International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 14-11-2024, Accepted: 15-12-2024, Published: 30-12-2024
Volume 8, Issue 2, 2024, Page No. 81-84

Fatty acid profiling through GCMS-FID from custard apple seeds oil for non-edible purpose

PJ Rathod[1], S K Bhuva[2], HP Gajera[3]
[1] Associate Professor, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India
[2] Assistant Professor, Department of Horticulture, College of Horticulture, Junagadh Agricultural University, Junagadh, Gujarat, India
[3] Professor and Head, Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

Abstract
Seed kernels oil of *Annona squamosa* Linn. were extracted through Soxhlet's and gas chromatography–mass spectrometry (GC/MS) applied to determines FAME presence qualitatively in seed oils. Extracted oils were ranges between 17.5 to 23.2% of crude oil on a dry weight basis from local variety. Eighteen peaks detected with fourteen compounds of fatty acids and their derivatives with phenolic acids too. These were constituting 100 % of the oil, The major components in sitafal (Custard apple) seeds oil are six fatty acids which were more than 1% and remaining are in below 1% were 12 compounds. Out of them Area % in chromatograms showed oleic acid (44.4%) with RT of 16.127, 17.849 and 16.129, linoleic acid (24.4%), alpha linoleic acids 1.0%, Palmitate (18.12%) at RT of 14.136 and 24.836, 24.057. Literature searched from each fatty acids with AI tools and search engines with experimentally confirmed potential role of these bye product of custard apple in industrial use too.

Keywords: *Annona squamosa* Linn, seed kernels oil, soxhlet extraction, Gas chromatography–mass spectrometry (GC/MS), crude oil yield

Introduction
Custard apple seeds (*Annona squamosa*) is already recognized for the health benefits, as well as functional foods, but their lipid content and composition has been poorly examined. Fatty acid profiling was one of the strategies to use lipid structure in a variety of bye products. Though there are various sources for obtaining fatty acids and oils, but custard apple seed which is routinely treated as waste products in the fruit industry So this will appears to be a potential source worth investigating oil analysis. This research, intended to use for assessment of fatty acid in custard apple seed oil with the aid of GC-MS-FID instruments. Custard apple in most cases is regarded as waste as its seeds are not much costly for food use, the oil derived from these seeds may find applications beyond food, such as in the industrial, cosmetic, or biofuels. Therefore, the objective behind this study was to provide more insight into the diversified fatty acid profile of custard apple seed oil focusing on its potential applications above-mentioned and enhancing green handling of agricultural by-products as well as broadening their potential applications.

Materials and methods
Custard apple seeds were freshly collected from after edible pulp remove from each locules or areola. Fresh seeds were cleaned and dried in oven for 3 hrs at below 45^0C. Then weigh the 3 grams of raw crushed put in to thimble for Soxhlet extraction. After obtaining oils, the oils were subjected to FAME formation and then utilized and injected in GCMS-FID for fatty acids profiling. The protocol of FTL were used for analysis. The peak analysis was match with the preserved standard and measured the area by tick marks with red colour. The data were taken in both pdf and CSV format. The chromatogram compared with the data obtained peak also. Each Fatty acid synonymous were searched in google and other NIST library. The obtained data were subjected to search for industrial use for particular fatty acids**.**

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

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,
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	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"
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

Extracted seed oils compose of 17.5 to 23.2% on dry weight basis. The replicated data were changed in composition because of seed size variation in same fruits. The analysed through GCMS-FID chromatogram depicted in figure 1 and peak number with area % of each fatty acids are presented in table.1. The results showed that the major variation in the flow of detection in fatty acids of custard apple seeds. This data showed eighteen peaks with detection standard with RT of 7.377 to 24.832 min. Means run were completed in within 30 minutes. Fatty acids of *Annona squamosa* (Sitaphal) seed kernels were determined through GC MS. fourteen fatty acids compounds were reported. The peak of eighteen compound were detected but due to poor detection RT of certain fatty acids showed three times or more may be differentially expressed in seed size. GC-MS analysis results showed that the main chemical compositions of fatty acid of hexane extracted solvents and methyl ester peak were identified at 6,8,12 and 15. The maximum content of oleic acids was found in seeds that was oleic acid (44.4%) with RT of 16.127, 17.849 and 16.129, linoleic acid (24.4%), alpha linoleic acids 1.0%, Palmitate (18.12%) at RT of 14.136 and 24.836, 24.057. These results are in agreement with slight variation with Dalia *et al.* (2019) [2] reported dominant fatty acids of custard apple seed kernel oil were oleic (49.75%), Linoleic (22.50%), palmitic (15.06%) and stearic and (4.63%). The oil could be classified as a semi-dry oil. Total lipid fractions consisted mainly of nine classes in which triacylglycerols were the major class. In table 1 Peak no showed 11-Octadecenoic acid, methyl ester also known as trans-Vaccenic acid had not found in any study though it was confirmed two times in seeds of custard aplle in these experiments.

