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GCMS, LCMS AND HS-SPME/GCMS METHODS TO DETECT ADULTERATION OF ARGAN OIL.

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by

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A Thesis
Submitted in Partial Fulfillment of the Requirements for the
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Approved by:

Dr. Gary Kinsel, Chair

Dr. Mary Kinsel

Dr. Boyd Goodson

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Afeez Olisa, for the Master of Science degree in Chemistry, presented on December 16, 2021, at Southern Illinois University Carbondale.

TITLE: GCMS, LCMS AND HS-SPME/GCMS METHODS TO DETECT ADULTERATION OF ARGAN OIL.

MAJOR PROFESSOR: Dr. Gary Kinsel

Methods for identifying argan oil adulteration have become important due to the exponential increase in the use of argan oil in food and cosmetics products over the years. Argan is the most expensive vegetable oil, has a tedious extraction process and is endemic to Morocco. This paper focuses on identifying markers that can be used to identify adulteration of argan oil with cheaper vegetable oils. Authentic argan oil from Morocco, commercially available argan oil, adulterant oil (sunflower and olive oil) and admixtures of argan oil with each adulterant oil at 98:2, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, and 40:60 v/v were analyzed. GCMS, LCMS and HS-SPME/GCMS methods were used. The GCMS method focused on the phenolic components of the argan oil, and it was also used to compare the authentic argan oil and the commercially available argan oil. This showed that both oil samples were comparable and similar. The LCMS method focused on the TAG profile of the authentic argan oil and the oil showed the characteristic hand-shaped like profile of TAGs of argan oil as reported in literature. The HS-SPME/GCMS method focused on the volatile organic compounds in the argan oil sample and the adulterant oils. Admixtures of the argan oil and the adulterant oils were evaluated for the presence and absence of volatile organic compound markers. In this method, the presence of the marker compound(s) could be detected with as little as 5% (w/w) adulteration level using olive oil as adulterant oil and 10% (w/w) using sunflower oil as the adulterant oil.

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I would like to acknowledge my advisor Dr. Gary Kinsel for giving me the opportunity to work in his lab. I would also like to thank Dr. Mary Kinsel for being a great mentor and providing guidance in every step of my graduate research; her patience despite my numerous mistakes and questions was vital in my growth as a scientist.

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CHAPTER 1

INTRODUCTION

1.1 ARGAN OIL

Argan oil is a vegetable oil extracted from the kernels of the fruits of the argan tree (*Argania spinosa*).[1] This tree is a member of the Sapotaceae family and its specie grows exclusively in the southwest region of Morocco where it plays a large environmental and economical role and is protected by UNESCO.[1,2] The argan tree has a slow growth rate, less than one foot per year.[3] Trees with this characteristic generally live longer, and the argan tree can live for more than 150 years.[2] The argan tree resists adverse weather conditions due to its deep root system, which makes it serve as a barrier against erosion and the encroaching desert.[4]

Figure 1.1: Goats climb the argan tree to gain access to the edible fruits.

Besides argan oil, the argan tree provides a source of timber, firewood, ornament, and animal fodder. Goats love to eat the fruit as shown in **Figure 1.1**.

Argan oil has been a staple in Moroccan societies and households for centuries where it has traditionally been used for culinary, cosmetics and medicinal purposes.[5] The world took notice after various scientific research showed argan oil has higher dietary and pharmacological quality compared to other vegetable oils.[6]

Argan oil is used in two forms: edible and cosmetic. The edible argan oil is extracted from slightly roasted kernels and has a nutty flavor, rich distinctive smell and is bronze-colored.[7] For the argan oil to meet the demands of the cosmetic industry, it needs to be odorless and tasteless. These qualities are achieved by extracting fresh or unroasted kernels.[7] Argan oil extraction is a laborious, multi-step process. After the fruits are picked from the argan tree, the fruits are dried, peeled and the nuts are extracted from the dried fruits. The nuts are then cracked to get the kernels, which are then processed (roasting, grinding, churning) to get the oil.[8]

1.2 ARGAN OIL PRODUCTION

For hundreds of years, Moroccan women have prepared argan oil on a subsistence level as shown in **Figure 1.2.1**. The production of argan oil is a tedious, repetitious, and yet delicate process. The familial method which involves several stages of preparation is the traditional method (Expertise, patience and experience are required to get argan oil of optimum quality). The mechanized form of oil extraction involves less steps and offers improvements on the traditional method.[9]

1.2.1 Traditional Method

The steps involved in the traditional method of argan oil production are shown in **Figure 1.2.2**. Ripe fruits from the argan tree are gathered and dried in the sun for several days. The dried fruits are then peeled to obtain the argan nuts.[10] A stone is used to break the nuts to obtain argan kernels. In the production of edible argan oil, the kernels are roasted using plates made of clay, after which a millstone is used to smash the kernels into a dough form.[11] Water is added to the dough and mixed by pressing with hands (malaxed) for 30 to 40 minutes. Malaxing is the slow mixing process that allows small oil droplets to aggregate and be more easily separated. The aggregated oil is then decanted.[6]

Figure 1.2: Moroccan women grinding argan kernel to extract argan oil

This traditional method of argan oil production has several disadvantages, such as the method being time consuming, having a low yield and involving labor-intensive processes. For example, the procedure requires about 220lbs of dried argan fruits to obtain 64fl oz of oil after almost three days of work.[12] Also, the step involving the addition of water to the dough during kneading can be a source of contaminants as the water used is usually of poor microbial condition.[12]

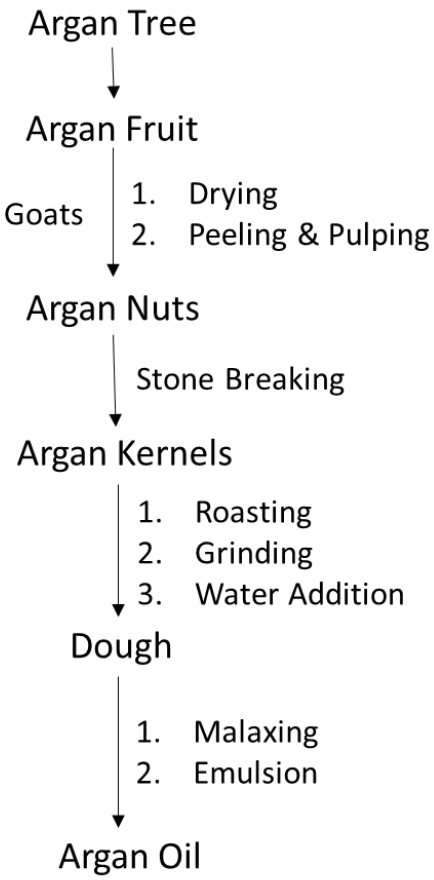


Figure 1.3: Traditional method used for edible argan oil production.

1.2.2 Mechanical / Industrial Method

With the formation of various women cooperatives, stringent regulations for the extraction of argan oil have been introduced.[10] Developments were made to eliminate uncertainties involving the quality of the argan oil, such as use of mechanical presses in oil extraction which eliminated the need to add water to the dough and simultaneously increased oil yield. Manual fruit peeling was replaced with scratching machines, and kernel roasting with clay plates was replaced with gas roasters which made the hazelnut taste of the edible argan oil reproducible.[13] All these changes have significantly reduced the time required for oil production.

In the laboratory and industry, argan oil can also be extracted by adding lipophilic solvents, such as hexane to crushed argan kernels. The argan oil is then recovered after solvent evaporation.[4] This method is commonly used in the industrial setting to produce argan oil for cosmetics purpose.[4]

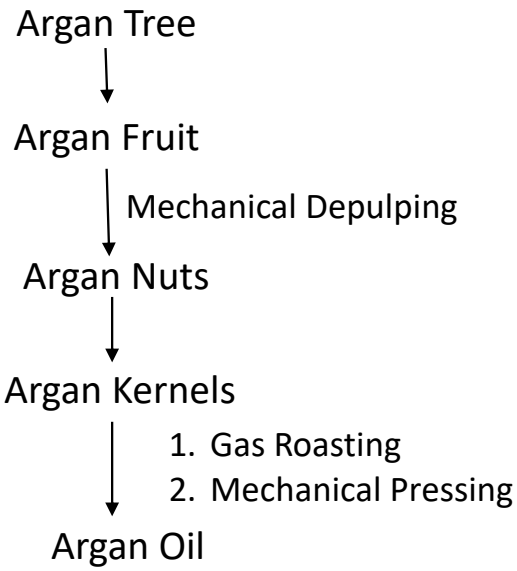


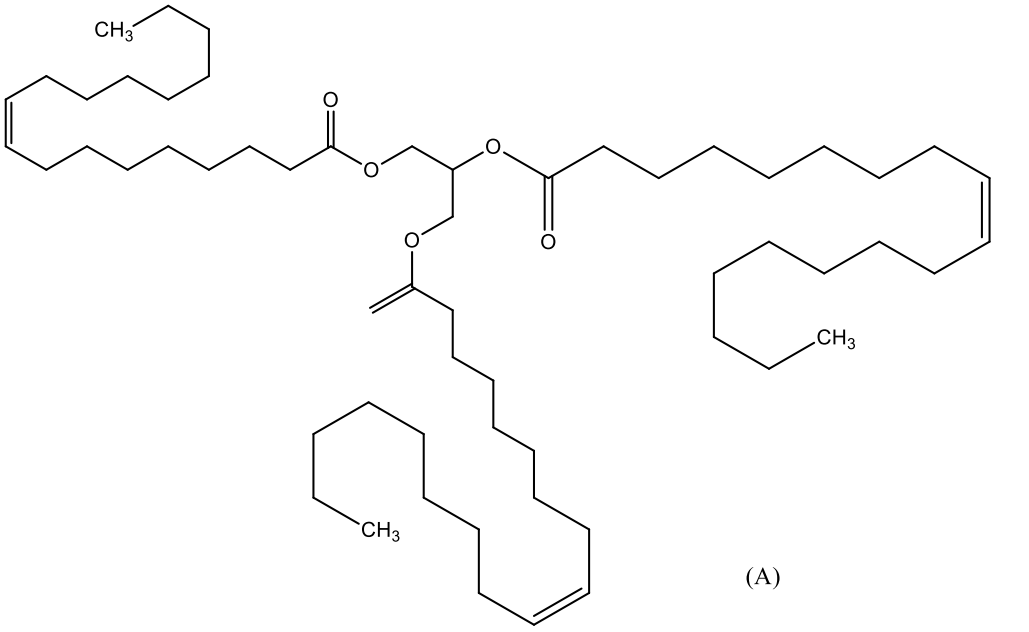
Figure 1.4: Mechanical method for edible argan oil production.

1.3 ARGAN OIL COMPONENTS

1.3.1 Triacylglycerols

95% of argan oil is made up of triacylglycerols (TAGs),[11] which are compounds having long chain fatty acids composed of 12 – 22 carbon units, along with a backbone of glycerol bounded at the carboxyl ends by ester bonds. The most prevalent triacylglycerols in argan oil are those containing two or three oleic acid units. Other commonly detected triacylglycerols in argan oil contain one oleic acid unit and two linoleic acid units or one palmitic acid unit and two oleic acid units.[11] The major triacylglycerol constituents are OOO (3 Oleic acid units), OOL (2 Oleic acid units and 1 Linoleic acid unit), OLL (1 Oleic acid unit and 2 Linoleic acid units) and POL (1 Palmitic acid, 1 Oleic acid & 1 Linoleic acid units).[11] Argan oil has its own unique set of

triacylglycerol constituents and the profile of TAGs has been recommended as a promising authentication marker of argan oil adulteration.[1,14] High-performance thin layer chromatography (HPTLC) assays have also been proposed +.for evaluating triacylglycerol profile to identify adulterated argan oil and its products.[59]



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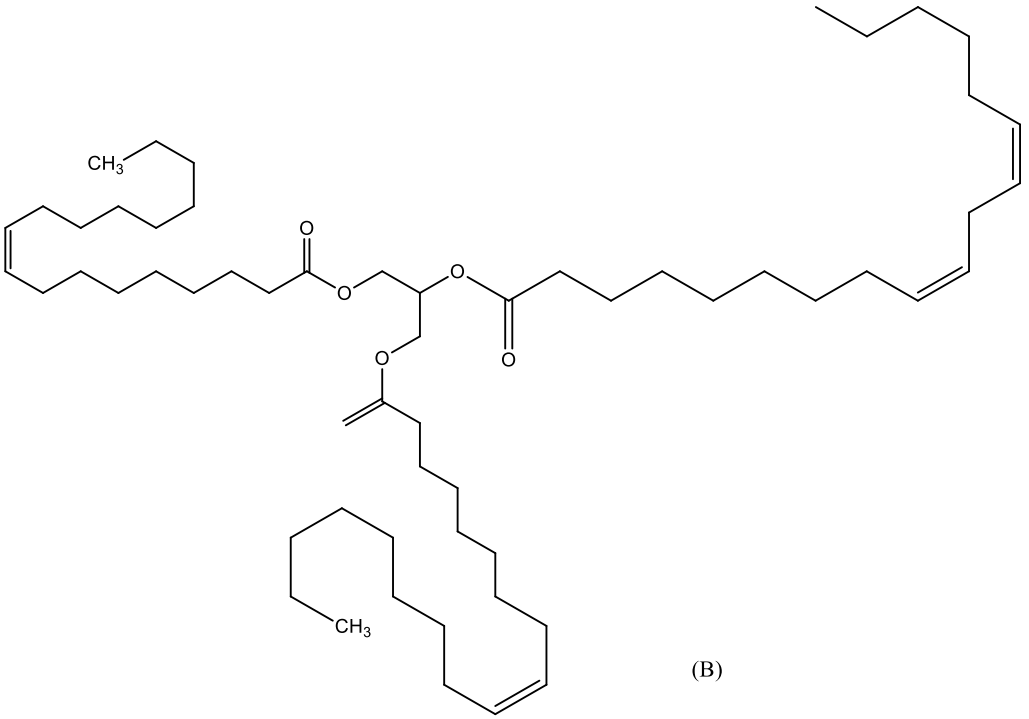


(B)

Figure 1.5: Structures of the major TAGs found in argan oil, (A) OOO, (B) OOL, (C) OLL, and (D) POL.

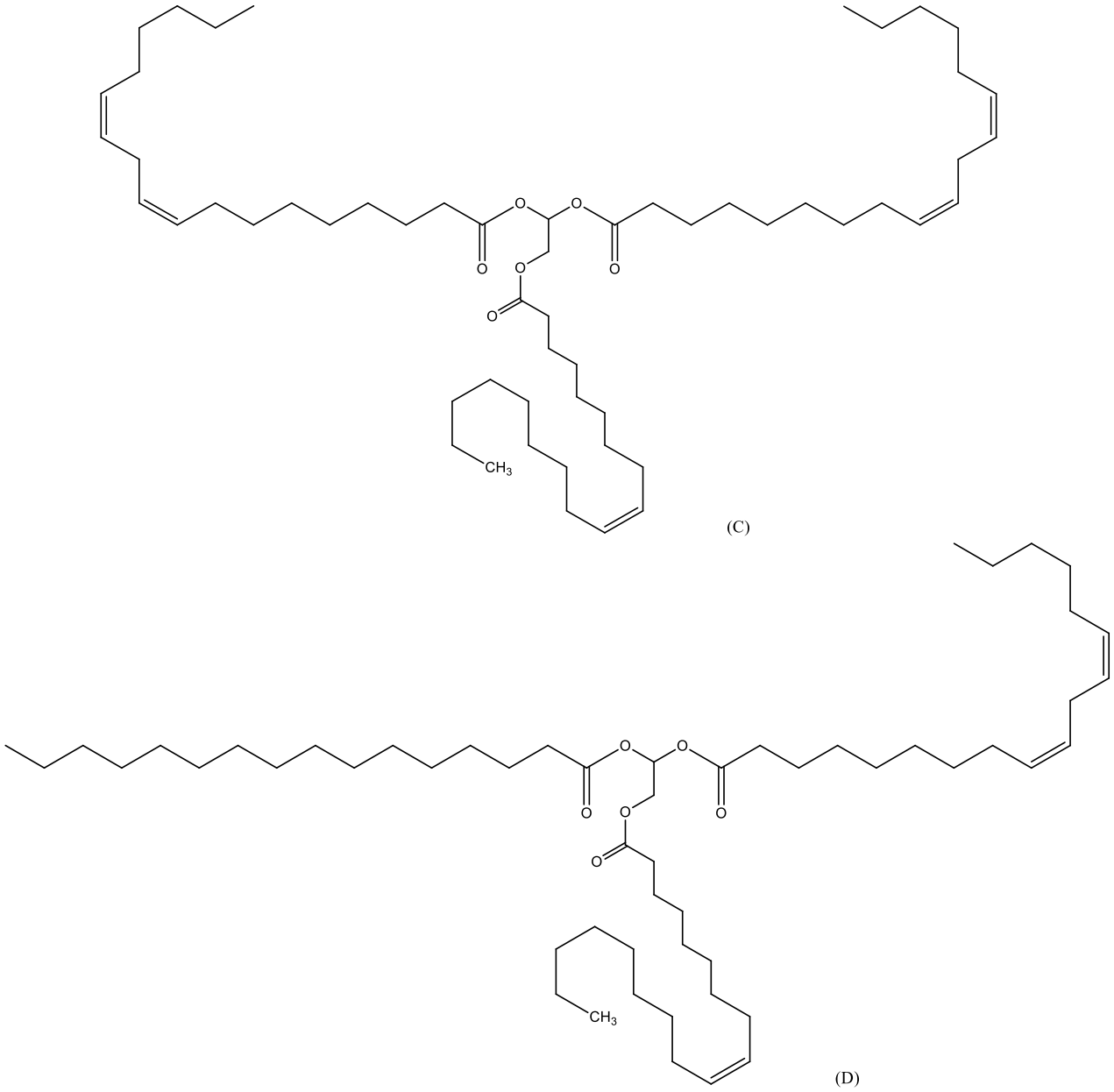


Figure 1.5: Structures of the major TAGs found in argan oil, (A) OOO, (B) OOL, (C) OLL, and (D) POL (cont'd.)

1.3.2 Volatile Organic Compounds

Volatile organic compounds in argan oil consist of a broad number of compounds of various classes, including ketones, alcohols, esters, aldehydes, terpenes, carboxylic acids, furan derivatives and thiophene derivatives to name a few.[16] The majority of these compounds are

formed by fatty acid oxidation after enzymatic reactions which takes place in the presence of oxygen.[17] Volatile organic compounds that come from primary or secondary lipoxygenase pathways, are especially recognized to participate in the aroma of edible oils.[17-20] Various enzymes are involved in the lipoxygenase pathway such as hydroperoxidelyase, alcohol acetyltransferase, alcohol dehydrogenase, isomerases and lipase and the process generates an array of volatile organic compounds.[19] The activity of the lipoxygenase pathway is contingent on the presence of the substrate and the connection of the substrate and enzyme; hence, it specifically occurs at the malaxation and crushing step during extraction.[20-24] Also, extended storage of the argan fruits assists the formation of the volatile organic compounds.[25] Increased temperature highly favors the formation of volatile organic compounds by lipoxidation.[26] Carbon-carbon cleavage can result to the formation of acids, esters and aldehydes, while other compounds are formed by more complicated methods which may require isomerization.[26,27]

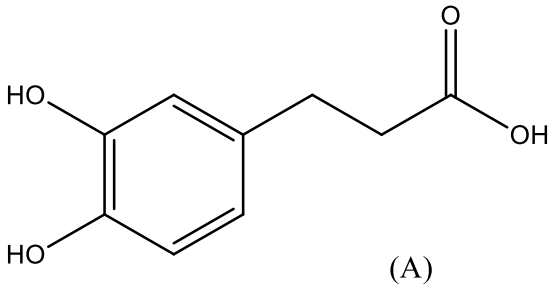
1.3.3 Phenolic Compounds

Phenolic compounds are the phytochemicals found in most vegetable oils. They possess several bioactive properties and their dietary intake has health benefits such as prevention of cardiovascular diseases and cancer.[1] The content of phenolic compounds in argan oil is low.[30] Nevertheless, due to the pharmacological actions of some phenolic compounds, they have important significance on the biological properties and taste of argan oil.[31] The analysis of phenolic compounds in argan oil requires the elimination of the large excess of fatty acid and the makes obtaining the trace amount of the phenolic compounds challenging.[31]

The phenolic compounds in argan oil are considered to be largely responsible for the antioxidant properties of the oil.[32] Compounds that show antioxidant properties are

increasingly regarded as valuable food components.[33] Argan oil has received great attention as a result of this trend, as well as other products such as teas, dark chocolate, and red wine. Manufacturers of argan oil and consumers alike are interested in the phenolic content of the oil.[34-36]

Chimi et. al. [31] showed that the polyphenol level in argan oil is 56.3ppm. This group was also able to identify two phenol derivatives, caffeic acid and oleuropein. Rojas et. al. [34] identified several additional phenol derivatives in argan oil such as vanillic acid, p-hydroxybenzoic acid, syringic acid, vanillin, 3,4-dihydroxybenzoic acid and tyrosol.



