



Fatty acids detection in dry seed oil of Citrus by utilizing hexane as extracting as solvents through gas chromatography mass spectrometry

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Abstract

Citrus limon (L.) contained 3.5-5.7 % seed oil and oils were extracted and analysed quantitatively through Gas Chromatography (GC-MS) technique composed of more than 16 compounds presence available in dryseed oils. Citrus seeds showed fourteen fatty acids, two steroid derivatives and one acid compounds were found total seventeen compounds were detected with 29.36 % area covered by linoleic followed by Hexadecenoic acid i.e. 20.64 % while oleic acids showed 17.51 ± 1.07 %. The composition indicated that more than 80% showed fatty acids and nearly 15% showed steroids compounds a. Out of these, unsaturated fatty acids composed of 55.21% while 28.1% saturated fatty acids. It showed almost double polyunsaturated fatty acids in lemon seed oils. It is acidic in nature because of 1,2-Benzenedicarboxylic acid with a greater number of fatty acids in nature.

Keywords: Fatty acids, *Citrus limon*, seed oil, gas chromatography mass spectrometry (GC-MS), fatty acid composition

Introduction

Citrus fruit contains vitamins, minerals, and antioxidants that help protect against heart disease and support your immune system. These fruits are also mostly water, which can help keep you hydrated as well as full of antioxidants. But seeds are discarded or wasteful products. Many literatures showed healthier oil (Adeyeye and Adesina, 2015.)^[1] Recently, there has been a great deal of attention on usage, byproducts, and wastes of the food industry. There have been many studies on the properties of citrus seeds and extracted oil (Reazai *et al.* 2014)^[6] Detection of fatty acids in dry seed oil of citrus using hexane as a solvent through gas chromatography-mass spectrometry (GC-MS) is significant for many reasons but one of them is to determines the quality triglycerides in dry seeds. Additionally, it supports research into the potential therapeutic properties of citrus seed oils there by using in functional foods and natural products. As the many important compounds were detected in seeds of lemon or citrus fruits. The experiment was aim to assess the valorisation of discarded seeds of citrus after juice extracts. The hypothesis based on utilization of this seed extract as important bioactive compounds for human health as well as to identify and quantify the beneficial fatty acids, which are crucial for nutritional and health applications. This study uses hexane as a solvent to extract fatty acids, which are subsequently evaluated using gas chromatography-mass spectrometry (GC-MS). Hexane is often used owing to its effectiveness in dissolving non-polar substances, which renders it useful for extracting lipophilic chemicals such as fatty acids. GC-MS is a sensitive and exact technology for detecting and measuring these substances while allowing understanding into the oil's composition. The results show an opportunity to boost the utilization of citrus seed oils as

ingredients in food, cosmetics, and biofuels, so helping to increasingly ecological agro-industrial processes.

Materials and Methods

Oil Extraction

Citrus seeds waste collected through extracted juice through mechanical press and collected and dried in sun lights in Petri plates. Collect the seeds, crushed with mortar pastel with coarse powder. The thimble was filled with the one to three grams of seed coarse material and performs simple oil extraction in Soxhlet apparatus. Oil extracted through hexane with 70°C for 4 hours. Extracted hexane with oils puts in small Petri plates and evaporates the hexane through hot air oven for over nights below 55°C. Weights the oils % with both extracted oils weights as well as removed from weights of thimble too.

GCMS conditions

Extracted oils was used for derivatives and make methyl ester and applied in autosampler tubes. GC-MS analyses were performed using a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). The chromatographic runs were started as per the following details conditions [GC-2010]. The GCMS conditions as per table indicated: ionization mode, electron ionization (EI) mode; ionization current, 60 μA; ionization voltage, 70 eV. Each sample was analysed. Eluted compounds were identified using the NIST (<http://www.nist.gov/srd/nist1a.cfm>), the Wiley NBS, the mass spectral data as well as used pubmed and other data sources for detection.

Fatty acids and compounds were used for google search, NIST library for the potential application for food and industrial application.

Instruments protocol for analysis of GCMS-FAME

Column Oven Temp.	40.0 °C	MS table: Start Time :3.50min, End Time :54.67min, ACQ Mode: Scan, Event Time :0.50sec, Scan Speed 1428, Start m/z :50.00, End m/z :700.00, Sample Inlet Unit: GC " [GCMS-QP2010 Plus], Ion Source Temp :200.00 °C, Interface Temp.:290.00 °C, Solvent Cut Time :2.00 min, Detector Gain Mode: Relative Detector Gain: +0.00 kV, Threshold 100"
Injection Temp.	:250.00 °C	
Injection Mode	: Split	
Flow Control Mode	: Pressure	
Pressure	:49.5 kPa	
Total Flow	:54.0 mL/min	
Column Flow	:1.00 mL/min	
Linear Velocity	:36.1 cm/sec	
Purge Flow	:3.0 mL/min	
Split Ratio	:50.0	
High Pressure Injection	: ON	
High Press. Inj. Pressure	:250.0 kPa	
High Press. Inj. Time	:1.00 min	
Carrier Gas Saver	: OFF	
Splitter Hold	: OFF	
Oven Temp. Program		
Rate	Temperature(°C)	Hold Time(min)
-	40.0	3.00
6.00	290.0	10.00

