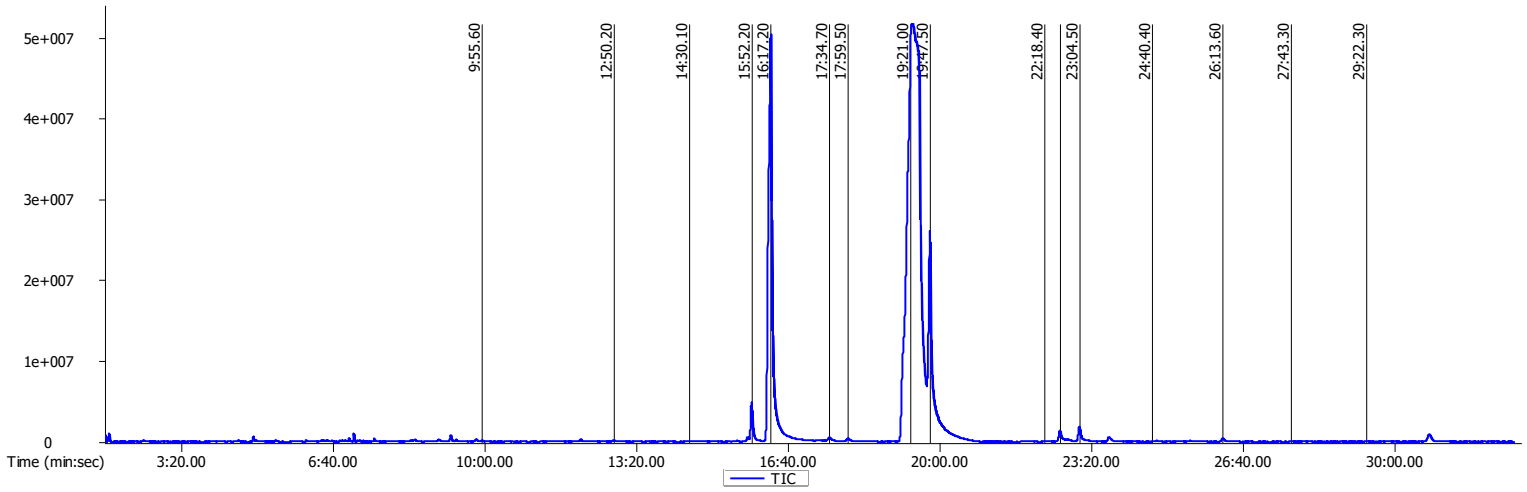

Figure 80: Gas chromatogram of Essentially Natural Moringa oil

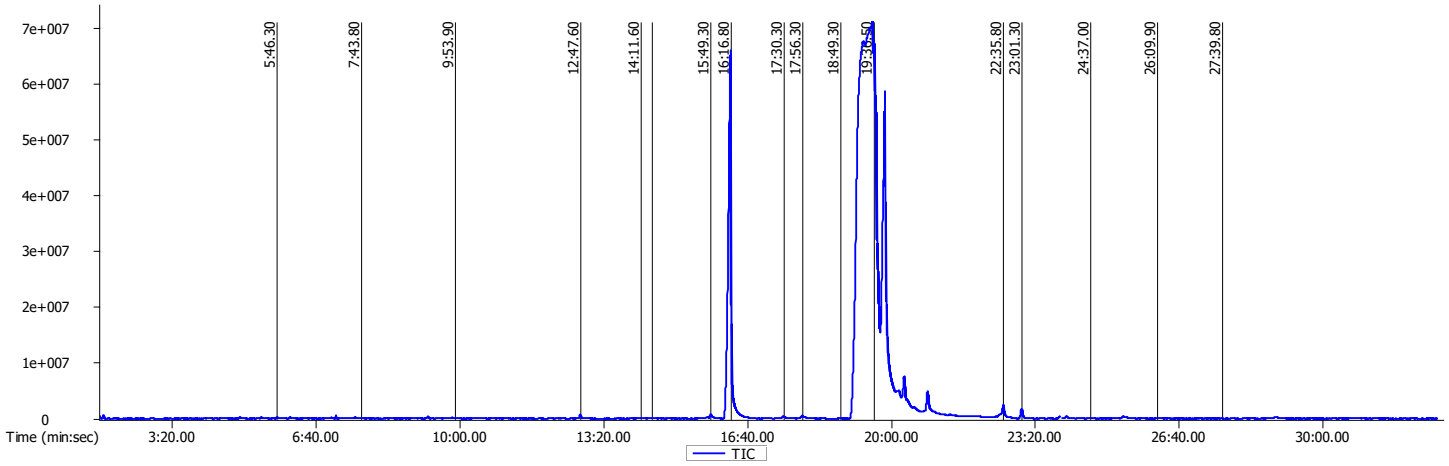

Figure 81: Gas chromatogram of Grapeseed oil

C1 - Internal use

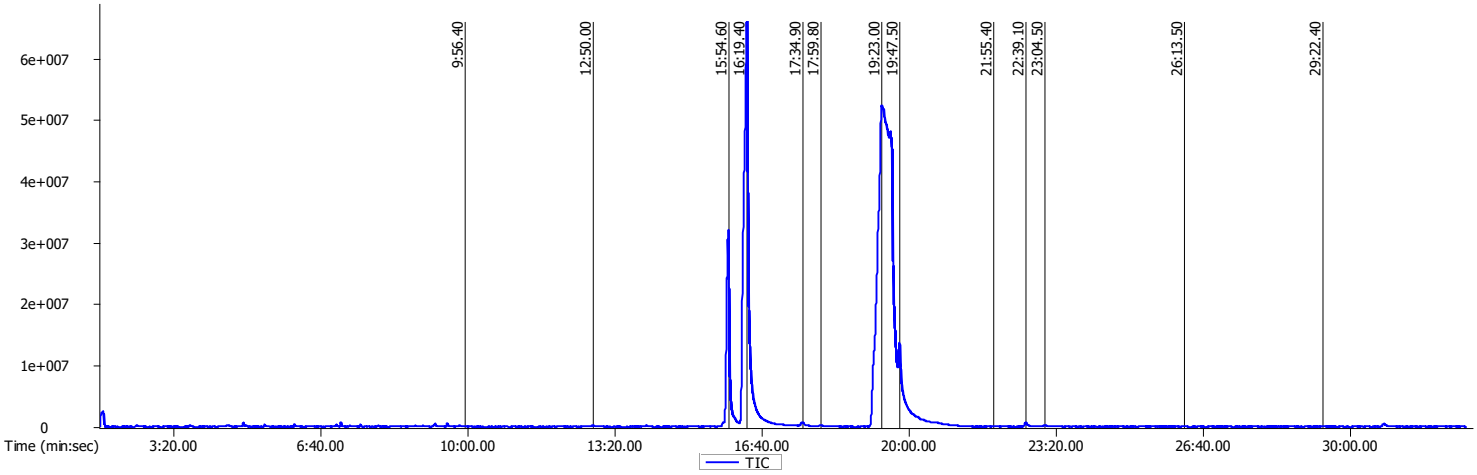

Figure 82: Gas chromatogram of Avocado oil

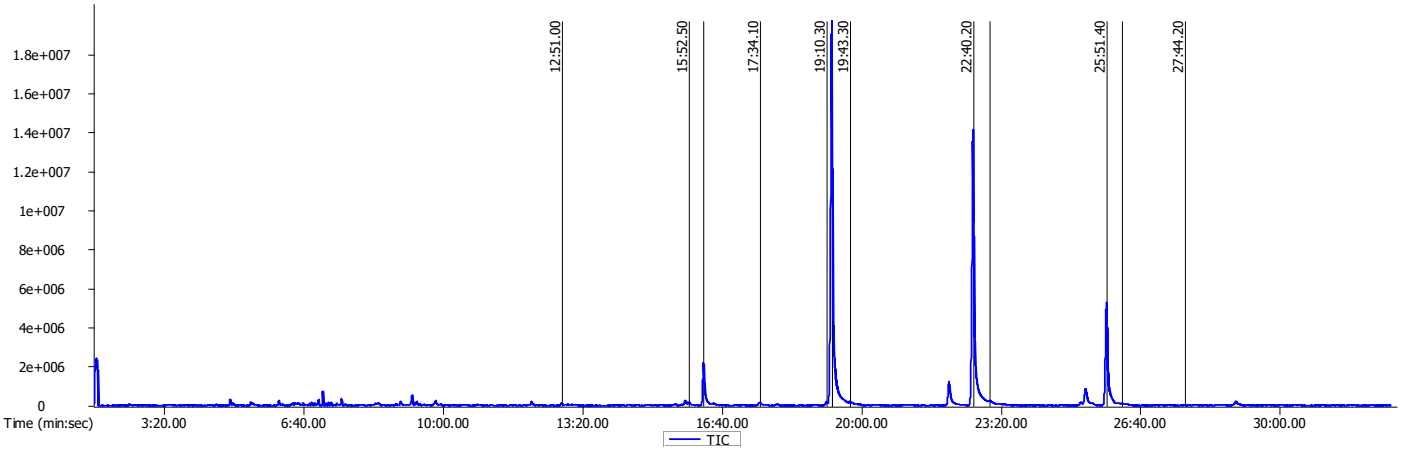

Figure 83: Gas chromatogram of Jojoba seed oil

C1 - Internal use

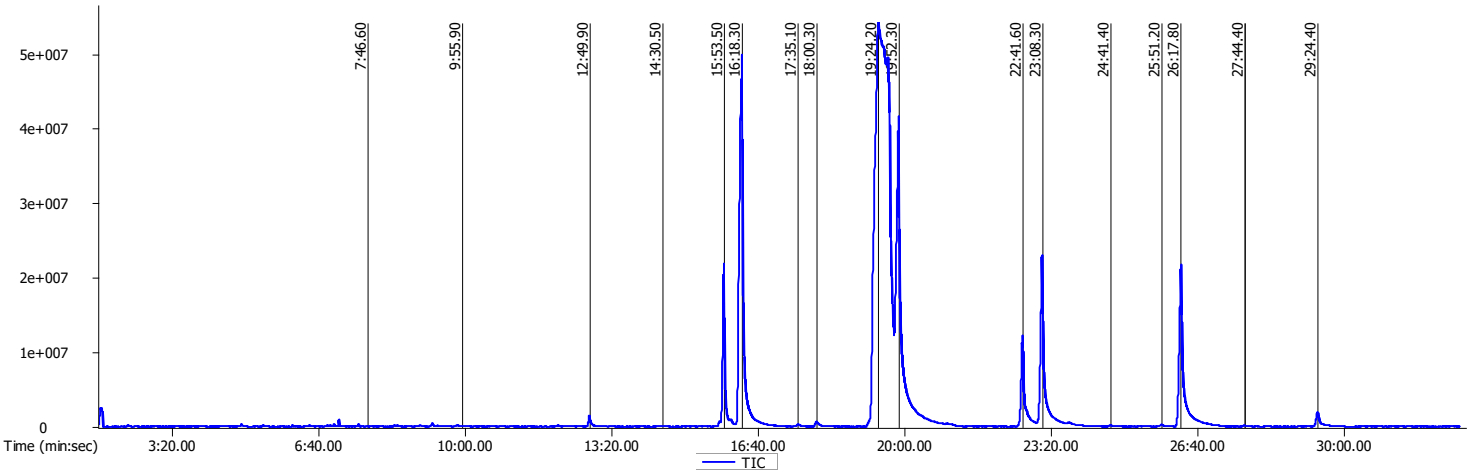

Figure 84: Gas chromatogram of Tree of life Pure organic moringa oil