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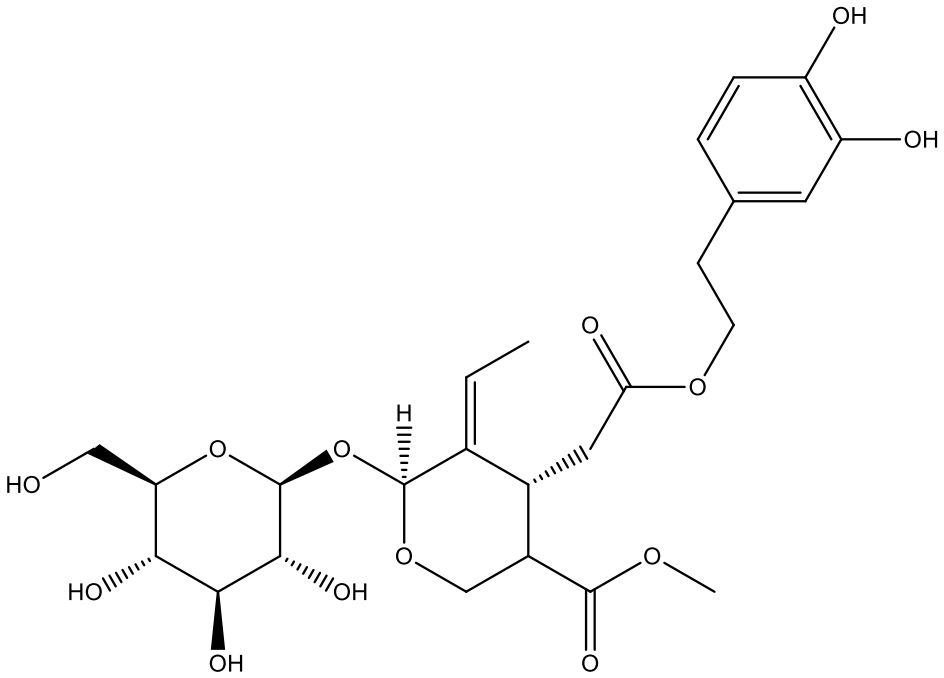

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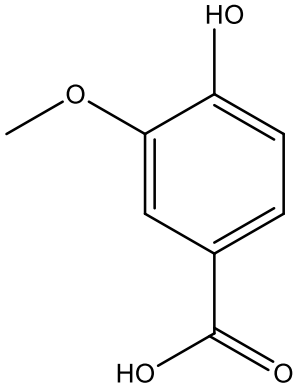


(C)

Figure 1.6: Phenolic compounds found in argan oil include (A) caffeic acid, (B) oleuropein, (C) vanillic acid, (D) p-hydroxybenzoic acid, (E) syringic acid, (F) 3,4-dihydroxybenzoic acid, and (G) tyrosol.

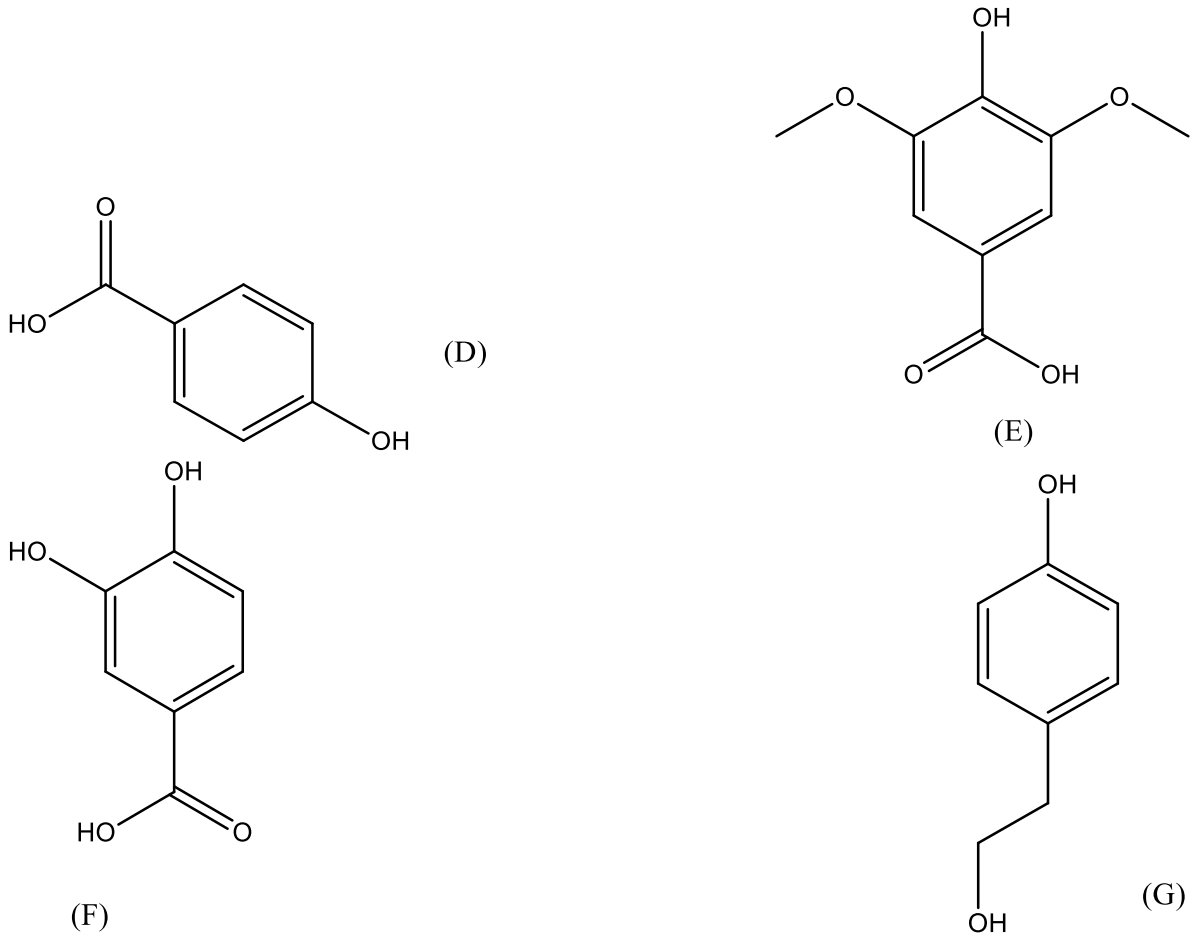
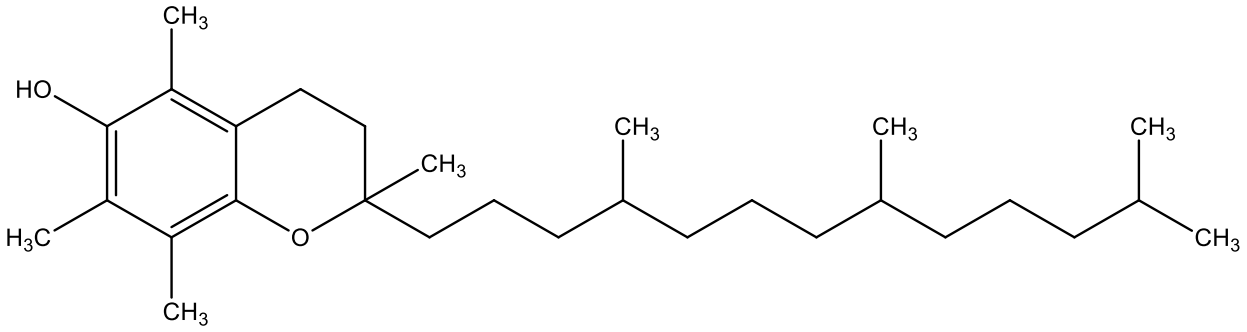


Figure 1.6: Phenolic compounds found in argan oil include (A) caffeic acid, (B) oleuropein, (C) vanillic acid, (D) p-hydroxybenzoic acid, (E) syringic acid, (F) 3,4-dihydroxybenzoic acid, and (G) tyrosol (con't.)

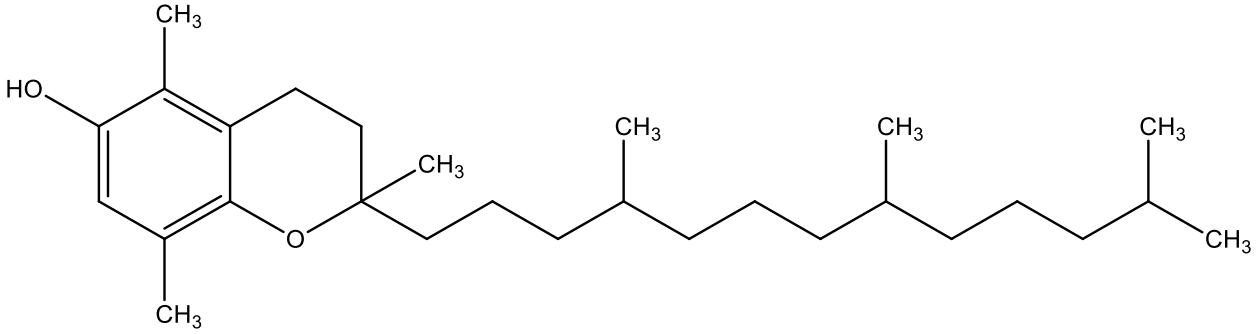
1.3.4 Tocopherols

The tocopherol content of argan oil is of great value. Tocopherols make up a significant group of compounds due to their powerful vitamin and antioxidant properties.[32,38] In argan oil, the total tocopherol level is in the range of 630 - 750 ppm.[37] This is four times the level that is obtainable in olive oil and twice the level in hazelnut oil.[37] The major tocopherol found in argan oil is γ-Tocopherol and its presence varies between 580 – 700 ppm, β -Tocopherol is essentially

non-existent in argan oil, while α and δ-Tocopherols are shown to occur in approximately the same amount.[37]

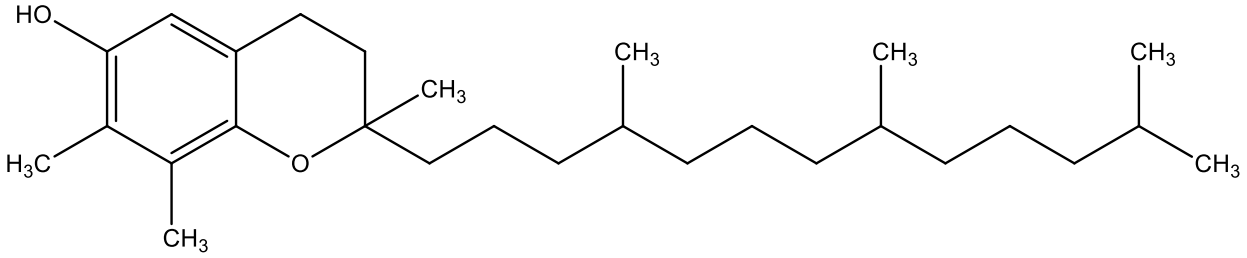


(A)



(B)

Figure 1.7: Tocopherol compounds found in argan oil include (A) α-tocopherol, (B) β-tocopherol, (C) γ-tocopherol, and (D) δ-tocopherol.



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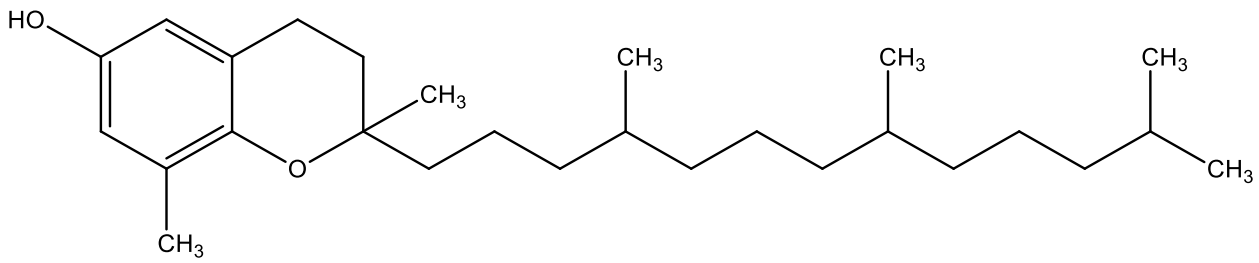


(D)

Figure 1.7: Tocopherol compounds found in argan oil include (A) α-tocopherol, (B) β-tocopherol, (C) γ-tocopherol, and (D) δ-tocopherol (cont'd).

1.3.5 Squalenes

Squalenes are part of the unsaponifiable component of argan oil which constitutes about 1% of the argan oil composition.[1] Unsaponifiable refers to all fatty substances remaining after alkaline hydrolysis that are soluble in organic solvents. Squalene content in argan oil was detected by gas chromatography – mass spectrometry (GCMS) and gas liquid chromatography (GLC). The GCMS method involves the saponification of the fatty acids and the unsaponifiable components are left which are mainly squalenes and sterols.[39] In comparison to other vegetable oils, argan oil and olive oil are known to have a relatively rich squalene content which is recommended to protect against skin cancer.[40] The squalene content is 315 mg/100g in argan oil. This amount is lower than what is seen in olive oil at 499 mg/100g but significantly more than the content in sunflower oil which is 6 mg/100g.[1]

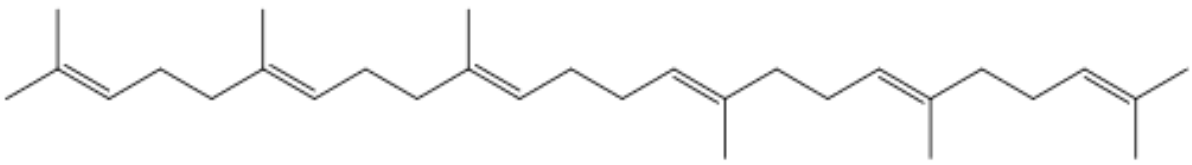


Figure 1.8: Structure of Squalene.

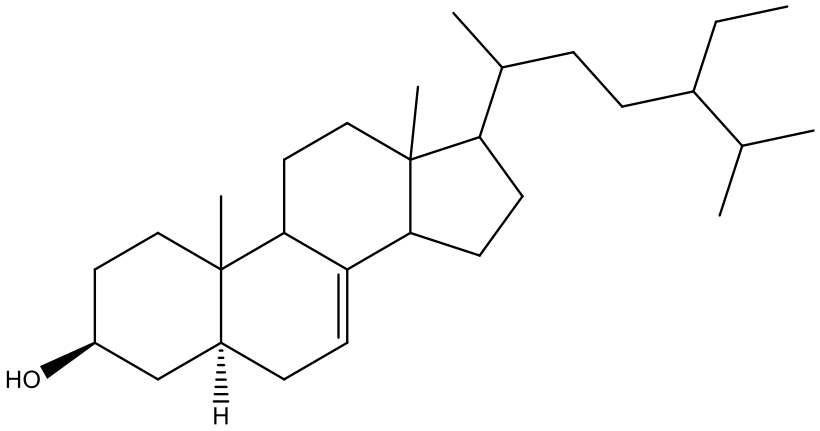
1.3.6 Peroxides

Peroxides are produced in the initial phase of lipid oxidation as primary oxidation compounds, and they may undergo further oxidation reactions to produce additional volatile and non-volatile secondary products.[41] The peroxide content or value is a conventional chemical method for evaluating oxidative deterioration of oil and is commonly applied as an indicator of quality in the food industry.[42,43]

Lipid oxidation limits the shelf life of edible oils.[44] The peroxides content is usually correlated alongside the rancidity of fat in edible oils and the threshold of this peroxide content depends on the fat material.[44,45] In almost all edible vegetable oils, rancid taste becomes evident at peroxide values in the range of 20 – 40 meq O_2/kg.[1] It has been shown that the storage conditions of the argan oil affects the peroxide value of the oil.[45]

1.3.7 Sterols

Sterols from plants are commonly called phytosterols and they play similar physiological roles to what cholesterols play in animals.[46,47] These plant sterols exists in several forms including, acylated glycosides, esterified/free with fatty acids and steryl glycosides.[48] Sterols have gained huge interests in recent times due to their promising contribution to reduced risk of cardiovascular diseases, anti-inflammatory effects, cancer preventive effects, hypocholesterolemic ability and immunomodulatory capacity.[49,50] The major sterol compounds found in argan oil are schottenol and spinasterol with trace amount of stigmasta-7,24-diene-3-ol, stigmasta-8,22-diene-3-ol and campesterol respectively. Unlike argan oil the major sterol in sunflower oil and olive oil is β-sitosterol.[37,51]



(A)

Figure 1.9: Sterol compounds found in argan oil include (A) schottenol, (B) spinasterol (C) stigmasta-7,24-diene-3-ol, (D) stigmasta-8,22-diene-3-ol, and (E) campesterol. (F) β-sitosterol is a major sterol in olive oil and sunflower oil.

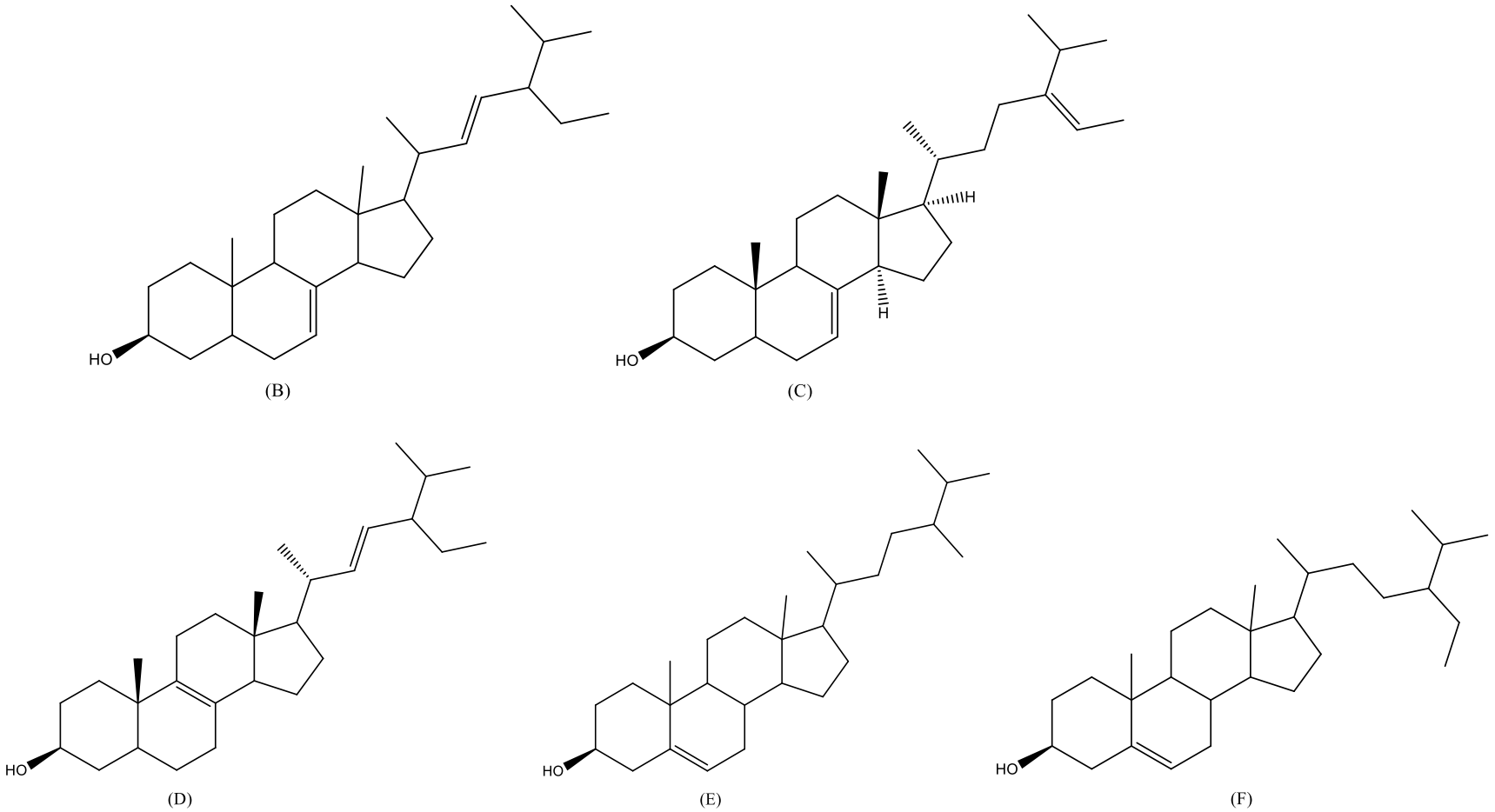


Figure 1.9: Sterol compounds found in argan oil include (A) schottenol, (B) spinasterol (C) stigmasta-7,24-diene-3-ol, (D) stigmasta-8,22-diene-3-ol, and (E) campesterol. (F) β-sitosterol is a major sterol in olive oil and sunflower oil (cont'd).