Results and discussions

The Fatty acids profiling of oils extracted from dried citrus seed oils through GC MS showed the seventeen compounds. The peak area distribution and compound description are presented in table.1. Total seventeen peak were identified and Area %, RT (minute) are given in Chromatographic presentation of FAME detection in seed oil of citrus. The results showed Citrus seeds showed fourteen fatty acids, two steroids' derivatives and one acid compounds were found total seventeen compounds were detected with 29.36 % area covered by linoleic followed by Hexadecenoic acid i.e 20.64 % while oleic acids showed 17.51 ± 1.07 %. Similar findings also obtained by park *et al* (2021) [5]. Oleic and linoleic acids consisted of principal fatty acids, which were significantly higher in oil citrus seed. As shown in table 2. The calculated fatty acids class indicated that unsaturated fatty acids composed of 55.21% while 28.1% saturated fatty acids. The composition indicated that more than 80% showed fatty acids and nearly 15% showed steroids compounds a. Out of these, unsaturated fatty acids composed of 55.21% while 28.1% saturated fatty acids. It showed almost double polyunsaturated fatty acids in lemon

seed oils. It is acidic in nature because of 1,2-Benzenedicarboxylic acid with a greater number of fatty acids in nature. These results are in line with Ijeoma and Christian (2020) [3] who study on lemon essential oil metabolites. As peak no. 14 showed the plant sterol and first time reported in these seeds. The steroid compound of cholesten reported for Antimicrobial, Diuretic, Anti-inflammatory, anti-asthmain acetone extract of *Cenchrusse tigrus*. Iqbal *et al* (2021) [4]. 26-Nor-5-cholesten-3. beta. -ol-25-one is a steroid compound that belongs to the class of steroids known as sterols. It is derived from cholesterol, which is a fundamental molecule in biological systems and involved in cell membrane structure, signalling pathways, and as precursors to hormones such as corticosteroids and sex hormones. Peak no 14 and 15 never presence in plans but similar structure can be possible in plants. Of course, it can be found in infected with fungal organism. (Ridho *et al*.2020, Asmaa *et al*. 2021) [2, 7] So, it is possible that the extracts may come from infected lemon seed extracts. Or it needs to reanalysed several times. But this study showed the peaks of sterols compounds of this saponin derivatives too.

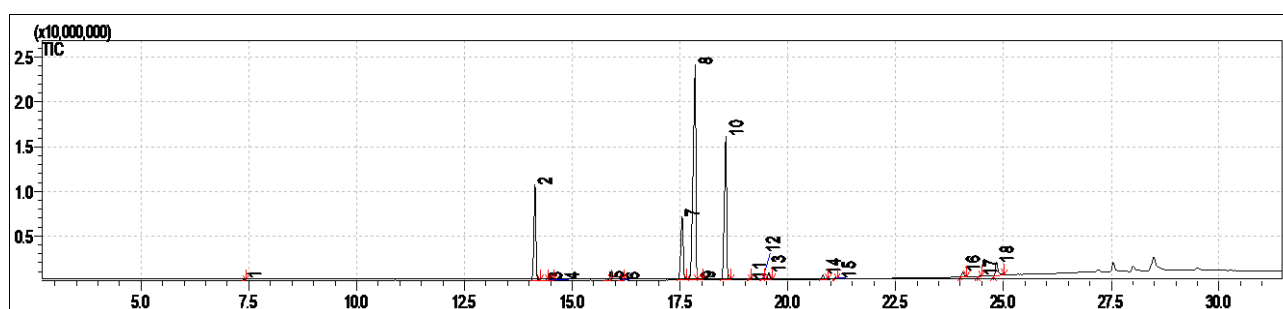


Fig 1: Chromatographic presentation of FAME detection in seed oil of citrus

Table 1: Compound identified in oils extracted from seeds of citrus and analysed through GC MS-FID

Peak No	RT (min)	Area (%)	Compound Identified	
1	7.377	0.35	Naphthalene	Plant hormones derivatives
2	14.146	20.64	Hexadecanoic acid, methyl ester	FA
3	14.501	0.37	9-Hexadecenoic acid, methyl ester, (Z)-	FA
4	15.247	0.19	Hexadecanoic acid, 14-methyl-, methyl ester	FA
5	17.515	3.78	Octadecanoic acid, methyl ester	FA
6	17.805	17.51	9-Octadecenoic acid (Z)-, methyl ester	FA

7	17.909	1.07	9-Octadecenoic acid (Z)-, methyl ester	FA
8	18.56	29.36	9,12-Octadecadienoic acid, methyl ester	FA
9	19.102	0.11	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	-
10	19.565	6.45	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	FA
11	20.813	0.26	Eicosanoic acid, methyl ester	FA
12	24.063	0.7	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	FA
13	24.838	1.7	n-Hexadecanoic acid	FA
14	25.961	4.8	26-Nor-5-cholesten-3. beta. -ol-25-one	Steroids
15	26.094	10.92	Cholestane-3,5-diol, 5-acetate, (3. beta.,5. alpha.)-	Steroids
16	27.545	0.96	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	FA
17	28.004	0.83	Octadecanoic acid (Stearic acids)	FA

Table 2: Bioactive compounds class and their Area % in seed oils of citrus spp.