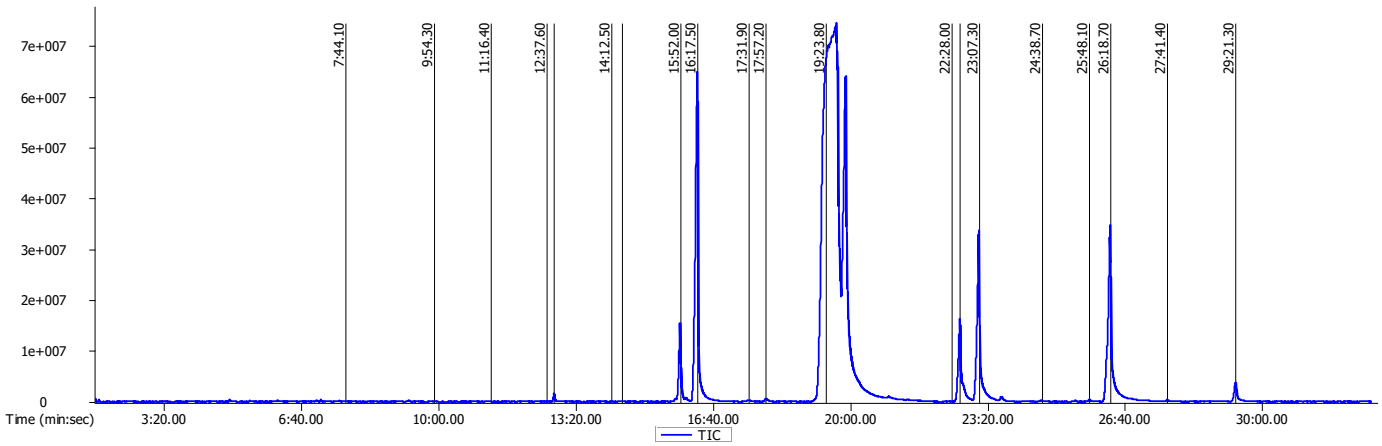

Figure 85: Gas chromatogram of Moringa 5000 Pure Moringa Ben oil

C1 - Internal use

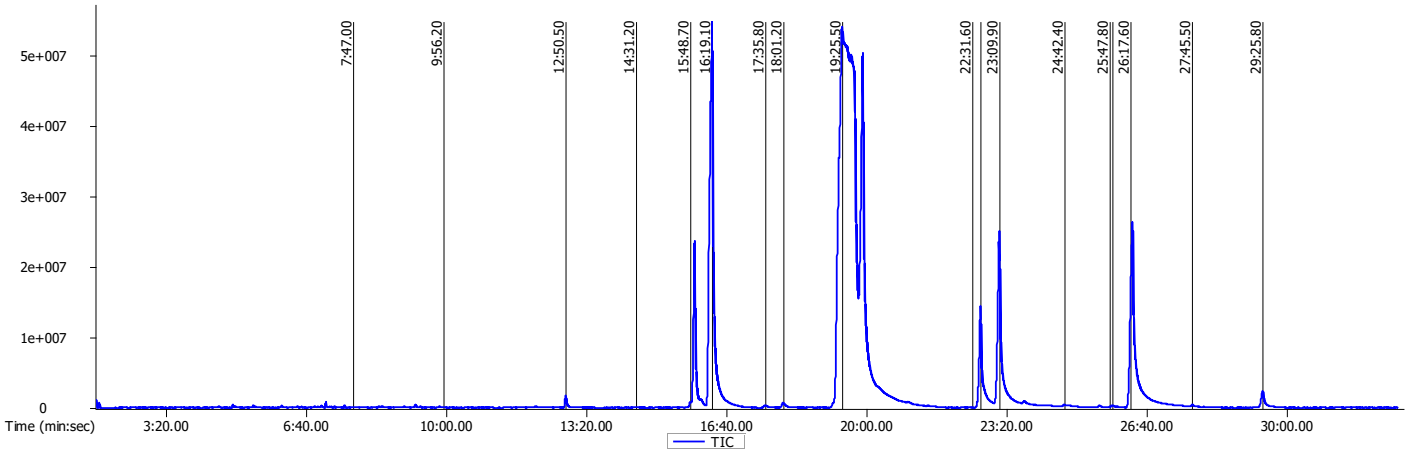

Figure 86: Gas chromatogram of Leaf- Moringa seed oil

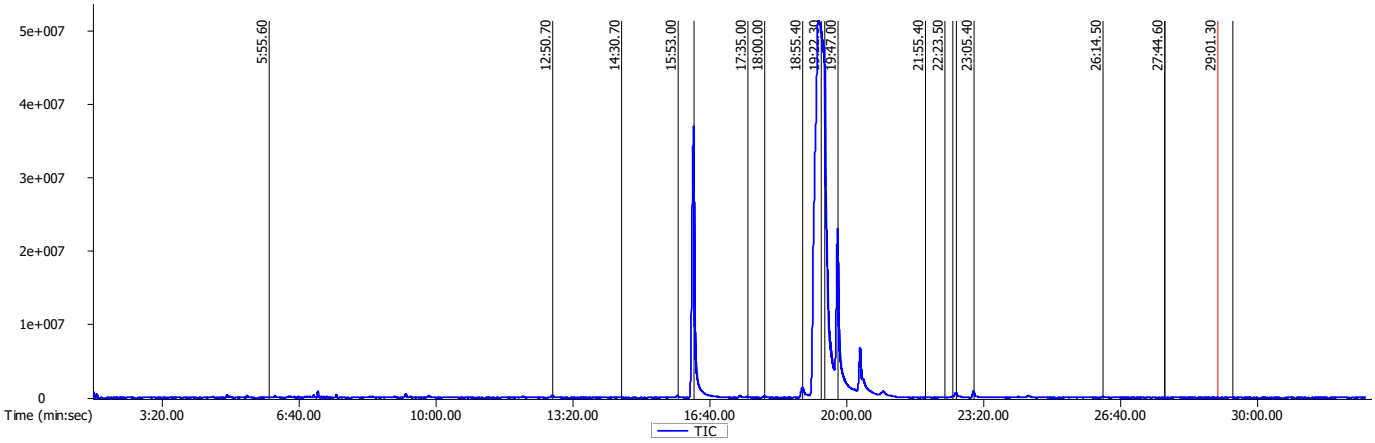

Figure 87: Gas chromatogram of Hemp seed oil

C1 - Internal use

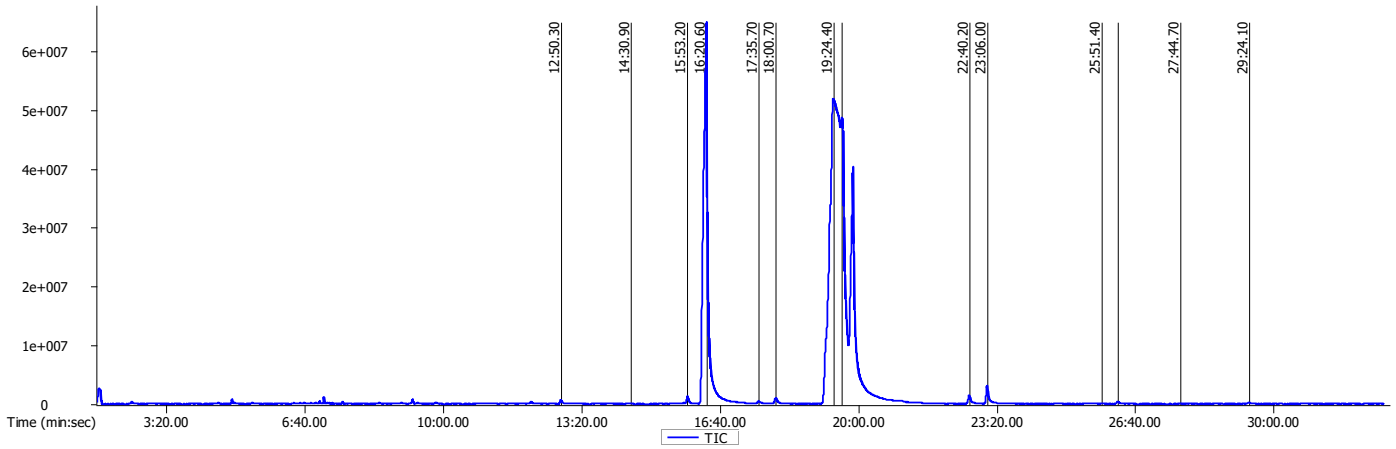

Figure 88: Gas chromatogram of Marula seed oil

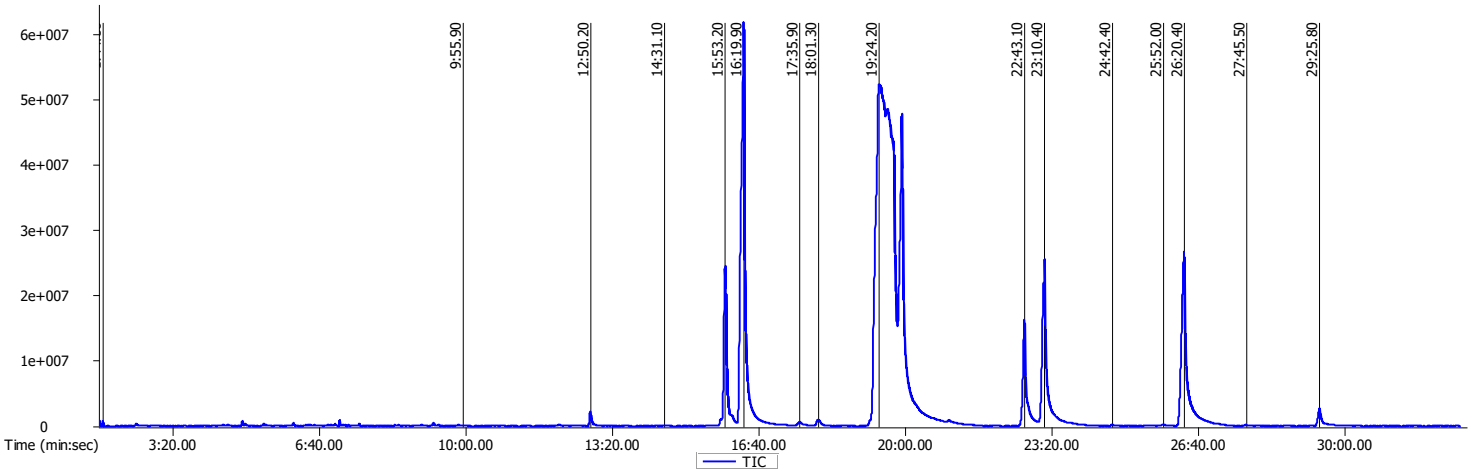

Figure 89: Gas chromatogram of Lefakong Cold-pressed MO oil