1.4 COMPARISON OF AUTHENTIC ARGAN OIL AND COMMERCIALLY AVAILABLE ARGAN OIL

Before purposely adulterating argan oil with the cheaper vegetable oils, a comparison of a commercially available argan oil with the authentic argan oil from Morocco will be undertaken. These studies will confirm that the commercially available argan oil is chemically comparable and can be substituted as a replacement for the authentic argan oil. Specifically, the phenolics will be extracted from the commercially available argan oil and compared to the phenolics extracted from the authentic argan oil.

1.5 ADULTERATION & DETECTION

The low yield, time consuming production and endemic to Morocco nature of the argan tree makes argan oil particularly susceptible to counterfeiting and adulteration.[5] Adulteration is the process of reducing the quality of a substance by adding an inferior or alien substance and / or the elimination of an important component of the substance.[52] Argan oil is usually adulterated with cheaper vegetable oils and the detection of this adulteration has become a huge quality assurance problem.[53,54]

Several groups have come up with various approaches for detection of argan oil adulteration using different markers such as sterol composition, triacylglycerol profile and phenolic acids content. Campesterol is a sterol compound which is present in low quantity in argan oil, however this sterol has been shown to be present in high quantity in cheaper vegetable oils such as sunflower oil, soybean oil, sesame oil etc. This large difference in campesterol concentration makes it a marker for detection of argan oil adulteration with these less expensive oils.[37] Hence, the use of gas chromatography for campesterol measurement has been applied for detecting argan oil adulteration as argan oil that has been mixed or replaced

with cheaper vegetable oils will present an unusually high level of campesterol.[37] Unfortunately, olive oil also contains low quantities of campesterol making this test less effective for determining olive oil adulteration.[55]

The evaluation of the triacylyglycerol profile of argan oil can also be used as an indicator of argan oil adulteration with cheaper vegetable oils.[14,15] Gas chromatography,[14] high-performance liquid chromatography-refractive index detection (HPLC-RID)[54], high-performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD)[56] and ultra high-performance liquid chromatograph-photodiode array-electrospray ionization-time of flight-mass spectrometry (UHPLC-PDA-ESI-TOF/MS)[61] have all proven useful to separate and characterize TAG profiles.

The phenolic acid content has also been used as an identification marker, particularly ferulic acid.[37] Ferulic acid was chosen as an authentication marker because it is highly present in argan oil as it accounts for about 95% of the phenolic acid content in the oil. However, some argan oil samples were shown to only contain trace amounts of ferulic acid and suggests a cause for concern about using ferulic acid as a potential authentication marker.[57]

A host of other approaches have also been applied to this issue of adulteration including inductively coupled plasma optical emission spectroscopy (ICP-OES) coupled with different chemometric approaches for trace element profiling was used for authentication of argan oil against adulteration.[58] Voltametric e-tongue and e-nose methods which imitates the taste buds and nose of humans have been employed to characterize vegetable oils and deal with the counterfeiting problems.[59,60] These e-tongues or e-noses can differentiate between vegetable oils and discover adulteration. This method was applied for identification and quantification of

adulteration in argan oil by adding known quantities of sunflower oil, a common adulterant of argan oil.[61-62]

This work proposes to use headspace - solid phase microextraction (HS-SPME) coupled to GCMS to analyze the volatile compounds in the commercially available argan oil and samples of the commercially available argan oil deliberately adulterated with different volumes of extra virgin olive oil and sunflower oil. The resultant chromatograms will be used to analyze the profile of the volatile compounds in each sample. Data obtained for the HS-SPME GCMS will be used to identify potential markers of the argan oil adulteration with olive oil and sunflower oil. If successful, HS-SPME GCMS would prove a significant improvement over other adulteration detection methods because HS-SPME requires no sample extraction thus eliminating this time-consuming aspect of sample analysis.

CHAPTER 2

GCMS EVALUATION OF PHENOLIC COMPOUNDS IN ARGAN OIL

2.1 MATERIALS AND REAGENTS

Authentic argan oil was a gift from Kamal Aberkani, a professor at Université Mohammed Premier in Nador, Morocco. This argan oil was extracted via the procedure of Yang and Kallio.[63] Briefly, 15g of powdered argan seed was extracted by refluxing with 200mL hexane over 8 hours. Extract was filtered and rotovapped to remove the solvent. The argan oil was then stored at -4°C.

Methanol, hexane, 2-propanol, bis(trimethylsilyl)trifluoroacetamide (BSTFA), acetonitrile, acetone and C9-C40 saturated alkanes standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the solvents used were HPLC grade.

2.2 EXTRACTION OF PHENOLIC COMPOUNDS

5mL of methanol was added to 2 grams of argan oil. The mixture was vortexed for two minutes and centrifuged at 5000rpm for ten minutes. The methanol extract containing phenolic compounds and polar and neutral lipids was removed. The addition of methanol followed by vortexing and centrifuging was repeated a total of three times. The methanol extracts were combined and stored at -20°C overnight. Visible oil droplets were filtered from the methanol. The methanol was then evaporated using nitrogen gas to yield a syrupy residue which was dissolved in 3mL acetonitrile.

To remove the remaining lipids, 5mL hexane was added, vortexed for two minutes and centrifuged at 5000rpm for ten minutes. The acetonitrile layer containing phenolic compounds

was removed. The hexane washing step was repeated three times. The acetonitrile extracts were combined, the solvent was evaporated using nitrogen gas, and then the residue was dissolved in 3mL acetone. A 1mL acetone aliquot was then derivatized with 10µL of BSTFA at room temperature for thirty minutes. Derivatization with BSTFA added trimethylsilane (TMS) to analyte hydroxyl groups which increased analyte volatility as well as improved chromatographic separation and peak shape.

2.3 GCMS INSTRUMENTATION

GCMS (**Figure 2.1**) was carried out on a Thermo Finnigan TraceGC PolarisQ quadrupole ion trap mass spectrometer equipped with a J&W Scientific DB5 column (30m X 0.25mm i.d.; 0.25µm film thickness). The oven temperature program was as follows: 100°C was held for 2 minutes, ramped to 270°C at 4°C/min and then held isothermally for 20 minutes. The transfer line was set to 275°C. The inlet temperature was set to 250 °C and the ion source was set to 240°C. 1µL injections were made in splitless mode. The carrier gas was helium (constant flow 1mL/min). The mass spectrometer was used in full scan electron impact (EI, 70eV) mode and positive ions were detected in the mass-to-charge range from 40 to 650 amu.

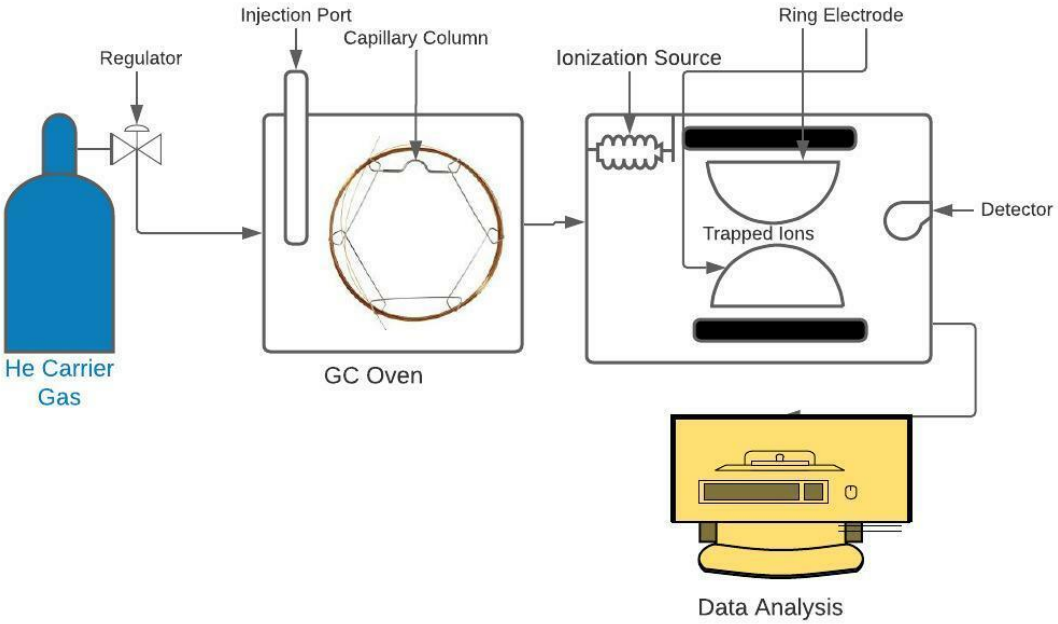


Figure 2.1: Schematic diagram of a GCMS system

2.4 RESULTS AND DISCUSSION

Argan oil phenol content is low, which makes phenolic extraction challenging. The phenolic extract of argan oil tends to be complex and contains interferences in the form of free fatty acids and monoglycerides. The major fatty acids in argan oil are palmitic acid (16:0), oleic acid (C18:1 n-9), and linoleic acid (C18:2 n-6) present in approximately 12.7%, 45.6%, and 34.6%, respectively.[65] The removal of the huge quantity of these interferants was also difficult in these studies.

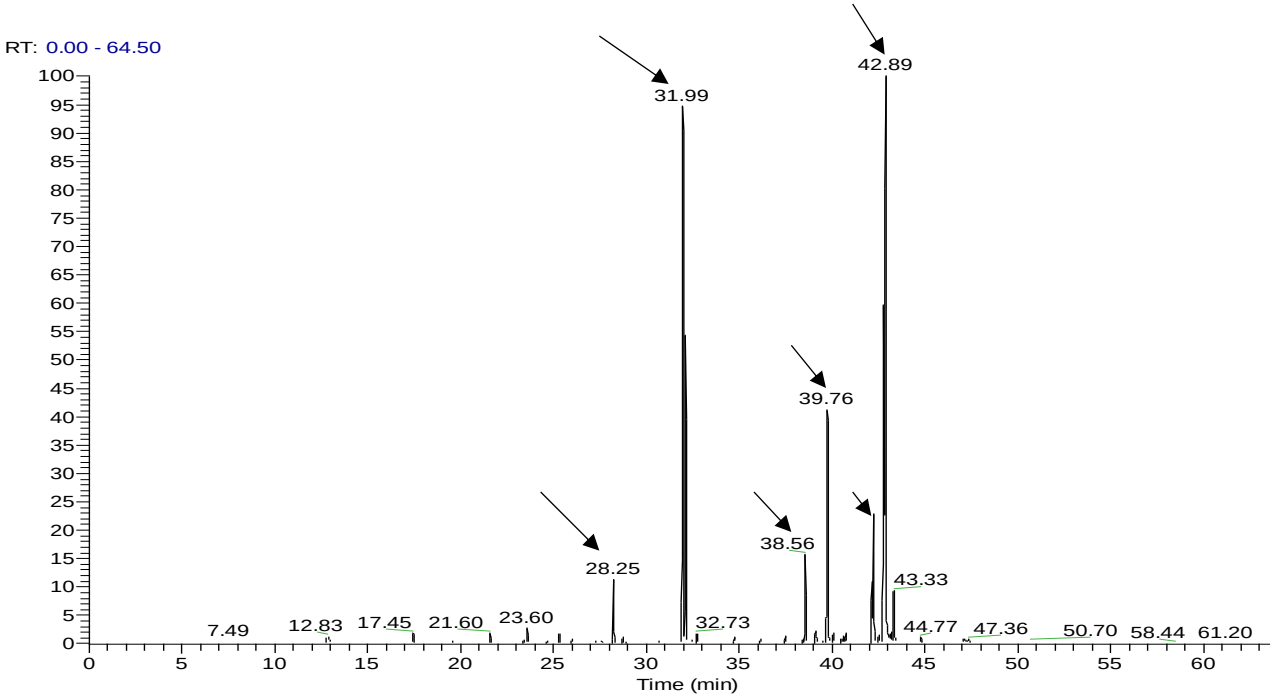


Figure 2.2: Total ion chromatogram of authentic argan oil from Morocco.

The chromatographic peaks appearing at retention times 28.25, 31.99, 38.56, 39.76, 42.16, and 42.89 minutes (highlighted with arrows) were identified as interferants palmitic acid (3.53%), linoleic acid (37.61%), phthalic acid, di(oct-3-yl)ester (0.22%), 1-monopalmitin (16.13%), 2-linoleolylglycerol (2.10%) and 1-monooleoylglycerol (28.27%), respectively. Hexane washing was most successful at removing free oleic acid. Linoleic acid and 1-monooleoylglycerol are the most abundant remaining interferants and were identified as the TMS- and 2TMS- derivatives with molecular weights of 350 and 500, respectively.

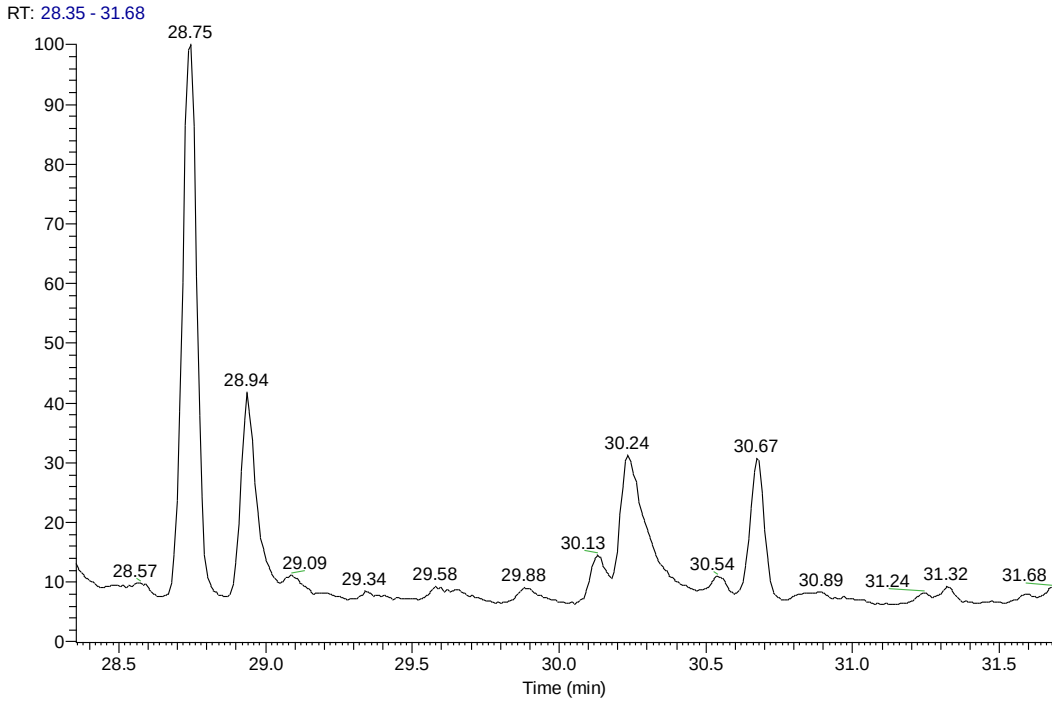


Figure 2.3: Showing Low abundance peaks between RT of 28.35 – 31.68 mins.

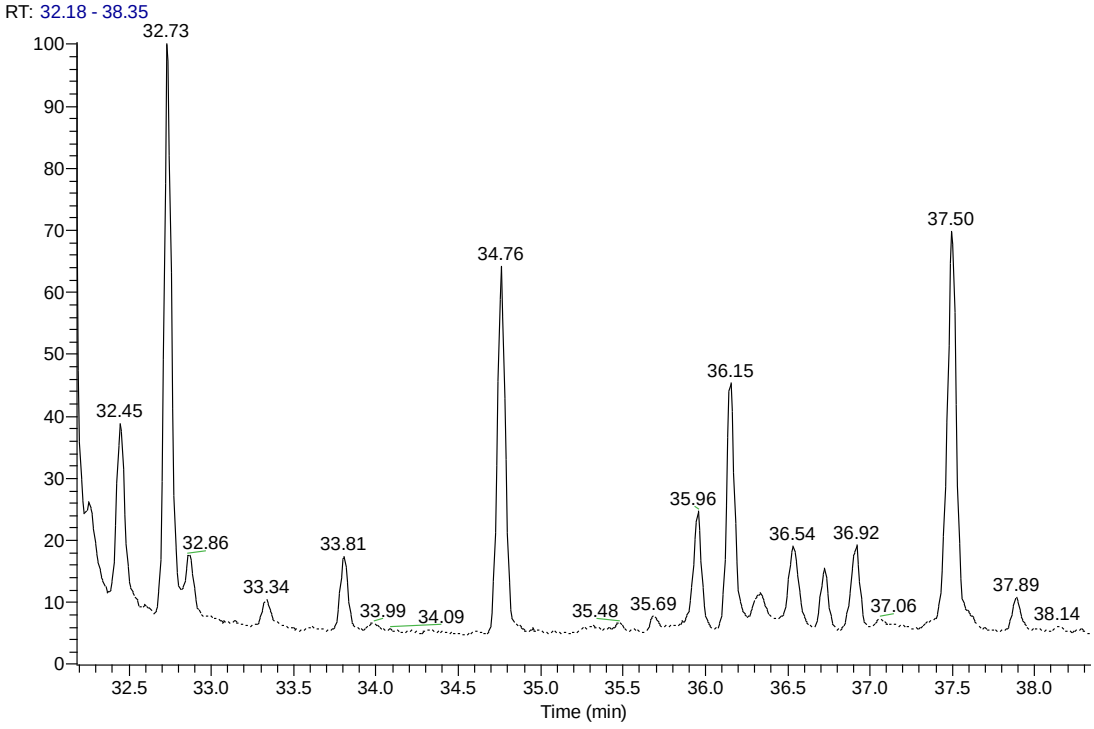


Figure 2.4: Showing low abundance peaks from RT 32.16 – 38.35 mins.