Bioactive compound name	Area %	Sub Class	Standardized name	Formula	Exact mass
Naphthalene	0.35	Hormones derivatives	Naphthalene	C ₁₀ H ₈	128.0626
Hexadecanoic acid	20.64	SFA	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.2402
9-Hexadecenoic acid	0.37	UnFA	Palmitoleic	C ₁₆ H ₃₀ O ₂	254.411
Hexadecanoic acid	0.19	SFA	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.2402
Octadecanoic acid	3.78	SFA	Stearic acid	C ₁₈ H ₃₆ O ₂	284.2715
9-Octadecenoic acid	17.51	UnFA	Elaidic acid	C ₁₈ H ₃₄ O ₂	282.2559
9-Octadecenoic acid	1.07	UnFA	Elaidic acid	C ₁₈ H ₃₄ O ₂	282.2559
9,12-Octadecadienoic acid	29.36	UnFA	Linoleic acid	C ₁₈ H ₃₂ O ₂	280.2402
1,2-Benzenedicarboxylic acid	0.11	Acid	Phthalic acid	C ₈ H ₆ O ₄	166.0266
9,12,15-Octadecatrienoic acid	6.45	UnFA	A-linoleic	C ₁₈ H ₃₀ O ₂	278.4
Eicosanoic acid	0.26	SFA	Arachidic acid	C ₂₀ H ₄₀ O ₂	312.3028
Hexadecanoic acid	0.7	SFA	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.2402
n-Hexadecanoic acid	1.7	SFA	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.2402
26-Nor-5-cholesten-3.beta.-ol-25-one	4.8	Sterol	Steroids	C ₂₆ H ₄₂ O ₂	386.3184
Cholestane-3,5-diol, 5-acetate, (3.beta.,5.alpha.)-	10.92	Sterol	Steroids	C ₂₉ H ₅₀ O ₃	446.3759
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	0.96	UnFA	Oleic acid triglyceride	C ₅₇ H ₁₀₄ O ₆	885.453
Octadecanoic acid	0.83	SFA	Stearic acid	C ₁₈ H ₃₆ O ₂	284.2715

Table 3: Applications of detected compounds in various fields such as pharmaceuticals, food industries and materials science

Compound name	Uses
Naphthalene	Naphthalene is an aromatic hydrocarbon commonly used in the production of mothballs and as a precursor to other chemicals. It serves as a solvent in chemical reactions and is utilized in the synthesis of phthalic anhydride, which is important for producing plastics and resins
Hexadecanoic Acid (Palmitic Acid)	saturated fatty acid found in palm oil and animal fats. & widely used in the cosmetic industry for its emollient properties & also used as a thickening agent in creams and lotions. It also serves as a surfactant in detergents and can be used in the production of biodiesel.
9-Hexadecenoic Acid (Palmitoleic Acid)	MUFA has applications in nutrition due to its potential health benefits, including anti-inflammatory properties. & also used in cosmetics for skin conditioning and moisturizing formulations
Octadecanoic Acid (Stearic Acid)	use in the production of soaps, cosmetics, and candles due to its emulsifying properties. It also acts as a lubricant
9-Octadecenoic Acid (Oleic Acid)	MUFA use as an emulsifier in food products, & dietary supplements for cardiovascular health benefits
6. 9,12-Octadecadienoic Acid (Linoleic Acid)	EFA plays significant roles in cell membrane structure and function and is often included in dietary supplements
9,12,15-Octadecatrienoic Acid (Alpha-Linolenic Acid)	Omega-3 fatty acid has role in heart health and anti-inflammatory effects
Eicosanoic Acid (Arachidic Acid)	can be used as a surfactant or emulsifier in cosmetic formulations due to its ability to stabilize mixtures.
Cholestane-3,5-diol, 5-acetate (Cholesterol Derivative)	pharmaceutical research & cell membranes or potential therapeutic uses.
26-Nor-5-cholesten-3. beta. -ol-25-one	Applications related to steroid hormone synthesis or research into cholesterol metabolism due to its structural similarities with cholesterol
1,2-Benzenedicarboxylic Acid (Phthalic Acid)	Phthalic acid is primarily used to produce plasticizers
9-Octadecenoic Acid, 1,2,3-Propanetriyl Ester (Glycerol Trioleate)	triglyceride form of oleic acid finds extensive use within the food industry as cooking oil or fat substitute

Conclusions

The Fatty acids profiling of oils extracted from dried citrus seed oils through GC MS showed the seventeen compounds and these oils also composed of Naphthalene, Palmitic acid,

Palmitoleic, Oleic acid triglyceride, Stearic acid, Elaidic acid, Linoleic Phthalic acid, α -linoleic acids, Arachidic acid and Steroidal compounds too in varying proportion. It has many applications in foods and pharmaceuticals industries.

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