C1 - Internal use

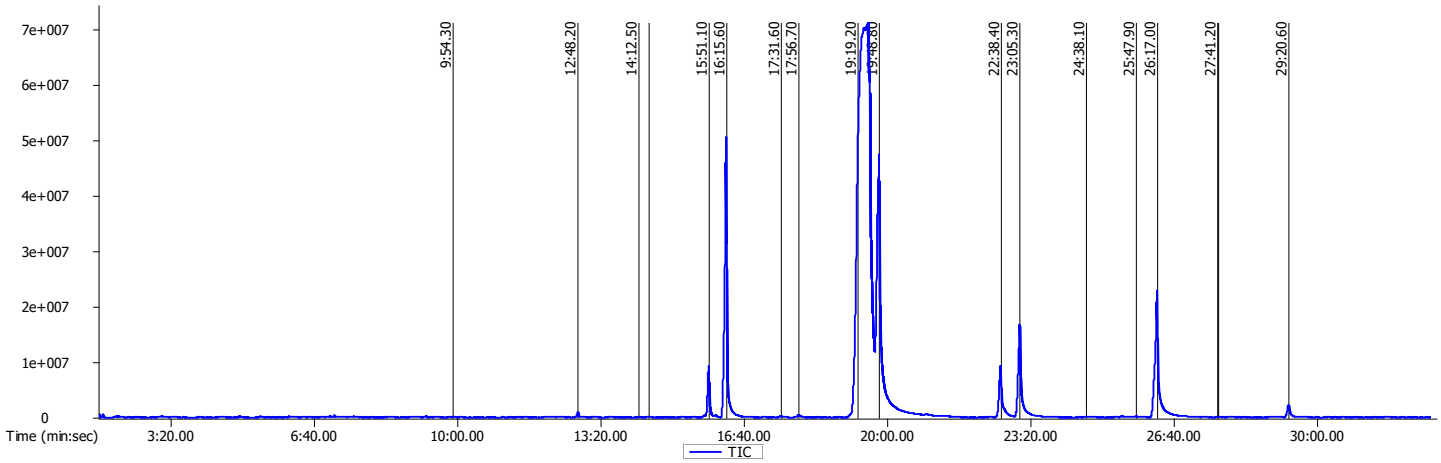

Figure 90: Gas chromatogram of Soxhlet extracted MO oil

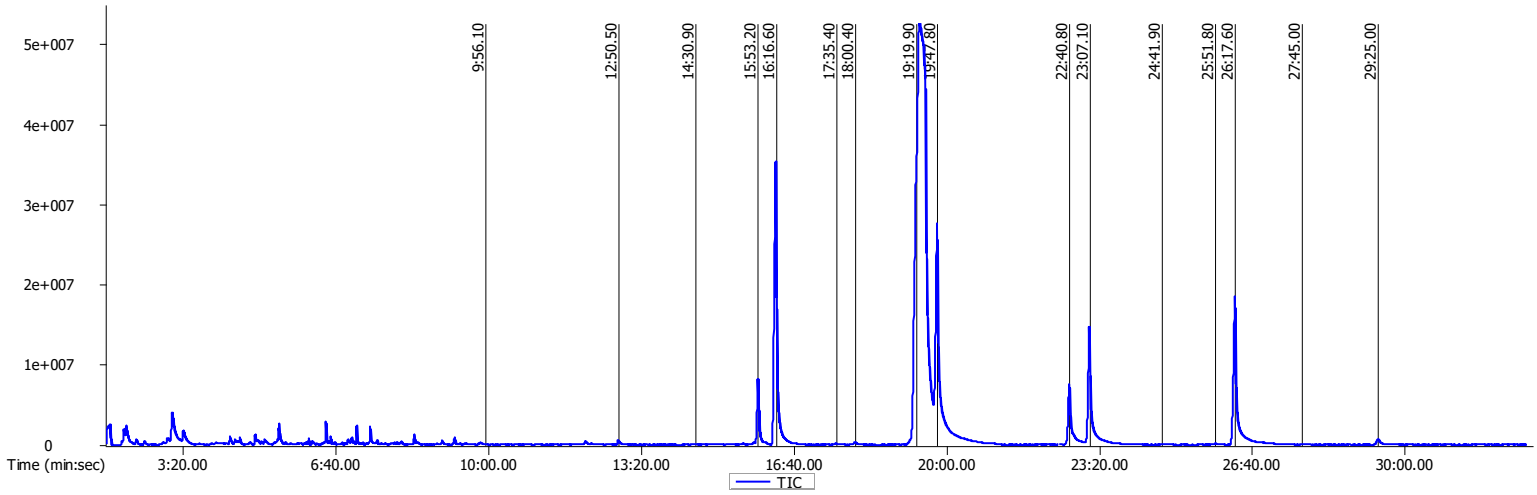

Figure 91: Gas chromatogram of Ultrasound extracted MO oil

C1 - Internal use

Table 20: Additional relative fatty acid content of various oils

Fatty acid name	Baobab seed oil		Kalahari melon seed oil		Marula seed oil		Hemp seed oil		Bright mountain - Moringa Seed Oil		Moyo moringa SA- Moringa Behen oil for skin		Moringa world-MO oil		Karibaa - Moriveda moringa basic oil	