Figure 0.3b:

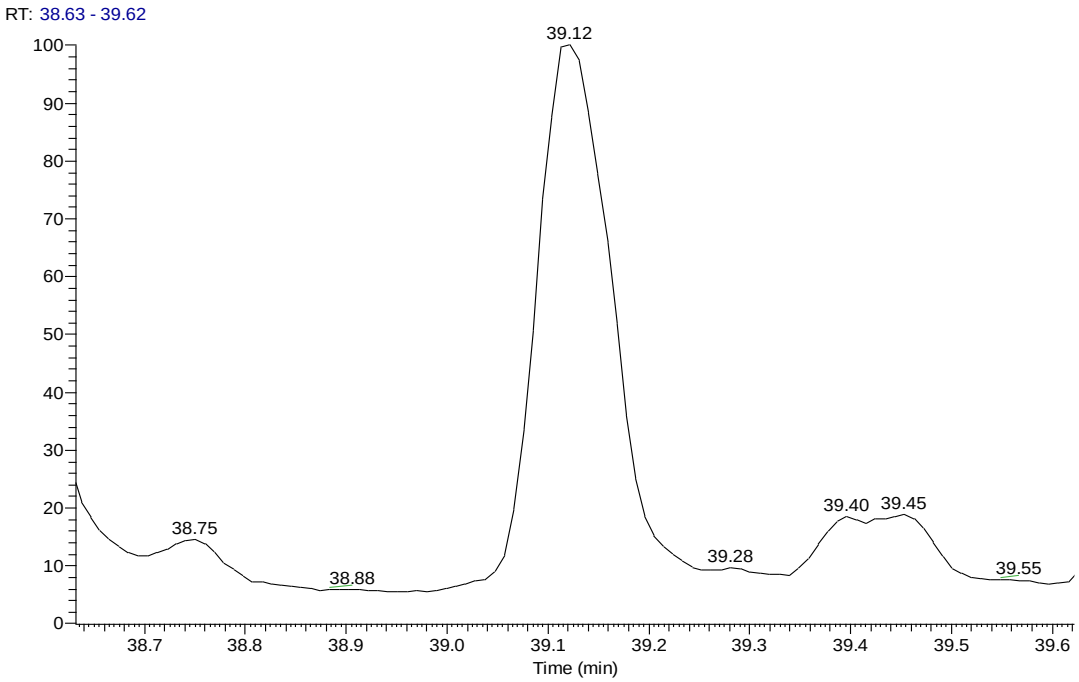


Figure 2.5: Showing low abundance peaks from RT 38.63 – 39.62 mins.

Low abundance peaks present in the total ion chromatogram are shown in greater detail in **Figures 2.3–2.5** by expanding the regions between the interferant peaks. The mass spectra of the TMS derivatives allow for preliminary identification of chromatographic peaks belonging to phenolic compounds. TMS derivatives have common losses such as 15 amu (methyl group), 29 amu (-CHO), 30 amu (HCHO), 89 amu (-OTMS), and 103 amu (-CH_2OTMS). Example mass spectra are shown in Figure 2.4 for vanillic acid (2TMS), γ-Tocopherol (2TMS), δ-Tocopherol (2TMS), 1-Monomyristin (2TMS), Stigmasta-7,22-dien-3-ol (TMS) and 5α-stigmast-7-en-3β-ol (TMS).

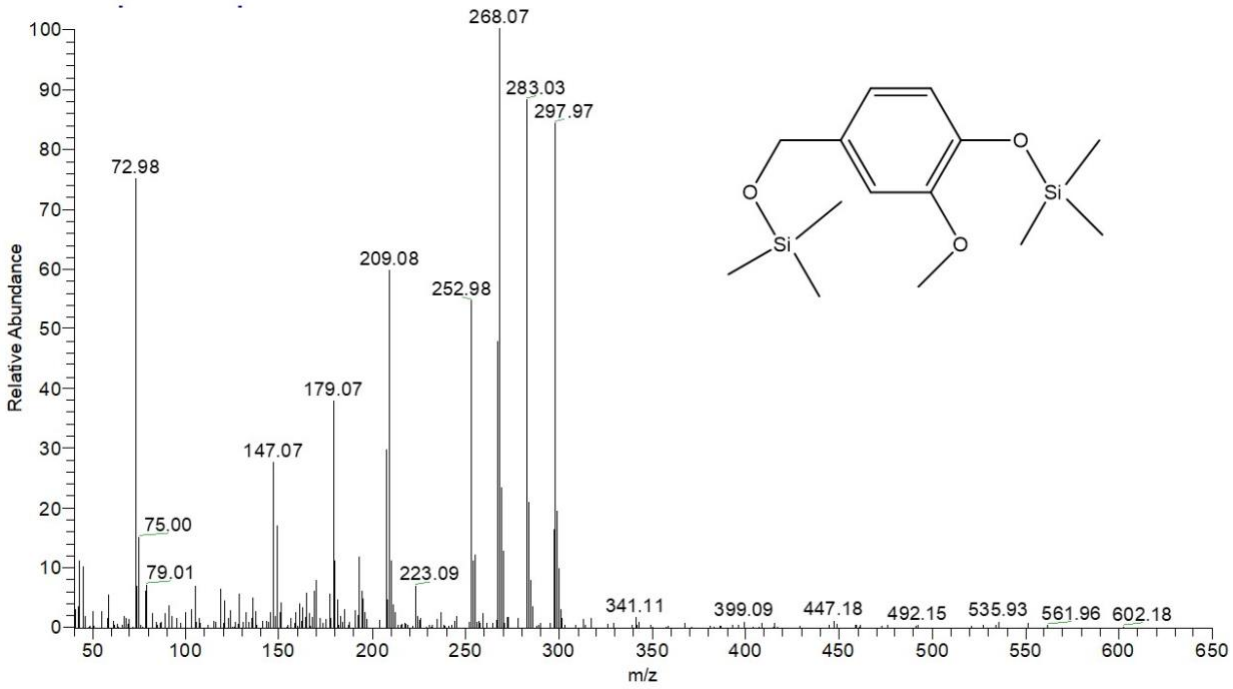


Figure 2.6: Electron impact mass spectrum of Vanillyl Alcohol, 2TMS derivative.

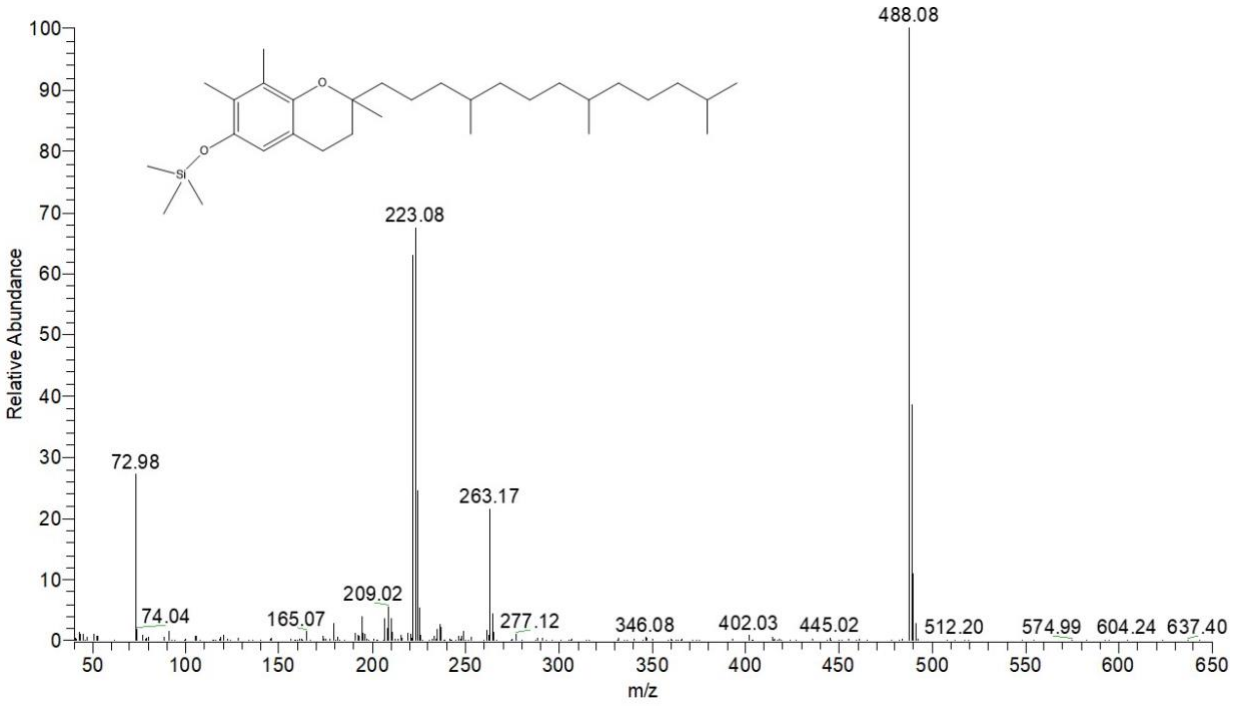


Figure 2.7: Electron impact mass spectrum of γ-Tocopherol, TMS derivative.

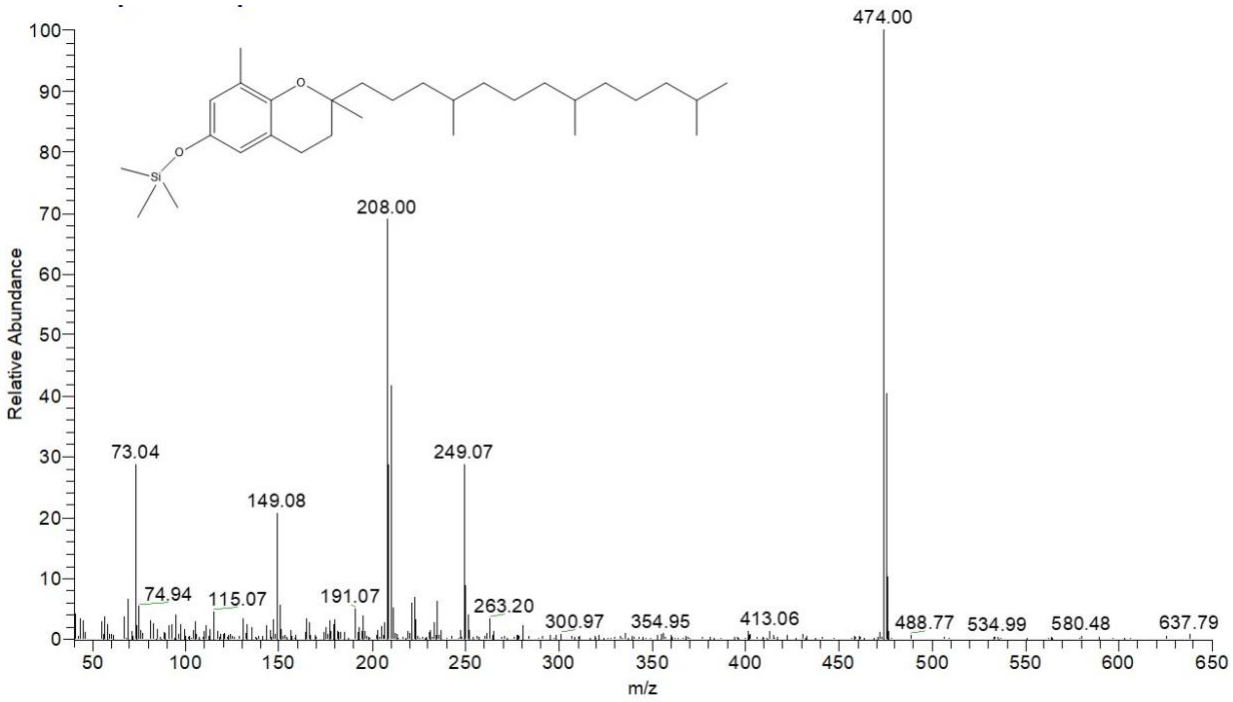


Figure 2.8: **El**ectron impact mass spectrum of δ-Tocopherol, TMS derivative.

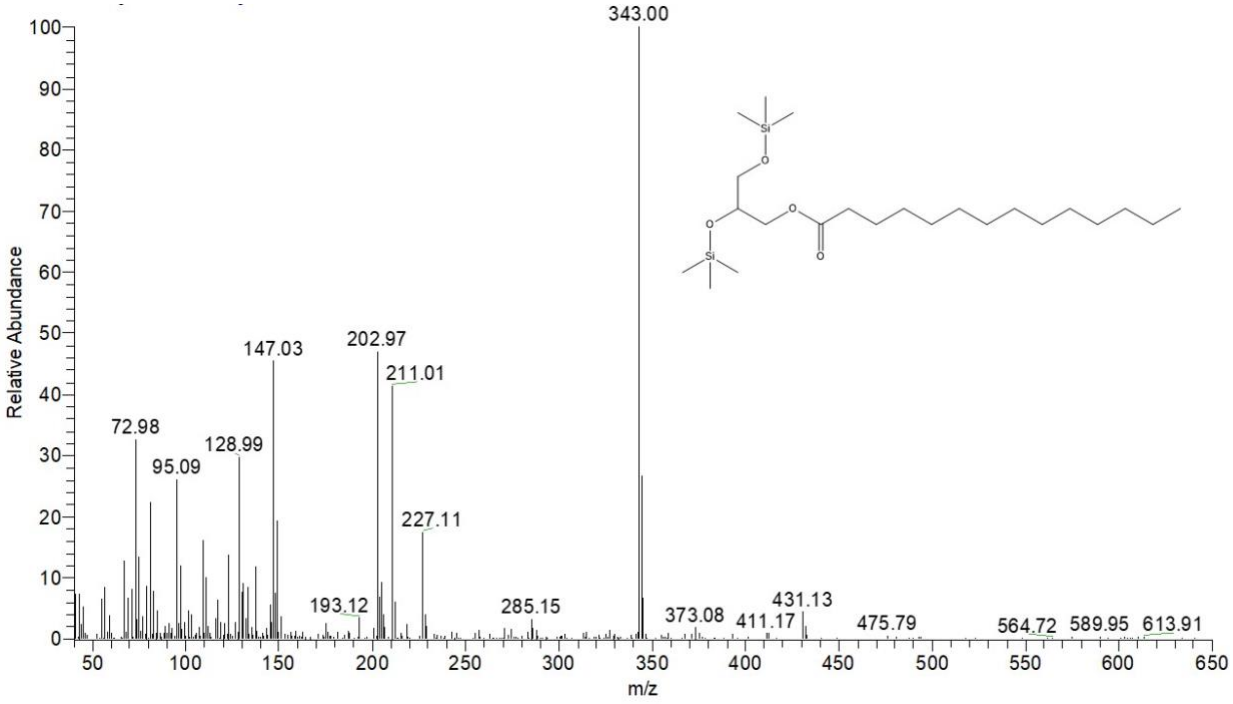


Figure 2.9: Electron impact mass spectrum of 1-Monomyristin, 2TMS derivative.

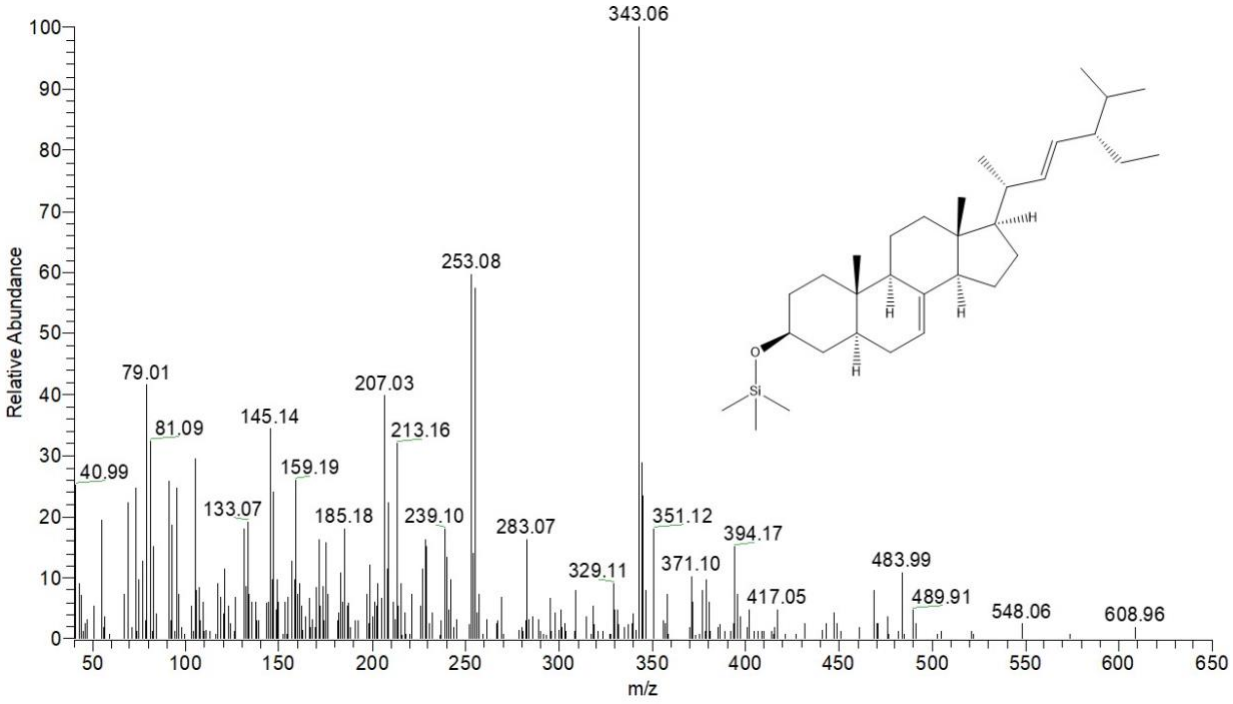


Figure 2.10: Electron impact mass spectrum of Stigmasta-7,22-dien-3-ol, TMS derivative.

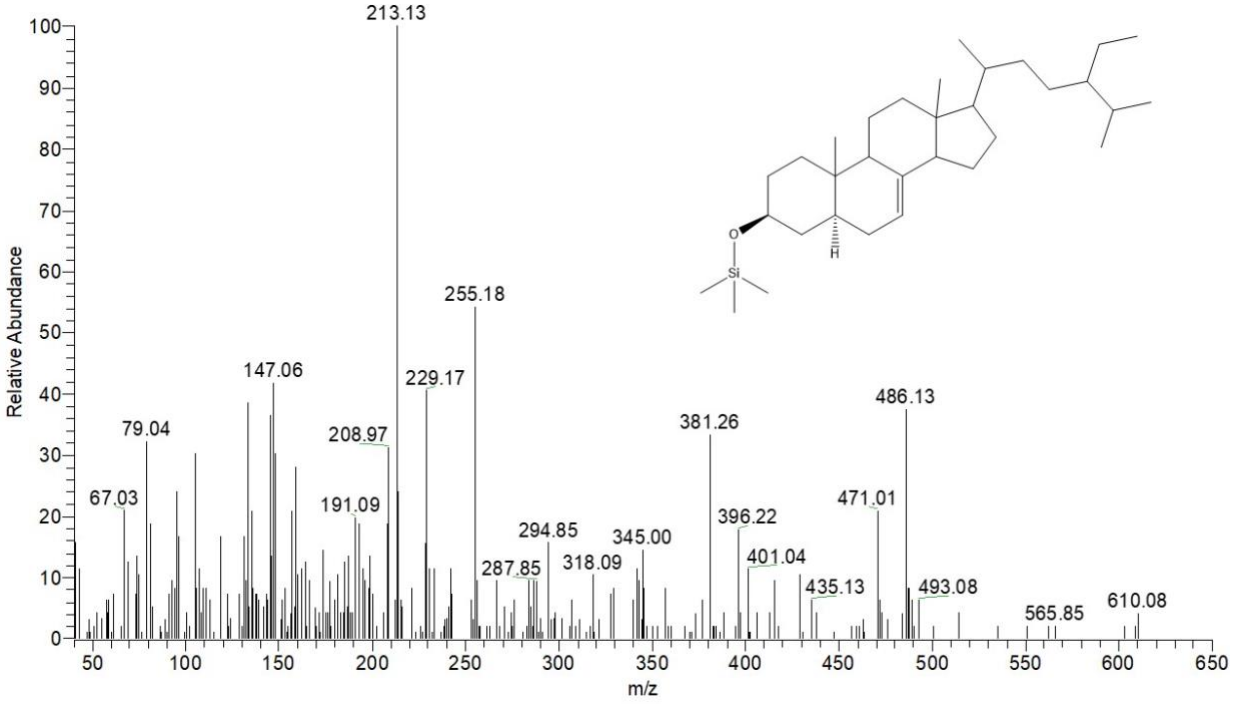


Figure 2.11: Electron impact mass spectrum of 5α-stigmast-7-en-3β-ol, TMS derivative

The peaks identification was carried out by importing each sample data file into the AMDIS software program to extract background free EI mass spectra that were then searched against the NIST 20 MS database. This method depends on the reference spectral library used and it cannot be used to differentiate isomers. Retention indices have been used to increase accuracy for compound identification, and retention indices of isomers are different from each other. Mass spectrum matching and retention indices were combined for compound identification in this study.

The retention index (RI) was calculated using the Kovats/Lee retention index formula which is given as[66]:

$$RI = 100\left[\frac{(RT_U - RT_C)}{(RT_{C+1} - RT_C)}\right] + 100C$$

where, RI is the retention index, RT_U is the retention time of the unknown, RT_c is the retention time of the n-alkane that elutes before the unknown, RT_{C+1} is the retention time of the n-alkane that elutes after the unknown, and C is the number of carbon atoms in the n-alkane that elutes before the unknown

Under the same chromatographic conditions as the extract, the n-alkane standard was analyzed and the peaks were identified to get retention times and the carbon numbers used in the retention index calculation.

Table 2.1: Table showing the compounds identified, molecular weight (MW), retention indices (RI) and %Area of peaks of authentic argan oil from Morocco.

Compound	MW	RT	RIa	DB1	DB5	%Area
Nonanoic Acid, (TMS)	230	9.91	1360	1355		0.092%
		12.05	1436			0.207%
Vanillyl Alcohol (2TMS)	298	17.65	1634	1633	n/a	0.040%
Dodecanoic Acid, (TMS)	272	18.17	1653	1651	1654	0.129
Levoglucosan (3TMS)	378	19.4	1697	n/a	1691	0.008%
4-tert-Octylphenol, TMS derivative	278	20.25	1729	1631	1600 ± 35	0.006%
Azelaic Acid (2TMS)	332	22.09	1797	1789 ± 2	1806 ± 6	0.024%
Myristoleic acid (TMS)	298	22.79	1825	1840	1839	0.005%
Myristic Acid (TMS)	300	23.40	1848	1845 ± 1	1850 ± 2	0.154%
		24.68	1898			0.092%
		25.2	1919			0.043%
		25.53	1933			0.040%
Pentadecanoic Acid (TMS)	314	25.88	1947	1944 ± 1	1949 ± 2	0.034%
		26	1952			0.199%
		27.49	2013			0.023%
Palmitelaidic Acid (TMS)	326	27.59	2017		2029 ± 1	0.095%
Hexanedioic Acid (2TMS)	378	28.13	2040			0.027%
Palmitic Acid (TMS)	328	28.25	2044	2042 ± 1	2049 ± 3	2.768%
Linoleic Acid (TMS)	352	31.99	2208	2201 ± 1	2210 ± 4	20.87%
		32.12	2214			10.81%
		32.26	2221			0.054%
6-Hydroxyhexanoic acid (2TMS)	276	32.45	2229			0.123%
Stearic Acid (TMS)	356	32.74	2243	2239 ± 1	2243 ± 4	0.392%
		33.34	2271			0.013%
1-Monomyristin (2TMS)	446	35.96	2395	2424	2418	0.067%
Ricinoleic Acid (2TMS)	442	36.15	2413		2403	0.187%
		36.53	2423			0.048%

Table 2.1: Table showing the compounds identified, molecular weight (MW), retention indices (RI) and %Area of peaks of authentic argan oil from Morocco (cont'd).

Compound	MW	RT	RIa	DB1	DB5	%Area
		36.92	2443			0.114%
Pentadecanoic acid,glycerine-(1)-monester, 2TMS	460	37.89	2491	2518	2512	0.017%
		38.43	2519			0.181%
		38.56	2525			3.234%
		38.75	2535			0.023%
		39.13	2555			0.368%
		39.4	2569			0.075%
1-Monopalmitin (2TMS)	474	39.76	2587	2613	2606 ± 5	7.556%
		40.76	2640			0.630%
		40.92	2649			0.006%
2-Linoleoylglycerol (2TMS)	498	42.16	2716	2740	2739 ± 33	2.119%
		42.25	2720			4.766%
1-Monolinolein (2TMS)	498	42.79	2751		2743	12.36%
1-Monooleoglycerol (2TMS)	500	42.88	2756	2784	2779 ± 9	28.70%
		43.1	2768			0.094%
		43.19	2773			0.310%
Glycerol Monostearate (2TMS)	502	43.33	2781	2808	2768 ± 31	1.798%
δ-Tocopherol (TMS)	474	45.24	2887	n/a	2900	0.036%
		46.45	2946			0.043%
22-Hydroxydocosanoic acid (TMS)		47.08	2975		2969	0.284%
γ-Tocopherol (TMS)	488	47.19	2980	n/a	2999	0.161%
		48.12	3019			0.020%
Stigmasta-7,22-dien-3-ol / Spinasterol (TMS)	484	57.12	3288	n/a	3331	0.238%
Stigmast-7-en-3β-ol / Schottenol (TMS)	486	59.87	3342	n/a	3391	0.139%

The spectra of some unidentified compounds in the table were observed to have characteristic ion losses shown for TMS derivatives in the literature. Compound with retention time 38.43 is an unidentified TMS-derivatized compound (molecular ion at m/z 451) with $[M - 15]^+$ at m/z 437 and $[M - OTMS]^+$ at m/z 361, , compounds with retention times 40.26 and 40.50 are an unidentified TMS-derivatized compounds (molecular ion at m/z 450) with $[M-OTMS]^+$ at m/z 361 and compound with retention time 41.56 is an unidentified TMS-derivatized compound (molecular ion at m/z 488) with $[M - 15]^+$ at m/z 474 and $[M - TMSOCH_2]^+$ at m/z 385.

Compound with retention time 48.12 had a molecular ion at m/z 486 and is tentatively identified as a tocomonoenol (possessing one double bond) related to either β- or γ-tocopherol. This is a new compound that has not been identified before in the literature. Peaks at m/z 72.98, 223.12 and 263.17 are all observed in the EI mass spectrum of β- or γ- tocopherol and arise from the fragmentation of the derivatized ring structure shown in **Figure 2.12**. The molecular ion at m/z 486.05 is 2 amu smaller than the molecular ion of β- or γ- tocopherol at m/z 488.08, which is due to the presence of double bond in the chain of tocomonoenol. One potential location of the double bond is shown in the structure in **Figure 2.12**. This is the first report of a tocomonoenol present in argan oil. Presence of a similar compound was reported in palm oil. [67]

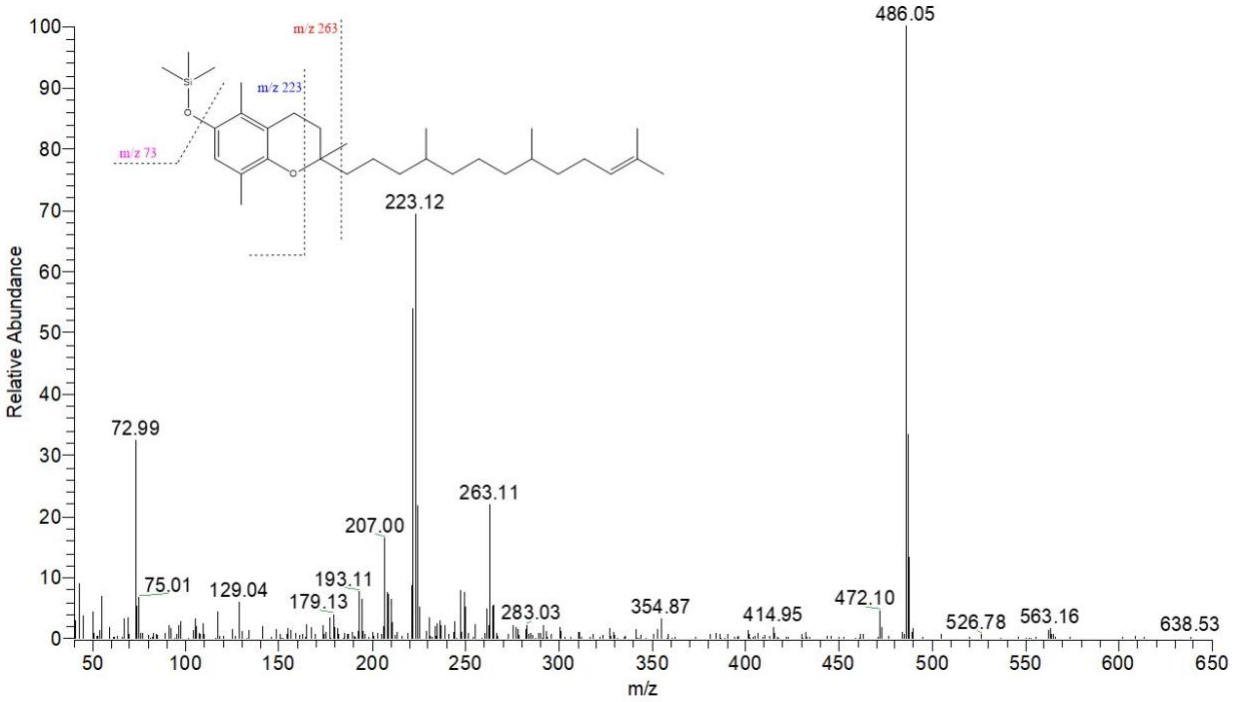


Figure 2.12: Electron impact mass spectrum of unknown Tocomonoenol, TMS derivative.

CHAPTER 3

LCMS EVALUATION OF TRIACYLGLYCEROL PROFILE OF ARGAN OIL

3.1 MATERIALS AND REAGENT

Authentic argan oil was a gift from Kamal Aberkani, professor at Université Mohammed Premier in Nador, Moracco. Acetonitrile, hexane, and 2-propanol used for the analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the solvents were HPLC grade.

3.2 SAMPLE PREPARATION

The authentic argan oil was prepared for LCMS profiling using the procedure of Pagliuca et al[61]. 50mg of argan oil was weighed and 1mL of 2-propanol: hexane (50:50, v/v) was added. The mixture was vortexed for one minute and diluted with the mobile phase 1:1000 (v/v). The sample was then filtered through a Whatman 42 Ashless 9.0cm filter paper and transferred to vials for LCMS analysis.

3.3 LCMS INSTRUMENTATION

LCMS (Figure 3.1) was carried out on a Shimadzu LCMS-2020 instrument equipped with an Agilent Zorbax 300 column (2.1mm i.d. X 150mm; 5µm particle size). 2-Propanol: acetonitrile: water with 0.2% formic acid (80:15:5, v/v/v) was used as mobile phase at a flow rate of 0.3ml/min. 10µL of the sample was injected and the single quadrupole was scanned from 800-1000m/z.

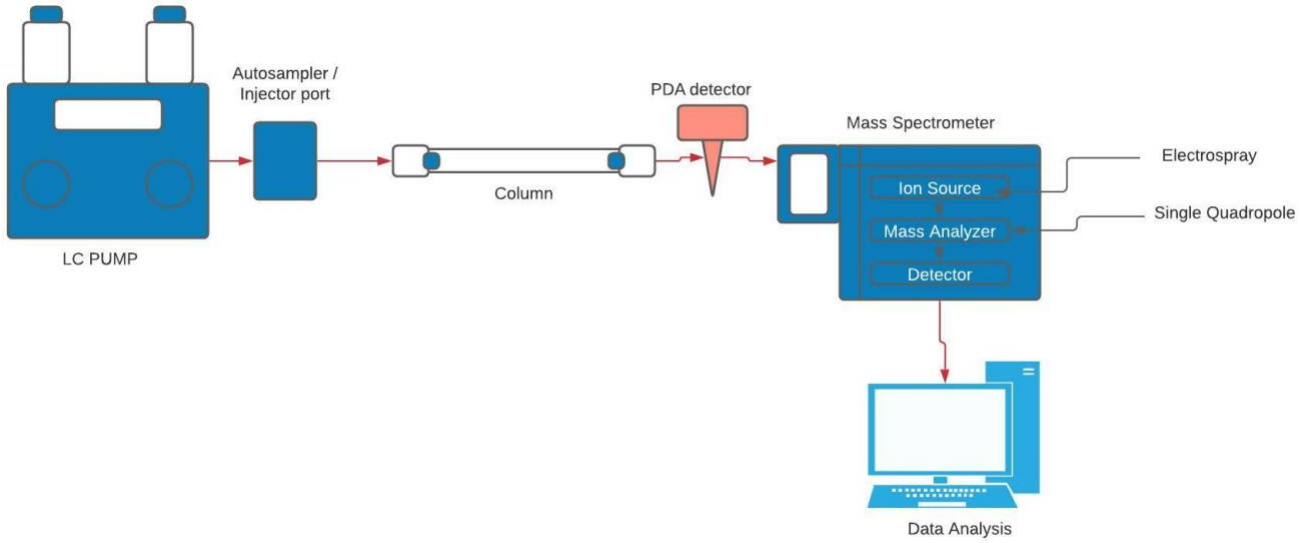
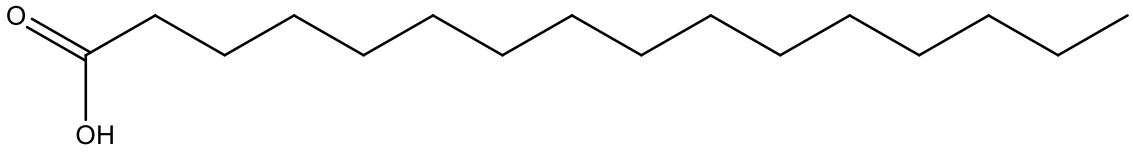


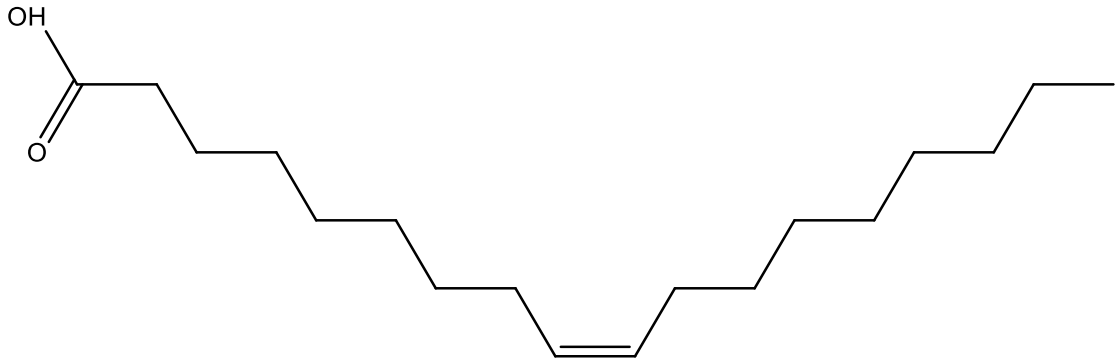
Figure 3.1: Schematic diagram of the LCMS instrument

3.4 RESULTS AND DISCUSSIONS

The individual fatty acids present in the triacylglycerol (TAG) mixture are represented as L – Linoleic acid, P – palmitic acid, O – oleic acid, Ln – Linolenic acid, S – stearic acid, and G – gadoleic acid (Figure 3.2) The major TAG components of argan oil are POO, OOO, OOL, POL and OLL. These TAGs are present in varying amount, with POO ranging from 11.2% - 17.49%, OOO 12.8% - 25.4%, OOL from 13.6% - 15.02%, POL from 7.1% - 13.4% and OLL from 13.6% - 16.67%.[14,37,57]

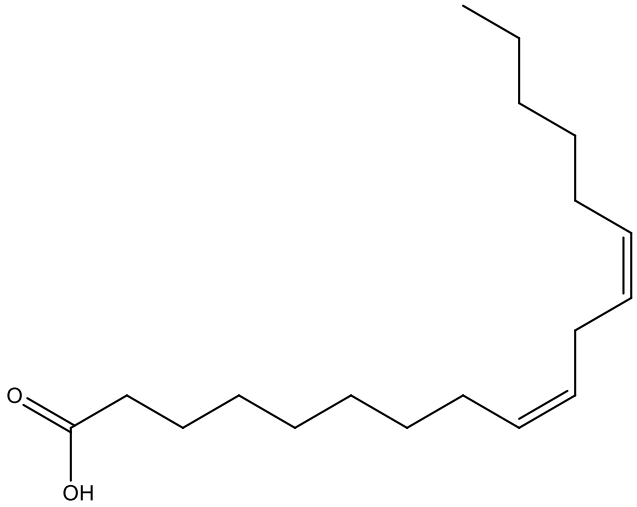


palmitic acid

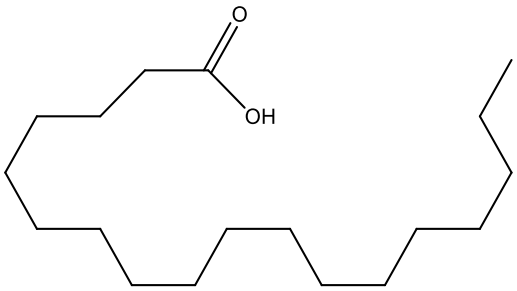


oleic acid

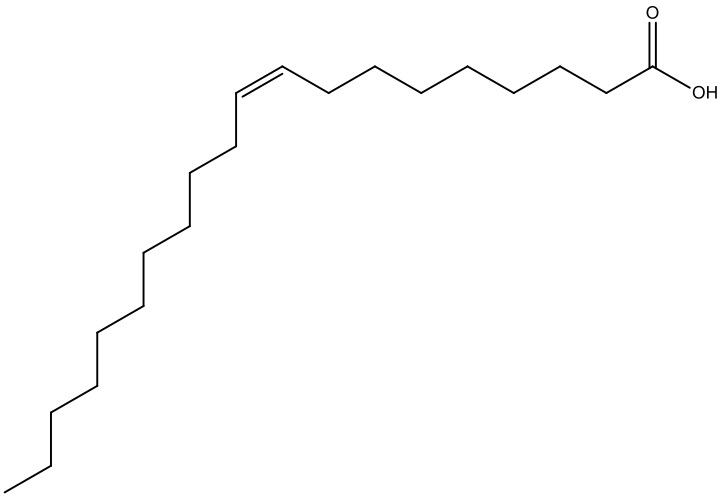
Figure 3.2: Structure of individual fatty acid components that make up the TAG compounds.



Linoleic Acid



stearic acid



Gadoleic Acid

Figure 3.2: Structure of individual fatty acid components that make up the TAG compounds (cont'd).

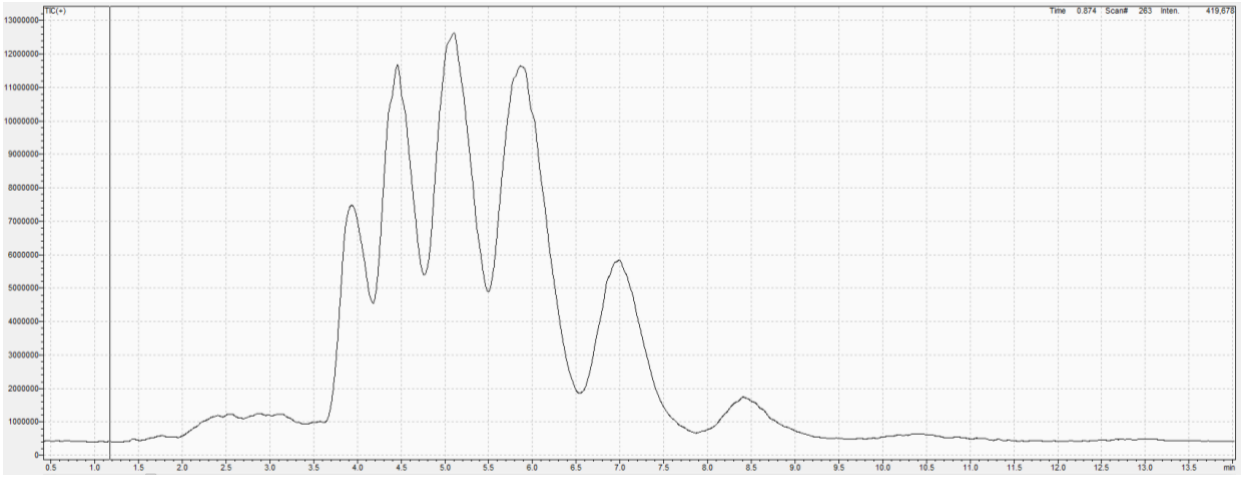


Figure 3.3: Total Ion Chromatogram showing triacylglycerol (TAG) profile of argan oil by LCMS.