	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
Methyl butyrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl hexanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl octanoate	nd	nd	nd	nd	nd	nd	0.01	0.01	nd	nd	nd	nd	nd	nd	nd	nd
Methyl decanoate	nd	nd	nd	nd	nd	nd	nd	nd	0.02	0.02	nd	nd	nd	nd	nd	nd
Methyl undecanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl laurate	nd	nd	0.01	0.01	nd	nd	nd	nd	0.06	0.04	0.05	0.02	0.03	0.02	0.02	0.00
Methyl tridecanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Myristoleic Acid Methyl Ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl tetradecanoate	0.24	0.08	0.34	0.07	0.19	0.04	0.14	0.06	0.83	0.30	0.59	0.15	0.37	0.15	0.18	0.03
cis-10-Pentadecenoic acid methyl ester	0.01	0.01	nd	nd	nd	nd	nd	nd	0.01	nd	0.01	0.01	nd	0.01	nd	nd
Methyl pentadecanoate	0.04	0.00	0.15	0.03	0.02	0.00	0.02	0.01	0.09	0.05	0.03	0.01	0.03	0.02	0.02	0.01
Methyl Palmitoleate	0.22	0.08	0.61	0.09	0.33	0.07	0.16	0.06	10.03	4.00	6.21	1.42	5.55	2.58	2.84	0.45
Methyl palmitate	24.05	18.01	5.91	1.44	31.44	5.90	21.85	6.58	10.91	13.34	25.39	5.52	14.36	10.03	18.65	3.04
cis-10-Heptadecenoic acid methyl ester	0.45	0.12	0.19	0.06	0.14	0.02	0.10	0.03	0.28	0.07	0.17	0.04	0.12	0.05	0.14	0.02
Methyl heptadecanoate	0.26	0.07	0.57	0.11	0.35	0.06	0.15	0.04	0.53	0.19	0.36	0.09	0.29	0.13	0.19	0.02
gamma-Linolenic acid methyl ester	nd	nd	nd	nd	nd	nd	0.89	0.29	nd	nd	nd	nd	nd	nd	nd	nd
Linolelaidic acid methyl ester	2.73	0.57	3.72	3.29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

C1 - Internal use

trans-9-Elaidic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	0.56	0.97	nd	nd	0.30	0.52	nd	nd
Methyl Linolenate	30.53	7.31	nd	nd	nd	nd	29.88	22.29	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Linoleate	nd	nd	67.95	19.32	nd	nd	nd	nd	nd	nd	nd	nd	31.35	30.01	nd	nd
cis-9-Oleic acid methyl ester	34.08	8.14	nd	nd	51.12	6.86	35.53	11.64	42.77	9.43	41.70	13.57	9.94	17.22	49.26	7.90
Methyl octadecanoate	5.96	1.45	15.97	13.79	14.78	12.92	10.08	3.22	nd	nd	nd	nd	11.87	5.61	17.14	2.28
Methyl cis-5.8.11.14-Eicosatetraenoic	nd	nd	nd	nd	nd	nd	0.01	0.01	nd	nd	nd	nd	nd	nd	nd	nd
Methyl cis-5.8.11.14.17-Eicosapentaenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-8.11.14-Eicosatrienoic acid methyl ester	0.04	0.01	2.74	0.38	nd	nd	0.02	0.02	nd	nd	nd	nd	nd	nd	nd	nd
cis-11.14-Eicosadienoic acid methyl ester	0.28	0.09	nd	nd	0.52	0.11	0.07	0.03	nd	nd	nd	nd	4.59	2.25	2.22	0.40
Methyl cis-11-eicosenoate	nd	nd	0.76	0.18	nd	nd	0.52	0.18	1.17	2.04	nd	nd	nd	nd	nd	nd
cis-11.14.17-Eicosatrienoic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	10.42	4.31	5.51	1.29	nd	nd	nd	nd
Methyl heneicosanoate	0.93	0.26	0.95	0.18	0.94	0.20	0.49	0.17	8.25	5.01	9.43	2.24	8.46	4.09	4.00	0.71
All cis-4.7.10.13.16.19-Docosahexaenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-13-16-Docosadienoic acid methyl ester (22:2)	nd	nd	nd	nd	nd	nd	nd	nd	0.11	0.09	nd	nd	nd	nd	nd	nd
Methyl Erucate	nd	nd	nd	nd	nd	nd	nd	nd	0.08	0.14	0.09	0.04	0.11	0.08	0.07	0.01
Methyl Arachidate	0.13	0.03	0.11	0.02	0.12	0.02	0.05	0.02	12.73	4.58	9.53	2.46	11.87	5.38	4.74	0.85

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Methyl docosanoate	0.02	0.02	0.01	0.01	0.01	0.00	nd	nd	0.07	0.04	0.05	0.02	0.03	0.02	0.03	0.01
Methyl tricosanoate	0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Nervonate	nd	nd	nd	nd	nd	nd	nd	0.01	nd	nd	nd	nd	nd	nd	nd	nd
Methyl lignocerate	0.03	0.01	0.03	0.01	0.04	0.04	0.01	0.01	1.00	0.51	0.83	0.28	0.68	0.30	0.49	0.09
Fatty acid name	**Essentially Natural Moringa oil**		**Tree of life Pure organic moringa oil**		**Moringa 5000 Pure Moringa Ben oil**		**Leaf- Moringa seed oil**		**Sunflower seed oil**		**Grapeseed oil**		**Avocado oil**		**Jojoba seed oil**	
	Mean	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**
Methyl butyrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl hexanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl octanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.16	0.03	nd	nd	nd	nd
Methyl decanoate	nd	nd	0.01	0.01	nd	nd	0.01	0.01	nd	nd	0.01	0.01	nd	nd	nd	nd
Methyl undecanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl laurate	0.07	0.01	0.03	0.00	0.02	0.02	0.03	0.02	0.02	0.01	0.03	0.01	0.02	0.01	nd	nd
Methyl tridecanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Myristoleic Acid Methyl Ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl tetradecanoate	0.10	0.02	0.32	0.01	0.26	0.23	0.46	0.07	0.29	0.04	0.34	0.02	0.07	0.01	0.09	0.08
cis-10-Pentadecenoic acid methyl ester	nd	nd	nd	nd	0.01	0.02	nd	nd	nd	0.01	0.06	0.01	0.00	nd	nd	nd
Methyl pentadecanoate	0.02	0.00	0.02	0.01	0.02	0.02	0.04	0.01	0.04	0.01	0.06	0.03	nd	nd	0.03	0.00
Methyl Palmitoleate	2.50	0.37	4.69	0.13	3.45	3.15	4.19	3.54	0.39	0.02	0.57	0.03	9.53	0.09	0.32	0.06
Methyl palmitate	41.93	6.97	19.20	0.92	20.16	18.36	19.51	16.24	25.47	1.70	58.65	0.68	32.52	0.34	3.41	0.33
cis-10-Heptadecenoic acid methyl ester	0.25	0.01	0.10	0.01	0.11	0.09	0.15	0.02	0.10	0.03	0.39	0.03	0.22	0.02	0.49	0.32
Methyl heptadecanoate	0.19	0.01	0.22	0.01	0.20	0.18	0.32	0.05	0.14	0.02	0.54	0.03	0.07	0.02	0.06	0.11
gamma-Linolenic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01	0.01	nd	nd	nd	nd