The total ion chromatogram in **Figure 3.3** shows the characteristic LCMS TAG "hand-shape profile" of argan oil.[61] Only the TAGs are visible in the chromatogram because the LCMS instrument was scanned in the mass range from 800-1000amu. The TAG chromatographic peaks (including coeluting TAGs) were all in agreement with literature[57,61] and are described in detail in **Table 3.1**, which lists the retention time (RT), the protonated molecule $[M + H]^+$, sodium adduct ions $[M + Na]^+$, potassium adduct ions $[M + K]^+$, and molecular formula of all TAGs observed. Briefly, the peak at retention time 3.935 minutes contained LLL and co-eluting LLP, the peak at retention time 4.457 minutes contained LLO with PPL and POL co-eluting, the peak at 5.101 minutes contained OOL with PPO and OOP co-eluting, the peak at 5.868 minutes contained OOO and co-eluting PSO and the peak at 6.990 minutes contained OOS and co-eluting OOG.

Table 3.1: Retention time, Protonated molecule, sodium & Potassium adduct ions (m/z) and Molecular formula of TAGs detected.[61]

RT	$[M + H]^+$	$[M + Na]^+$	$[M + K]^+$	TAG	Molecular Formula
3.935	879.6	901.5	917.5	LLL	$C_{57}H_{98}O_6$
	855.7	877.7	893.7	LLP	$C_{55}H_{98}O_6$
4.457	881.8	903.7	919.7	LLO	$C_{57}H_{100}O_6$
		853.7	869.7	PPL	$C_{53}H_{98}O_6$
		879.7	895.7	POL	$C_{55}H_{100}O_6$
5.101		905.8	921.7	OOL	$C_{57}H_{102}O_6$
		855.7	871.7	PPO	$C_{53}H_{100}O_6$
	859.8	881.8	897.7	OOP	$C_{55}H_{102}O_6$
5.868	885.8	907.8	923.7	OOO	$C_{57}H_{104}O_6$
		883.8	899.8	PSO	$C_{55}H_{104}O_6$
6.990	88 7.8	909.8	925.8	OOS	$C_{57}H_{106}O_6$
	913.8	935.8	951.8	OOG	$C_{59}H_{108}O_6$
8.413		911.8	927.8	SSO	$C_{57}H_{108}O_6$

Figure 3.4 shows the mass spectrum of the TAG trilinolein (LLL) eluting at retention time of 3.935 showing its protonated molecular ion peak ($[M + H]^+$) at m/z 879.6, sodium adduct ion peak ($[M+Na]^+$) at m/z 901.5 and potassium adduct ion peak ($[M+K]^+$) at m/z 917.7.

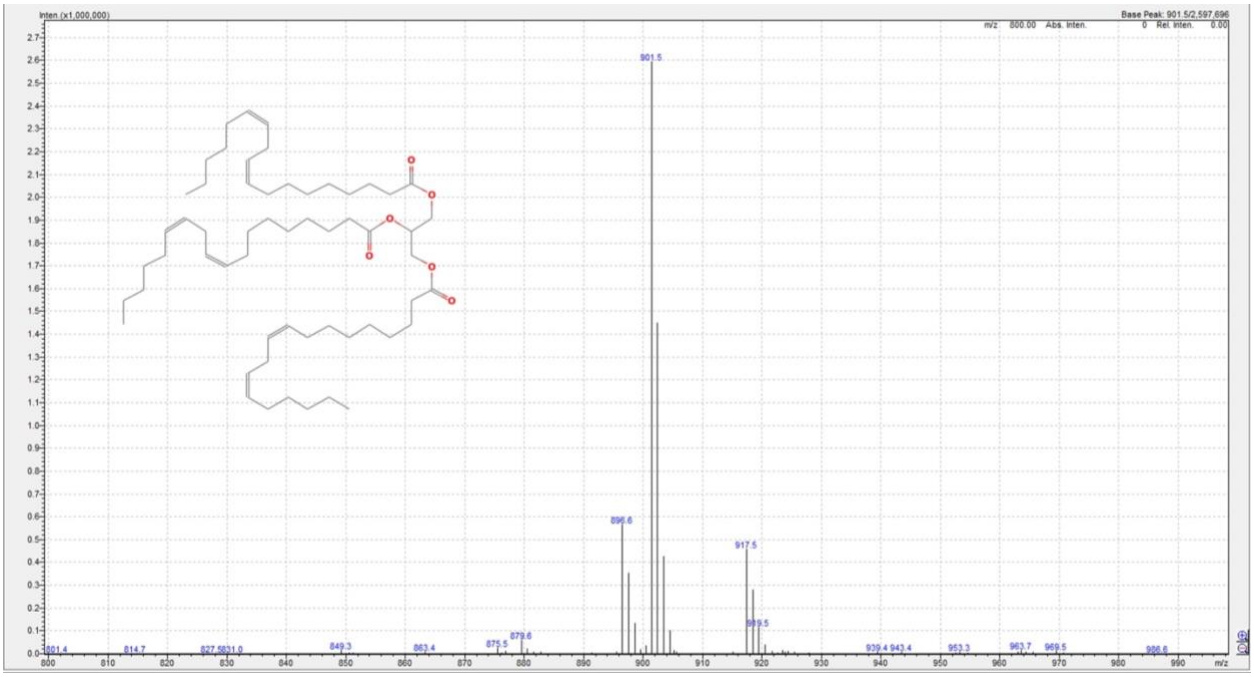


Figure 3.4: Mass spectrum of Trilinolein (LLL) where L is Lenoleic Acid ($C_{57}H_{98}O_6$).

CHAPTER 4

COMPARISON OF COMMERCIAL ARGAN OIL TO AUTHENTIC ARGAN OIL USING GCMS

4.1 MATERIALS AND REAGENTS

Authentic argan oil used was a gift from Kamal Aberkani, a professor at Université Mohammed Premier in Nador, Morocco. Commercially available argan oil (Cliganic 100% pure natural argan oil certified organic) was purchased online from Amazon.

Methanol, hexane, 2-propanol, bis(trimethylsilyl)trifluoroacetamide (BSTFA), acetonitrile, acetone and C_9-C_{40} saturated alkanes standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the solvents used were HPLC grade.

4.2 EXTRACTION OF PHENOLIC COMPOUNDS

The method that was used for the extraction of the phenolic compounds is the same as described in Chapter 2. Briefly, phenolic compounds were extracted using methanol. The extract was dried using a nitrogen stream and the syrupy residue dissolved in acetonitrile. Hexane washings were performed to reduce the fatty acid content. The remaining extract was dried, dissolved in acetone and derivatized with BSTFA.

4.3 GCMS INSTRUMENTATION

The GCMS instrumentation (Thermo Finnigan TraceGC PolarisQ quadrupole ion trap mass spectrometer equipped with a J&W Scientific DB5 column (30m X 0.25mm i.d.; 0.25µm film thickness)) and oven temperature program used for this comparative analysis was the same as described in Chapter 2.

4.4 RESULTS AND DISCUSSIONS

The phenolic compounds in the authentic argan oil were evaluated and discussed in Chapter 2. In this chapter the phenolic compounds in the commercially available argan oil were evaluated, using the same method and instrumental conditions used for the authentic argan oil. **Figure 4.1** compares the chromatogram of the phenolic extract of authentic argan oil to the chromatogram of the phenolic extract of the commercially available argan oil. The peak identifications were done by importing the sample data files into the AMDIS software program to extract background free EI mass spectra that were then searched against the NIST 20 MS database.

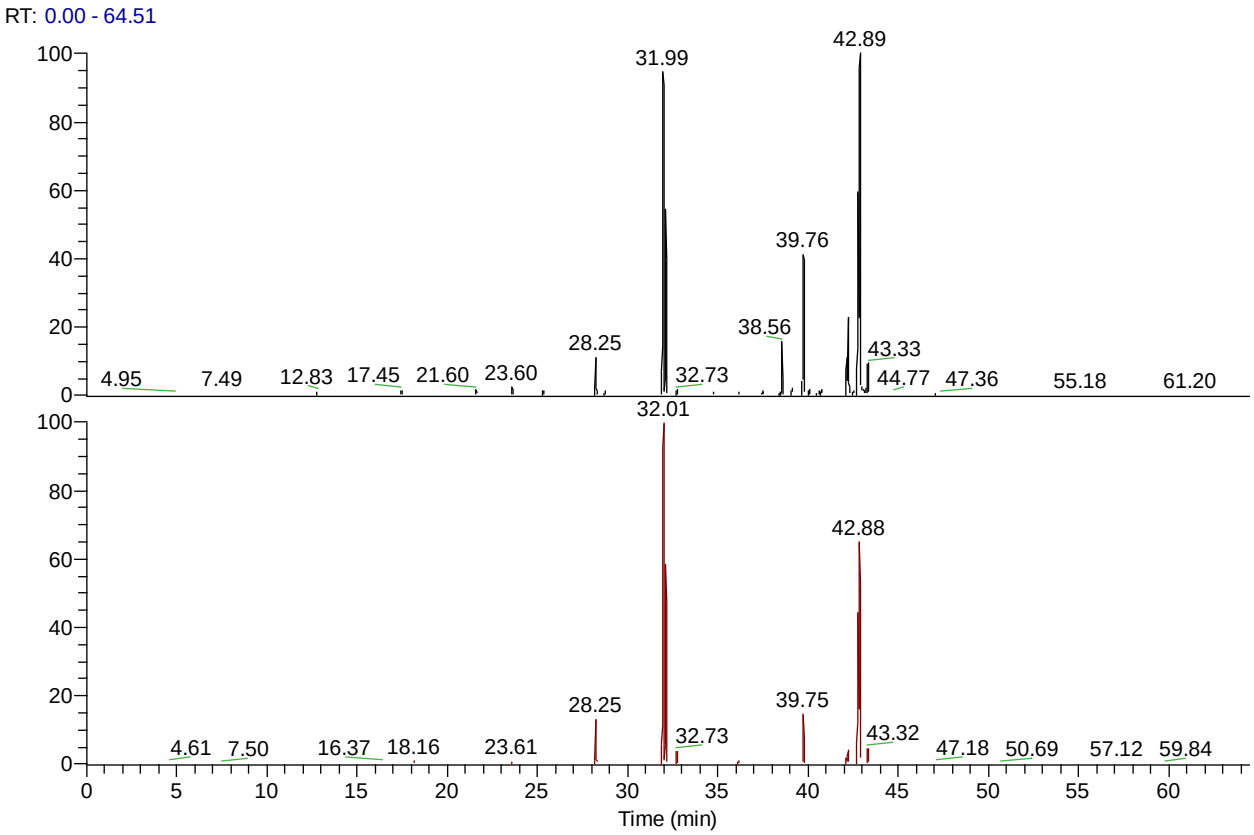


Figure 4.1: Stack plot showing the total ion chromatogram of the authentic argan oil (top) and the commercially available argan oil (bottom).

Table 4.1 shows the compounds identified and the yet to be identified peaks in both the authentic argan oil and the commercially available argan oil, molecular weight of compounds that were identified, retention index and their percentage peak area. Commercially available argan oil appears very similar to the authentic argan oil in terms of its phenolic, fatty acid, and sterol content. As discussed in Chapter 2, phenolic compounds are present in low quantity in argan oil.[4,30] As expected phenolic compounds represent a low percentage of the total peaks observed in both the authentic and commercially available argan oil. Two phenolic compounds derived from vanillin were identified in these experiments supporting reports in the literature of phenolic compounds found in argan oil.[30,33] Vanillic acid (0.038%), an oxidized form of vanillin was identified in the commercially available argan oil, and vanillyl alcohol (0.039%) was identified in the authentic argan oil.

The majority of peaks in both argan oils were identified as fatty acid compounds which, as discussed in Chapter 2, shows the difficulty of removing these compounds.[2,11,37] A large peak can be observed for linoleic acid in both the authentic argan oil and the commercially available argan oil at 20.719% and 39.806%, respectively. Other major fatty acid compounds identified in the commercially available argan oil such as stearic acid (1.187%) and palmitic acid (3.408%) were also reported to be present in the authentic argan oil at 0.389% for stearic acid and 2.748% for palmitic acid.[11&37] The variations in percent total peak area for these fatty acids are attributed to natural variations in argan oils and the extraction procedures.

Table 4.1: Table showing the compounds, RT, RI, and % Peak Area of all peaks in the authentic and commercially available argan oil.

Compounds	MW	RT (min)	RI	RI_{db}	A.A.O	C.A.A.O
Urea, 2TMS	204	6.76		1237		0.119% (± 0.041%)
		7.43				0.843% (± 0.113%)
Glycerol, 3TMS	308	7.49		1292±4		0.105% (± 0.023%)
Butanedioic acid, 2TMS	262	8.62	1312.45	1316		0.044% (± 0.009%)
Nonanoic acid, TMS	230	9.91	1359.71	1355±2	0.092% (± 0.08%)	
		12.05	1436.49		0.207% (± 0.023%)	
2-Methylbutanoic Acid, TMS		12.72	1460	999		0.048% (± 0.008%)
		15.84	1569.96			0.011% (± 0.005%)
		16.37	1588.69			0.288% (± 0.017%)
Vanillyl alcohol, 2TMS	298	17.66	1634.77	1633±1	0.040% (± 0.007%)	
Dodecanoic acid, TMS	272	18.17	1653	1654±4	0.129% (± 0.003%)	0.258% (± 0.019%)
Levoglucosan, 3TMS	378	19.41	1697.49	1691	0.008% (0.004%)	
4-Tert-octylphenol, TMS	278	20.25	1728.73	1600±35	0.006% (0.001%)	
Vanillic Acid, 2TMS	312	21.09	1760.07	1776±2		0.038% (± 0.004%)
Azelaic acid, 2TMS	332	22.08	1797.01	1806±6	0.024% (± 0.004%)	0.043% (± 0.002%)
Myristoleic acid, TMS	298	22.78	1824.22	1840	0.005% (± 0.003%)	
Myristic acid, TMS	300	23.4	1848.44	1850±2	0.154% (± 0.001%)	0.138% (± 0.002%)
		24.66	1897.66		0.092% (± 0.035%)	0.116% (± 0.007%)
		25.2	1919.43		0.043% (± 0.007%)	
		25.53	1932.79		0.040% (± 0.027%)	
Pentadecanoic acid, TMS	314	25.84	1945.34	1949±4	0.034% (± 0.001%)	0.034% (± 0.001%)
		26	1951.82		0.199% (± 0.009%)	0.196% (± 0.003%)
		27.49	2012.66		0.023% (± 0.005%)	
Palmitelaidic acid, TMS	326	27.59	2016.88	2029±1	0.095% (± 0.001%)	0.023% (± 0.002%)
13-methylpentadec-6-enoic acid, TMS	326	27.71	2021.94	2023		0.015% (± 0.001%)

Table 4.1: Table showing the compounds, RT, RI, and % Peak Area of all peaks in the authentic and commercially available argan oil (cont'd).

Compounds	MW	RT (min)	RI	RI_{db}	A.A.O	C.A.A.O
Hexanedioic acid, 2TMS	378	28.13	2039.66	1589	0.027% (± 0.002%)	
Palmitic acid, TMS	328	28.26	2045.15	2049±3	2.768% (± 0.173%)	3.419% (± 0.164%)
		28.95	2074.26			0.040% (± 0.001%)
		30.13	2125.33			0.025% (± 0.001%)
		30.27	2131.56			0.033% (± 0.002%)
Linoleic acid, TMS	352	31.99	2208.29	2210±4	20.870% (± 2.755%)	39.937% (± 0.977%)
Unidentified fatty acid		32.16	2216.13		10.808% (± 1.409%)	21.190% (± 0.494%)
		32.26	2220.74		0.054% (± 0.021%)	
6-Hydroxyhexanoic acid, 2TMS	276	32.45	2229.49	1431±69	0.123% (± 0.015%)	
Stearic acid, TMS	356	32.74	2242.86	2243±4	0.392% (± 0.006%)	1.191% (± 0.123%)
		33.34	2270.51		0.013% (± 0.002%)	
		35.57	2376.08			0.009% (± 0.005%)
		35.67	2380.86			0.126% (± 0.025%)
1-Monomyristin, 2TMS	446	35.94	2392.78	2338	0.067% (± 0.007%)	0.096% (± 0.004%)
Ricinoleic acid, 2TMS	442	36.15	2404	2403	0.187% (± 0.014%)	0.333% (± 0.011%)
		36.3	2411.5			0.152% (± 0.005%)
Sebacic acid, 2TMS	346	36.55	2424	1899±5	0.048% (± 0.001%)	0.189% (± 0.008%)
		36.92	2442.5		0.114% (± 0.076%)	
Pentadecanoic acid, glycerine-(1)-monoester, 2TMS	460	37.89	2489.5	2482	0.017% (± 0.003%)	
		38.43	2518.56		0.181% (± 0.065%)	
		38.59	2526.8		3.234% (± 0.445%)	0.077% (± 0.003%)

Table 4.1: Table showing the compounds, RT, RI, and % Peak Area of all peaks in the authentic and commercially available argan oil (cont'd).

Compounds	MW	RT (min)	RI	RI $_{db}$	A.A.O	C.A.A.O
		38.75	2535.05		0.023% (± 0.001%)	
2-Palmitoylglycerol, 2TMS	474	39.15	2555.67	2558±18	0.368% (± .0183%)	0.275% (± 0.001%)
		39.38	2567.53		0.075% (± 0.001%)	0.015% (± 0.001%)
1-Monopalmitin, 2TMS	474	39.77	2587.63	2606±5	7.556% (± 0.793%)	3.888% (± 0.033%)
		40.76	2640.11		0.630% (± 0.258%)	
		40.91	2648.13		0.006% (± 0.001%)	0.011% (± 0.001%)
2-Linoleoylglycerol, 2TMS	498	42.16	2715.56	2739±33	2.119% (± 0.424%)	0.345% (± 0.001%)
	500	42.24	2720	2742	4.766% (± 0.804%)	0.828% (± 0.026%)
1-Monolinolein, 2TMS	498	42.78	2750	2743	12.358% (± 2.415%)	10.415% (± 0.101%)
1-Monooleoylglycerol, 2TMS		42.88	2755.56	2779±9	28.703% (± 2.468%)	12.784% (± 0.222%)
		43.1	2767.78		0.094% (± 0.045%)	
		43.19	2772.78		0.310% (± 0.171%)	
Glycerol monostearate, 2TMS	502	43.33	2780.56	2768±31	1.798% (± 0.445%)	1.572% (± 0.009%)
δ-Tocopherol, TMS	474	45.25	2887.22	2900	0.036% (± 0.007%)	0.045% (± 0.001%)
		46.45	2945.54		0.043% (± 0.001%)	0.007% (± 0.001%)
22-Hydroxydocosanoic acid, TMS	500	47.08	2975.12	2969	0.284% (± 0.043%)	0.065% (± 0.001%)
γ-Tocopherol, TMS	488	47.19	2980.28	2987	0.161% (± 0.036%)	0.167% (± 0.001%)
		48.12	3019		0.098 (± 0.031%)	
Stigmasta-7,22-dien-3-ol, TMS	484	57.12	3288.14	3331	0.238% (± 0.009%)	0.276% (± 0.004%)
5α-stigmast-7-en-3β-ol, TMS	486	59.86	3343.27	3391	0.139% (± 0.019%)	0.173% (± 0.001%)

Stigmasta-7,22-dien-3-ol and 5α-stigmast-7-en-3β-ol are reported as the major sterols in argan oil.[71] Both sterols are observed in the commercially available argan oil at 0.275% and 0.173%, respectively. Percentages of these sterols observed in the authentic argan oil were 0.236% and 0.138%, respectively. γ-Tocopherol has been reported as the major tocopherol present in argan oil with δ-Tocopherol also present in a smaller quantity. β-Tocopherol is virtually undetected in argan oil.[30&37] Both γ-Tocopherol (0.166%) and δ-Tocopherol (0.045%) appear in the commercially available argan oil with comparable percentages observed for the authentic argan oil.

Table 4.2 summarizes the percentage of each compound class present in the authentic argan oil and the commercially available argan oil. The presence of 93.4% and 93.38% of total fatty acids in both argan oils and other compounds such as phenols and sterols present in smaller percentages agrees with literature, which reports that argan oil comprises of 95+% acylglycerols and free fatty acids and other compounds constitute the remaining percentages.[37] No unexpected compounds are observed that would indicate that the commercially available argan oil is anything other than argan oil. Therefore, adulteration studies described in Chapter 5 will use the commercially available argan oil due to its ease of sourcing.

Table 4.2: Number of class of compounds and their percentage (%) present in both authentic argan oil and commercially available argan oil.

Compounds	AAO		CAAO	
	Number	%	Number	%
Phenols	21	5.87	15	3.1
Fatty Acids (dicarboxylic)	2	0.05	2	0.25
Fatty Acids (saturated)	17	49.13	14	21.43
Fatty Acids (unsaturated)	5	33.28	4	50.54
Unknown Fatty Acids	2	10.94	2	21.16
Total Fatty Acids	26	93.4	22	93.38
Sterols	4	0.75	4	0.67

CHAPTER 5

HEADSPACE - SOLID PHASE MICROEXTRACTION (HS-SPME) COUPLED TO GCMS ANALYSIS OF VOLATILE COMPOUNDS IN ARGAN OIL ADULTERATED WITH OLIVE OIL AND SUNFLOWER OIL

5.1 MATERIALS AND REAGENTS

Commercially available argan oil (Cliganic 100% pure natural argan oil certified organic) was obtained using Amazon. Olive oil (Lucini extra virgin) and sunflower oil (Spectrum culinary organic) were purchased locally (Carbondale, IL). All oil samples were placed in 16mL clear glass vials closed by TEF/SIL septa.

5.2 HEADSPACE - SOLID PHASE MICROEXTRACTION SAMPLING CONDITIONS

A manual solid phase microextraction (SPME) device and fiber were purchased from Supelco (Bellefonte, PA). The fiber used for the extraction of the volatile compounds was a Supelco 50/30µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber[68].

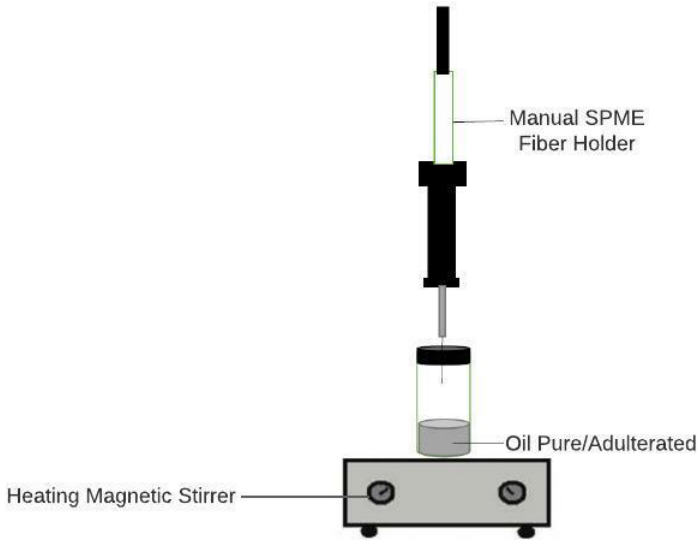


Figure 5.1: Schematic diagram of SPME Set-up for Equilibration of Oil.

The SPME fiber was conditioned as recommended by the manufacturer before use by exposing it in the GC inlet at 250°C for 30 minutes. After oil equilibration, the SPME fiber was exposed to the oil headspace for thirty minutes. The oil temperature was maintained at 60°C throughout fiber exposure. After exposure the fiber was thermally desorbed into a GC inlet (78.5mm x 6.33mm OD and 0.75mm ID inlet liner) for five minutes. GC analyses were carried out on a Thermo Finnigan TraceGC PolarisQ quadrupole ion trap mass spectrometer equipped with a J&W Scientific DB5 column (30m X 0.25mm i.d.; 0.25µm film thickness). The oven temperature program began by holding at 50°C for 2 minutes, ramping to 200°C at 4°C/min and then holding isothermally for 20 minutes. The transfer line was set to 275°C. The inlet temperature was set to 250 °C and the ion source was set to 240°C. The carrier gas was helium

(constant flow 1mL/min). The mass spectrometer was used in full scan electron impact (EI, 70eV) mode from 40 to 650 amu.

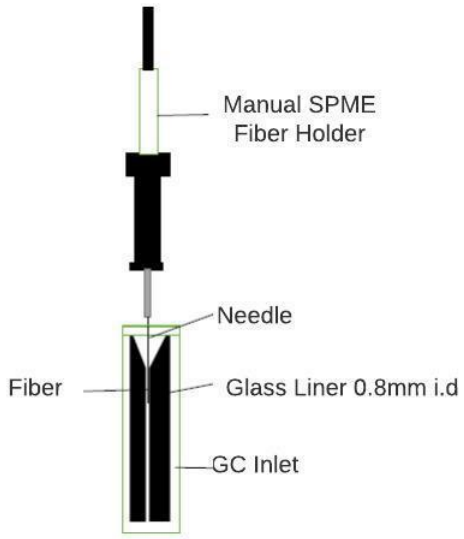


Figure 5.2: Schematic Diagram Showing GC Injector Port and Glass Liner 0.8mm i.d.

5.3 RESULTS AND DISCUSSION

The pure argan oil, olive oil, sunflower oil, and mixtures of the pure argan oil with adulterant oils (olive oil and sunflower oil) at different percentages were analyzed for volatile compounds using head-space solid phase microextraction (HS-SPME). Each experiment was repeated four times. Peaks from each analysis were identified by importing each sample data file into the AMDIS software program to extract background free EI mass spectra that were then searched against the NIST 20 MS database. Mass spectra of peaks that gave high probability against the NIST 20 MS database were identified while peaks with lower probability

were not identified. The retention index was calculated using the Kovats/Lee equation shown in Chapter 2 and the retention times of the alkane C_9 – C_{40} standard components.

Fifty-three volatile compounds were isolated in the pure argan oil; fifteen were identified based on matching electron impact mass spectra and nine tentatively identified based on matching retention index. The amount of each volatile compound was calculated using the peak areas. The compounds identified were monoterpenes (3-carene, 3-thujene and γ-terpinene), aromatic hydrocarbons (o-cymene, eucalyptol, cumene and o-xylene), an ester (3-methylcyclopentyl acetate), ketones (cyclohexanone and 2,2,5-trimethylhexane-3,4-dione), as well as hexanoic acid. **Table 5.1** lists the peaks identified by GC-MS showing both identified and yet to be identified volatile compounds. The major compounds identified were eucalyptol, 3-thujene, 3-methylcyclopentyl acetate, 3-carene and hexanoic acid based on the average of the peak areas of the four repeat injections. These compound structures are shown in **Figure 5.3**.

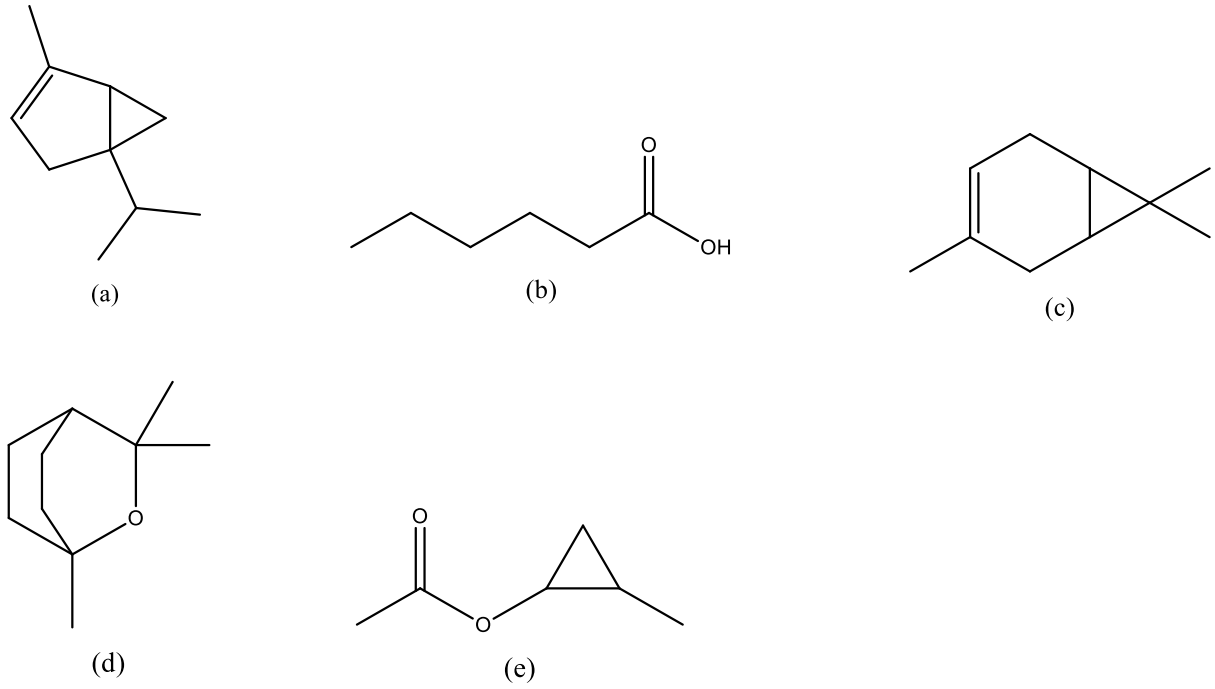


Figure 5.3: Structures of the major compounds identified in the commercially available argan oil, (a) 3-thujene, (b) hexanoic acid, (c) 3-carene, (d) eucalyptol and (e) 3-methylcyclopropyl acetate.

Other volatile compounds eluting at retention time of 6.69, 7.53, 8.59, 9.87 and 10.78 minutes are present in relatively high amount based on their large peak areas. Retention indices were used to tentatively identify the compound at 7.53 minutes as α-pinene and the compound at 9.87 minutes as ethylpyrazine, these compounds have been reported as components of argan oil in the literature.[72]

Table 5.1: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Argan Oil.

Compound	RT (min)	RI	RI_{db}	%	STDEV
	3.8			1.12%	0.20%
	3.93			0.52%	0.12%
	4.22			0.48%	0.04%
	4.37			0.64%	0.13%
	4.58			0.76%	0.04%
	4.75			1.54%	0.16%
2-Nitrobutane	5.26		776	0.81%	0.09%
o-Xylene	5.54		888 ± 8	1.03%	0.13%
Cyclohexanone	5.63		894 ± 3	0.51%	0.08%
3-Methylcyclopentyl acetate	5.75		905	1.10%	0.19%
3-Thujene	6.48	900	929 ± 2	6.22%	0.36%
α - Pinene*	6.69	907	937 ± 3	3.35%	0.26%
	7.53	932		3.98%	1.26%
Cumene	7.59	934	922 ± 9	0.12%	0.02%
3-Methylbutan-1-ol *	7.96	946	943	1.02%	0.15%
2-Hydroxypentan-3-one *	8.09	950	949	1.07%	0.08%
	8.47	962		1.25%	0.18%
	8.59	965		3.40%	1.11%
Hexanoic Acid	8.88	974	990 ± 16	1.08%	0.32%
3-Carene	9.14	982	1010	1.30%	0.08%
	9.45	992		0.77%	0.08%
2,5-Dimethylpyrazine *	9.66	998	999	0.13%	0.05%
o-Cymene	9.75	1001	1022 ± 2	4.54%	0.49%
Ethylpyrazine *	9.87	1004	1005	31.45%	2.62%
Eucalyptol	9.96	1007	1032 ± 2	11.91%	1.38%
2,2,5-trimethylhexane-3,4-dione	10.5	1019	1039	0.18%	0.02%
	10.65	1026		0.24%	0.03%

* Represents compound identified tentatively using retention index

Table 5.1: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Argan Oil (cont'd).

Compounds	RT (min)	**RI**	**RI_{db}**	**%**	**STDEV**
	10.78	1030		1.91%	0.15%
γ-Terpinene	10.91	1033	1060 ± 2	1.14%	0.23%
	10.99	1036		0.54%	0.19%
	11.38	1046		0.30%	0.01%
	11.56	1051		0.30%	0.02%
D-Limonene *	11.87	1060	1063	0.69%	0.05%
1-Octen-3-one *	12.12	1067	1068	0.25%	0.01%
	12.41	1075		1.52%	0.30%
2-Ethyl-5-methylpyrazine *	12.66	1082	1084	7.54%	1.12%
	13.37	1102		0.35%	0.04%
	13.65	1110		0.39%	0.04%
	14.08	1122		0.09%	0.01%
	14.19	1125		0.75%	0.08%
	14.42	1131		0.52%	0.10%
	14.57	1135		0.31%	0.04%
	14.77	1141		0.50%	0.07%
	14.99	1147		0.52%	0.07%
1-Methylpyrrole-2-carbaldehyde *	15.28	1155	1153	0.19%	0.01%
	16.06	1177		0.39%	0.10%
1,3-di-tert-butylbenzene	17.66	1222	1249	0.12%	0.02%
	18.61	1249		0.76%	0.09%
	18.93	1258		0.05%	0.01%
	19.11	1263		0.05%	0.01%
	19.37	1271		0.02%	0.01%
	22.48	1363		0.10%	0.01%
	23.02	1380		0.15%	0.02%
	25.61	1431		0.05%	0.01%

* Represents compound identified tentatively using retention index

Thirty-two volatile compounds were isolated in the pure olive oil, and thirteen were identified based on matching electron impact mass spectra and four were tentatively identified using matching retention indices. The compounds identified were alcohols such as 1-hexyn-3-ol, 2-hexen-1-ol, 2-phenylethanol and 2-methylbicyclo[2.2.1]hept-5-en-2-ol, esters such as methyl benzoate, methyl 3-methylbut-2-enoate and hexyl acetate, the aldehyde heptanal, sesquiterpenes such as sesquithujene and α-farnesene, monoterpenes such as 3-carene and o-cymene. **Table 5.2** lists the peaks identified by GC-MS showing both identified and yet to be identified volatile compounds. The major compounds identified were 2-hexen-1-ol, 1-cyclopropylpropane, hexyl acetate and α-farnesene, based on the average of the peak areas of the four repeat injections.

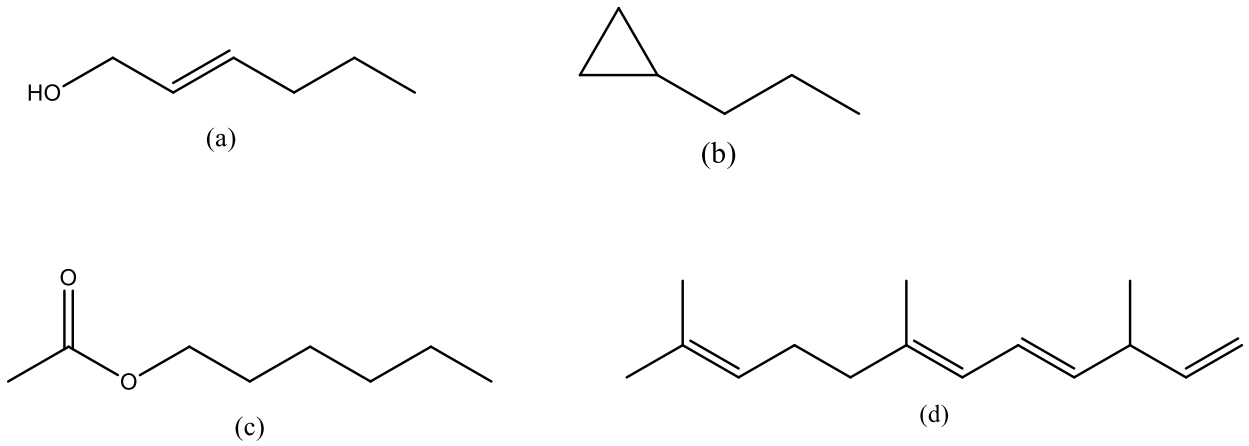


Figure 5.4: Structures of the major compounds identified in the olive oil, (a) 2-hexen-1-ol, (b) 1-cycloproplylpropane, (c) hexyl acetate and (d) α-farnesene.

Unidentified volatile compounds eluting at retention time of 8.75 and 16.2 minutes are present in relatively high percentage based on their large peak areas. The volatile compounds identified by matching electron impact mass spectra have been reported in the literature.[68,69]

Retention indices were used to tentatively identify compounds with retention times 9.09, 9.87, 12.64, and 25.92 minutes as octanal, (Z)-3-hexenyl acetate, nonanal, and trans-α-bergamotene, respectively. These compounds have also been reported as components of olive oil and have been identified using retention indeces in the literature.[73,74,75]

Table 5.2: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Olive Oil.

Compound	RT (min)	RI	RI_{db}	%	STDEV
	3.94			0.27%	0.05%
	4			0.34%	0.10%
Methyl 3-methylbut-2-enoate	4.38		842	0.15%	0.03%
1-Hexyn-3-ol	4.41		813	0.57%	0.10%
2-Hexen-1-ol	4.97		862 ± 5	20.84%	3.66%
1-Cyclopropylpropane	5.06		609	25.98%	4.39%
Heptanal	5.85		901 ± 2	0.40%	0.08%
	6.81	910		6.07%	0.26%
	7.04	917		6.12%	0.40%
	8.75	970		7.16%	1.10%
Octanal *	9.09	981	982	8.32%	0.76%
Hexyl Acetate	9.36	989	1011 $\pm$ 3	2.03%	0.37%
o-Cymene	9.73	1000	1022 $\pm$ 2	0.18%	0.03%
(Z)-3-Hexenyl acetate *	9.87	1005	1004	0.31%	0.05%

* Represents compound identified tentatively using retention index

Table 5.2: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Olive Oil (cont'd).

Compound	RT (min)	RI	RI_{db}	%	STDEV
	10.17	1013		0.45%	0.07%
	10.32	1017		0.43%	0.27%

3-Carene	10.51	1022	1011 ± 2	4.29%	0.47%
	11.54	1051		0.48%	0.02%
Methyl benzoate	12.35	1074	1094 ± 3	0.29%	0.09%
Nonanal *	12.64	1082	1079 ± 5	2.09%	0.24%
	12.91	1089		1.01%	0.08%
2-phenylethanol	13.04	1093	1116 ± 5	0.24%	0.12%
	14.99	1147		0.03%	0.01%
	16.04	1176		0.06%	0.02%
	16.2	1180		7.34%	1.06%
	21.68	1339		0.39%	0.02%
	21.97	1348		2.12%	0.11%
α-Copaene	23.74	1401	1398	0.02%	0.01%
Sesquithujene	23.88	1403	1393	0.07%	0.01%
trans-α-Bergamotene *	25.92	1436	1439	0.22%	0.03%
α-Farnesene	26.13	1440	1508 ± 3	1.60%	0.36%
	26.5	1446		0.05%	0.01%

* Represents compound identified tentatively using retention index

Forty-nine volatile compounds were isolated in the pure sunflower oil; seven were identified based on matching the mass spectra and eight were tentatively identified using retention indices. The compounds identified were 5-methyl-5-nitrohexan-2-one, hexanoic Acid, 2,3,5-trimethylhexane, 1,5-diisopropyl-2,3-dimethylcyclohexane, (3-methylpentan-2-yl)cyclohexane, kaur-15-ene and kaur-16-ene.