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Linolelaidic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	1.10	0.99	nd	nd	1.50	2.59	0.23	0.22
trans-9-Elaidic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	30.49	2.35
Methyl Linolenate	nd	nd	nd	nd	nd	nd	nd	nd	59.21	3.78	nd	nd	nd	nd	9.26	16.04
Methyl Linoleate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20.98	18.17
cis-9-Oleic acid methyl ester	36.33	15.71	47.60	4.22	10.97	18.99	49.64	11.06	nd	nd	nd	nd	54.68	2.25	nd	nd
Methyl octadecanoate	9.64	1.99	8.32	0.76	nd	nd	nd	nd	11.56	5.96	35.51	0.82	1.04	0.01	0.15	0.08
Methyl cis-5.8.11.14-Eicosatetraenoic	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.03	0.01	0.01	0.03
Methyl cis-5.8.11.14.17-Eicosapentaenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.03	0.05	0.01	0.02	nd	nd
cis-8.11.14-Eicosatrienoic acid methyl ester	0.01	0.01	nd	nd	nd	nd	nd	nd	0.06	0.01	nd	nd	nd	nd	nd	nd
cis-11.14-Eicosadienoic acid methyl ester	0.76	0.19	3.60	0.28	nd	nd	nd	nd	0.72	0.07	0.12	0.20	0.14	0.11	23.47	0.59
Methyl cis-11-eicosenoate	nd	nd	nd	nd	5.40	4.94	5.27	1.26	nd	nd	2.12	0.11	0.07	0.13	nd	nd
cis-11.14.17-Eicosatrienoic acid methyl ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl heneicosanoate	1.00	0.24	7.37	0.57	10.88	10.08	10.25	2.59	0.64	0.06	1.17	0.07	0.08	0.01	0.08	0.07
All cis-4.7.10.13.16.19-Docosahexaenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-13-16-Docosadienoic acid methyl ester (22:2)	nd	nd	nd	nd	nd	nd	0.05	0.08	nd	nd	nd	nd	nd	nd	6.16	5.35

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Methyl Erucate	nd	nd	0.06	0.02	0.09	0.08	0.07	0.02	nd	nd	0.01	0.02	nd	nd	4.67	8.08
Methyl Arachidate	0.23	0.04	7.73	1.52	13.78	12.63	8.88	4.83	0.84	0.07	0.10	0.01	0.01	0.01	0.12	0.07
Methyl docosanoate	0.01	0.01	0.04	0.00	0.07	0.06	0.06	0.04	0.02	0.01	0.01	0.00	nd	nd	nd	0.01
Methyl tricosanoate	nd	nd	nd	nd	nd	nd	0.01	0.03	nd	nd	nd	nd	nd	nd	nd	nd
Methyl Nervonate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl lignocerate	0.03	0.01	0.62	0.03	1.20	1.07	1.05	0.16	0.12	0.01	0.01	0.01	0.01	0.01	nd	Nd

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APPENDIX E : Cosmetic product questionnaire

Cosmetic product questionnaire

Thank you for taking part in this preference testing study, no personal information will be recorded. You will receive 3 types of cosmetics namely a liquid soap, lotion, and cream. Each type of product has 2 samples labelled with a letter to be used in filling in this questionnaire to differentiate them. Please evaluate each sample per cosmetic type on equal but opposite parts of the body i.e. one on your left wrist and the other on your right wrist etc. Please fill in this questionnaire based on your honest experience.

A. General information

What is your gender?	**What is your age?**
Describe your body skin type?	**Describe the type of body skin products you use and prefer in terms of cleansers and moisturizers?**

B. Cosmetic product comparison

Liquid soap	
Which sample spread more easily?	**Which sample foamed more?**
Which sample rinsed off more easily?	**Which sample left the skin feeling dry if any?**
Which sample left a cleaner feel?	**Which sample would you purchase?**
General comments on choice:	
Lotion	
Which sample spread more easily?	**Which sample absorbed the fastest?**
Which sample left the most shine?	**Which sample left the skin tacky if any?**
Which sample left the skin softer?	**Which sample would you purchase?**
General comments on choice:	
Cream	
Which sample spread more easily?	**Which sample absorbed the fastest?**
Which sample left the most shine?	**Which sample left the skin tacky if any?**
Which sample left the skin softer?	**Which sample would you purchase?**
General comments on choice:	

Figure 92: Cosmetic product questionnaire template

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