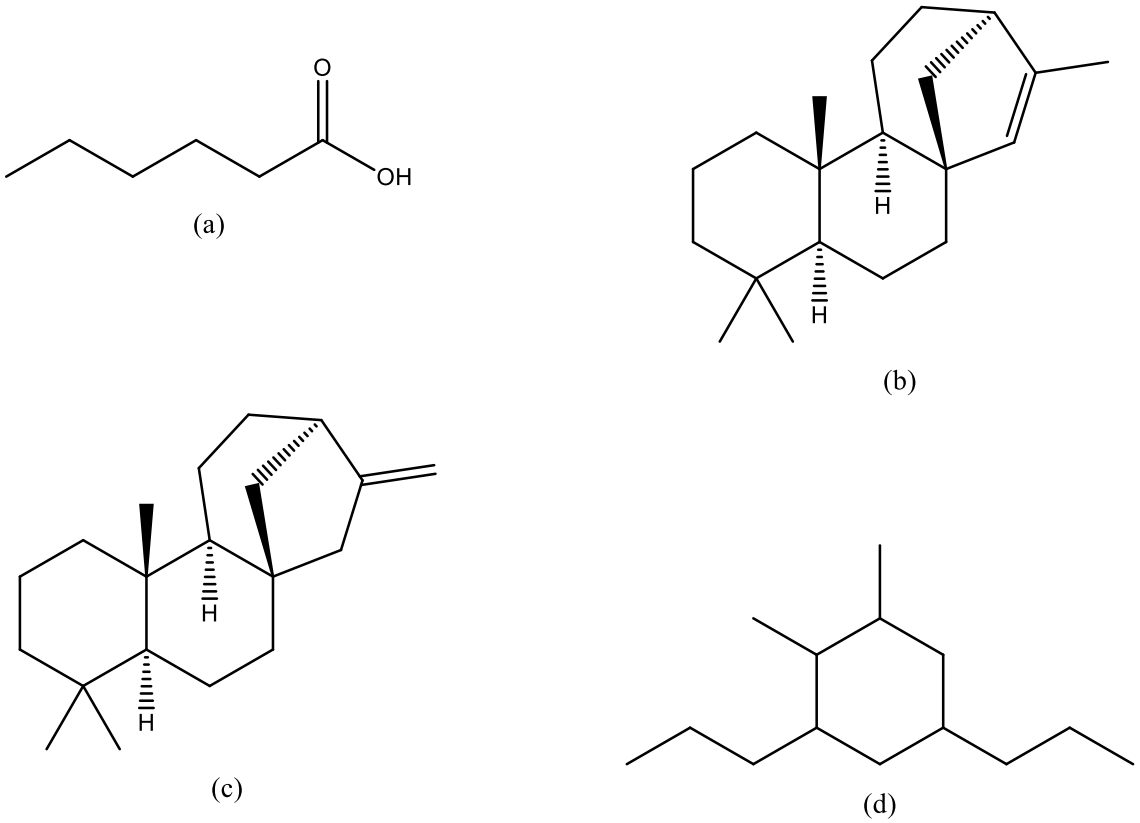


Figure 5.5: Structures of the major compounds identified in the sunflower oil, (a) hexanoic acid, (b) kaur-15-ene, (c) kaur-16-ene and (d) 1,5-dipropyl-2,3-dimethylcyclohexane.

Table 5.3 lists the peaks identified by GCMS showing both identified and yet to be identified volatile compounds. The major compound identified is hexanoic acid, based on the average of the peak areas of the four repeat injections. Unidentified volatile compounds eluting at retention times of 5.56, 6.64, 8.84, 9.55, 10.56 and 11.01 minutes are present in relatively high amounts based on their large peak areas. Retention indices were used to tentatively identify compounds with retention times 7.45, 8.84, 9.1, 9.34, 10.43, 11.11, 11.62, and 12.03 minutes as α - Pinene, 2-Ethyl-3-methylpyrazine, 3(E)-3-methyl-3-nonene, 4-decene, D-limonene, 1,4-Butanediol diacetate, 2-Ethyl-3,5-dimethylpyrazine and 2,4-Dimethyl-1-decene respectively. These compounds have been reported as components of olive oil and have been identified using retention indeces in the literature.[76]

Table 5.3: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Sunflower Oil.

Compound	RT (min)	RI	RI_{db}	%	STDev
	5.07			0.95%	0.12%
	5.2			0.57%	0.08%
	5.49			2.23%	1.20%
	5.56			2.57%	0.87%
	5.69			1.22%	0.15%
Heptanal	5.85		901 ± 2	1.50%	0.99%
	6.09			1.33%	0.07%
	6.39			0.07%	0.02%
	6.64	905		4.48%	0.25%
	6.75	908		0.11%	0.01%
	6.89	913		0.88%	0.07%
α - Pinene*	7.45	930	937 $\pm$ 3	2.58%	0.07%
	7.53	933		0.09%	0.05%
	7.62	935		0.17%	0.02%
5-methyl-5-nitrohexan-2-one	7.75	939	1150	0.15%	0.07%
	8.03	948		4.58%	0.35%
Hexanoic Acid	8.59	965	990 $\pm$ 16	12.01%	2.65%
2-Ethyl-3-methylpyrazine*	8.84	973	1004 $\pm$ 3	5.94%	0.83%
3(E)-3-methyl-3-nonene*	9.1	981	994	2.22%	0.36%
4-Decene*	9.34	989	1001 $\pm$ 4	0.13%	0.01%
	9.44	992		0.35%	0.13%
	9.55	996		4.79%	0.43%
	9.8	1003		2.57%	0.58%
	9.9	1005		9.30%	0.49%
	10.02	1009		1.68%	0.21%
2,3,5-Trimethylhexane	10.23	1015	816 $\pm$ 2	0.36%	0.11%
D-Limonene*	10.43	1020	1031 $\pm$ 1	1.09%	0.15%
	10.57	1024		7.69%	0.68%

* Represents compound identified tentatively using retention index

Table 5.3: Retention time, average area and standard deviation of compounds extracted by HS-SPME/GCMS in pure Sunflower Oil (cont'd).

Compound	RT (min)	RI	RI_{db}	%	STDev
	10.63	1026		1.26%	0.30%
	10.77	1030		1.20%	0.26%
	10.92	1034		0.71%	0.08%
	11.01	1036		5.55%	0.50%
1,4-Butanediol diacetate*	11.11	1039	1181	1.03%	0.20%
	11.38	1047		2.81%	0.32%
	11.5	1050		2.29%	0.25%
2-Ethyl-3,5-dimethylpyrazine*	11.62	1053	1084 ± 3	0.52%	0.10%
	11.75	1057		0.49%	0.11%
	11.83	1059		0.47%	0.19%
	11.93	1062		0.82%	0.18%
2,4-Dimethyl-1-decene*	12.03	1065	1117	0.95%	0.40%
	12.16	1068		0.24%	0.06%
	12.25	1071		1.13%	0.16%
	12.83	1087		1.38%	0.29%
	12.94	1090		2.00%	0.21%
	13.13	1095		0.52%	0.08%
(3-methylpentan-2-yl) cyclohexane	13.32	1101	1149	0.57%	0.11%
	14.35	1129		0.86%	0.20%
	15.72	1167		0.49%	0.13%
	15.99	1175		2.18%	0.51%
Kaur-15-ene	39.28	1955	1989 ± 6	0.51%	0.04%
Kaur-16-ene	40.5	2002	2040 ± 6	0.41%	0.03%

* Represents compound identified tentatively using retention index

5.3.1 Oil Adulteration

Olive oil and sunflower oil were used to adulterate argan oil initially using admixture ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70 and 20:80 (v/v). The goal was to identify adulteration using volatile compounds as marker compound(s) for the detection of the less expensive food oils added to expensive argan oil.

In the HS-SPME/GCMS analysis of adulterations using olive oil, two peaks at retention times of 4.97 minutes (2-Hexen-1-ol) and 5.06 minutes (unknown) appeared as abundant peaks in the HS-SPME analysis of the pure olive oil with peak area percentages of 20.84% and 25.98%, respectively. **Figure 5.6** shows the plot of the average absolute area versus the admixture ratio for both the 2-Hexen-1-ol and the unknown compound peak. 2-Hexen-1-ol was selected as a marker compound because the compound at retention time 5.06 minutes was not observed below the admixture ratio of 80:20 (v/v) argan oil: olive oil while the 2-Hexen-1-ol was observed until the admixture ratio of 95:5 (v/v) argan oil: olive oil. Importantly, 2-Hexen-1-ol is absent in the analysis of the pure argan oil. The 2-Hexen-1-ol peak was not observed with an admixture ratio of 98:2 (v/v) argan oil: olive oil. 2-Hexen-1-ol is more volatile than the unknown compound at retention time of 5.06 minutes which may favor its detection by HS – SPME at lower admixtures.

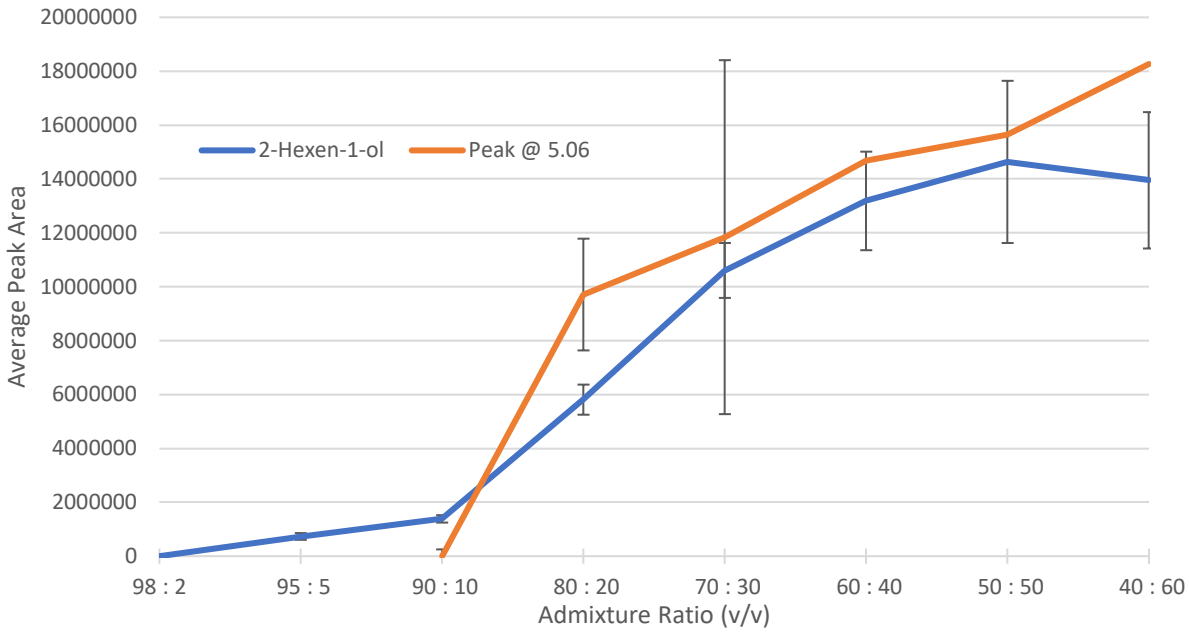


Figure.5.6: Plot showing trend of average absolute peak area of marker compounds in the Argan oil: Olive oil admixture.

In the HS-SPME/GC-MS analysis of adulterations using sunflower oil, unknown peaks at retention times 9.55, 10.57 and 11.01 minutes were identified as potential adulteration markers. These three peaks were prominent in the HS-SPME analysis of the pure sunflower oil with peak area percentages of 4.79%, 7.69% and 5.55%. Other more prominent peaks in the analysis of pure sunflower oil are the peaks eluting at retention times of 6.64, 8.03, 8.59 and 8.85 minutes. However the peaks at retention times of 6.64, 8.03 and 8.59 minutes could not be used as adulteration markers because they are also present in the HS-SPME analysis of the pure argan oil and the peaks at retention times of 8.59 and 8.85 minutes were no longer detected at the admixture ratio of 80:20 v/v argan oil: sunflower oil. The peak eluting at the

retention time of 6.64 can be tentatively identified as ∝-Pinene, but the search of its spectrum in the NIST database gave a low probability below 15%, hence it is yet to be confirmed. ∝-Pinene, a monoterpene compound has been used in previous literature as an adulteration marker for adulterating highly priced poppy seed oil with sunflower oil[70]. However, it cannot be used as a marker for argan oil because it is present in both oils.

Figure 5.7 shows the plot of the average absolute area versus the admixture ratio for all three peaks used as adulteration markers in the admixtures at retention times of 9.55, 10.57 and 11.01 minutes. The three peaks showed an increase in their peak areas as the ratio of adulterant oil increases above an admixture ratio of 90:10 (v/v). At admixture ratio of 95:5 (v/v) of pure argan oil and sunflower oil, all three peaks used as adulteration markers were no longer detected in the chromatogram.

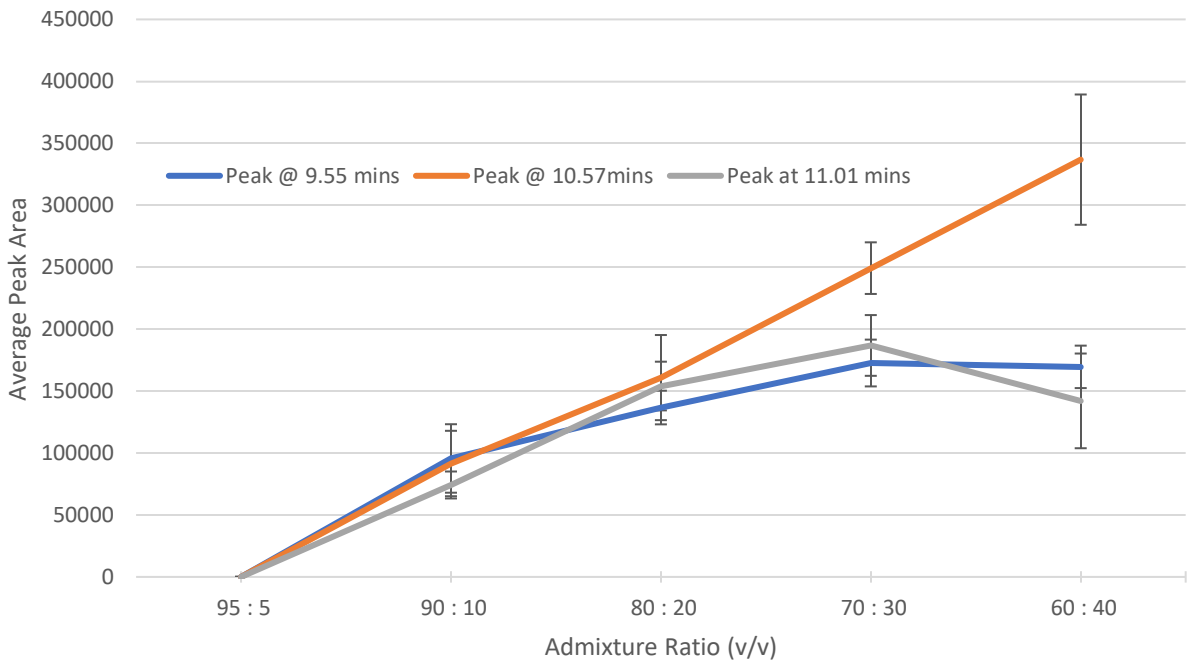


Figure 5.7: Plot showing the trend of the average absolute peak area of the marker compounds in the Argan oil: Sunflower oil admixtures.

From the results of the HS-SPME GCMS analysis of the volatile compounds in the adulterated argan oil using olive oil and sunflower oil, adulteration can be determined with as little as 5% (v/v) of olive oil and 10% (v/v) of sunflower oil. This result compares favorably with literature reports where SPME-MS was used in the detection of adulteration of olive oil using sunflower oil. In these studies sunflower adulteration was detected at 5% (v/v) using 2-heptanone as the adulteration marker.[77] Adulteration of argan oil using a campesterol rich adulterant allowed detection of adulteration at as little as 1% (v/v) and adulteration using a low campesterol adulterant allowed detection of adulteration at 5% (v/v).[53] Although this method was able to detect adulteration at s low as 1% (v/v) adulterant level, it would not be a suitable method in detecting adulteration using vegetable oils, such as olive oil and hazelnut oil, that also have similar campesterol composition as argan oil. The electronic nose and voltametric electronic tongue method for detection of adulteration of argan oil were able to detect up to 10% (v/v) of the adulterant oil using sunflower oil,[62] similar to the results of the HS-SPME/GCMS method contained herein.

CHAPTER SIX

CONCLUSION AND FUTURE WORK

In conclusion, in the GCMS evaluation of the phenolic compounds in the authentic argan oil from Morocco, twenty-eight compounds comprising of phenols, fatty acids, and sterols were identified and are in agreement with literature reported components of argan oil. A novel tocomonoenol was also tentatively identified based on fragmentation observed in the electron impact mass spectrum. Fatty acid contamination remains a significant challenge and characterization of the phenolic compounds would benefit from additional efforts at fatty acid removal. Optimizing the hexane washing step in the sample preparation to further remove traces of glycerides, reintroducing the freezing step after dissolving the residue with acetonitrile might be helpful to further remove residual oil and introducing a deacidification step into the sample preparation method might be able to solve this issue.

Evaluation of the triacylglycerol (TAG) content of the authentic argan oil using LCMS showed the characteristic hand-shape profile of the triacylglycerol of argan oil as reported in literature. This can be improved further by finding ways to quantitatively analyze the triacylglycerols of the argan oil. Gas chromatography has been used in quantifying TAGs and gives information on the fatty acid components of the oil.

The comparison between the authentic argan oil from Morocco and the commercially available argan oil showed that the commercially available argan oil used was a viable alternative to the authentic argan oil from Morocco. The GCMS analysis showed that both oils contained no major deviations in phenolics, fatty acids, and sterol content. Both oils had 29 identical peaks and 22 of those peaks were identified as compounds reported in literature as

components of argan oil. Variation in the percentages of the compounds present was expected as nothing is known regarding the method of collection of the argan oil used in the commercial product.

The HS-SPME GCMS analysis allowed for identification of adulteration markers in argan oil using olive oil and sunflower oil as adulterant oils. 2-Hexen-1-ol was reported as the adulteration marker to detect the adulteration of argan oil with olive oil. The peak of 2-Hexen-1-ol at a retention time of 4.9 minutes, was the main peak in the HS-SPME GCMS analysis of olive oil with 20.84% of the total peak area and was present in the oil admixture until it disappeared at 98:2 (v/v) admixture ratio. This shows that adulteration of argan oil with olive oil can be detected using this HS-SPME GCMS up to 5% (v/v) of adulterant olive oil. In the studies using sunflower oil as the adulterant the peaks in the HS-SPME GCMS chromatogram at retention times of 9.55, 10.57 and 11.01 minutes of yet to be identified compounds with 4.79%, 7.69% and 5.55% of the total peaks area are found to be the best adulteration markers to detect adulteration of argan oil with sunflower oil. These peaks were present in the oil admixture up to 90:10 (v/v) ratio. The HS-SPME GCMS method of detecting oil adulteration has the advantages of elimination of sample preparation and versatility over other methods such as using an electronic nose or a voltametric electronic tongue, both of which involve designing sensors and a method for identifying patterns.

As a future work, the HS-SPME GCMS method could be used to characterize argan oil from different regions and as obtained using different extraction methods because it has been reported that origin and extraction method plays a role in the components of the oil. Furthermore, the data collected could be examined using multivariate analysis to reveal

heretofore unseen patterns in observed compounds. Additional cheaper vegetable oils could also be examined as potential adulterants.

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