At peak no. 17 Fatty acids containing a cyclopropane ring in their structure (cyclopropane FA) have been found in a wide variety of bacteria, a number of protozoa, and Myriapoda. (Sledzinski *et al*., 2013). [3] Little is known about cyclopropane FA in especially in plant tissues. The present study deals with the identification of cyclopropane. FA may be due to poor seed quality or infected with bacteria may cause such identification. If it is true then it is discovery of compounds can possible. Author is not sure about this compound as sited in many literatures. This results are in agreements with GC-MS analysis of. (Chen *et al.*, 2016) [1] They showed that the main chemical compositions of fatty acid of ASO were palmitic acid (9.92%), linoleic acid (20.49%), oleic acid (56.50%) and stearic acid (9.14%). Their results demonstrated that ASO suppressed the H22 solid tumor development may due to its main chemical constituents unsaturated fatty acid and ACGs via IL-6/Jak/Stat3 pathway. ASO may be a potential candidate for the treatment of cancer. Literature searched from each fatty acids with AI tools and search engines with experimentally confirmed potential role of these bye product of custard apple in industrial use too. The finding suggested that these fatty acids can also use for various purposes (Table.2)

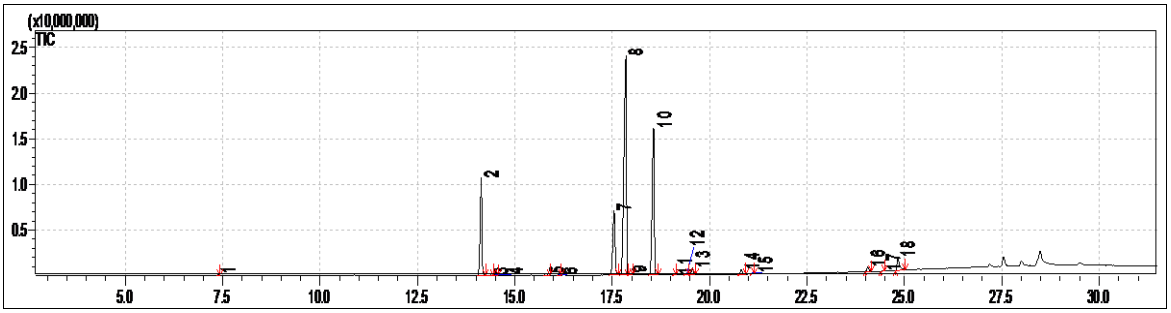

Fig 1: Total ion current GCMS chromatogram through fatty acids methyl ester of *Annona squamosa* Linn seed oils

Table 1: Qualitative analysis of FAME of Custard apple seed oils

Peak No	RT (min)	Area (%)	Class/Synonymous	Compound Identified
1	7.377	0.16	Plant hormones derivatives	Naphthalene
2	14.136	14.07	Saturated fatty acids (Palmitate)	Hexadecanoic acid, methyl ester
3	14.383	0.07	aldehydic type odor	UNDECANAL DB5-1156 (=HENDECANAL
4	14.499	0.15	Fatty acids (B)	7-Hexadecenoic acid, methyl ester, (Z)-
5	15.801	0.26	Margaric acid	Heptadecanoic acid, methyl ester
6	16.129	0.07	Oleic acids	9-Octadecenoic acid (Z)-, methyl ester
7	17.547	11.38	Saturated fatty acids	Octadecanoic acid, methyl ester
8	17.849	43.72	Oleic acids	9-Octadecenoic acid (Z)-, methyl ester
9	17.925	0.22	trans-Vaccenic acid	11-Octadecenoic acid, methyl ester

10	18.562	24.4	Linoleic acid	9,12-Octadecadienoic acid, methyl ester
11	19.104	0.07	Phenolic acids (C6-C1)	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
12	19.413	0.1	Oleic acids	9-Octadecenoic acid (Z)-, methyl ester
13	19.557	1	α-linolenic acid	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
14	20.814	0.74	arachidic acid	Eicosanoic acid, methyl ester
15	21.09	0.12	Oleic acids	9-Octadecenoic acid (Z)-, methyl ester
16	24.057	0.75	isomers of diacylglycerols (Palmitate)	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester
17	24.445	0.06	cyclopropane FA	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester
18	24.832	2.66	Palmitate	n-Hexadecanoic acid

Table 2: Potential and recognized application of custard apple seed oils

Naphthalene	used in the production of phthalic anhydride, including dyes, plasticizers, and resins. used in mothballs and as a solvent in various chemical applications.
Hexadecanoic Acid, Methyl Ester (also known as Methyl Palmitate):	Used in the production of biodiesel, as a lubricant, and in the formulation of various personal care products such as soaps and cosmetics. It's also used in the manufacture of surfactants and emulsifiers.
7-Hexadecenoic Acid, Methyl Ester (Z)-:	Utilized in the synthesis of flavors and fragrances, particularly in perfumes and food additives. It can also be used as a starting material for other chemical syntheses.
Heptadecanoic Acid, Methyl Ester:	used in the production of specialty chemicals, particularly in the synthesis of surfactants and lubricants. It is also used in the formulation of certain types of biodiesels.
9-Octadecenoic Acid (Z)-, Methyl Ester (also known as Methyl Oleate):	Used in the manufacture of surfactants and as an additive in industrial lubricants and coatings. It is also used in the production of certain biodiesels and as a flavouring agent.
11-Octadecenoic Acid, Methyl Ester (also known as Methyl Eicosenoate):	Used in the production of soaps, cosmetics, and as an emulsifier in various industrial processes. It also serves as a lubricant and a release agent in the manufacturing of rubber and plastics.
9,12-Octadecadienoic Acid, Methyl Ester (also known as Methyl Linoleate):	Used primarily as a biodiesel component, in surfactants, and as a lubricant. It is also used in the cosmetic industry and in the manufacture of plasticizers.
1,2-Benzenedicarboxylic Acid, Bis(2-methylpropyl) Ester (also known as Iso-Nonyl Phthalate):	Utilized in the production of biodiesel, and as an intermediate in the synthesis of surfactants and lubricants. It can also be used in various personal care products.
9,12,15-Octadecatrienoic Acid, Methyl Ester (Z, Z, Z)- (also known as Methyl Linolenate):	used in the production of biodiesel and as a component in various surfactants and emulsifiers. It is also used in the manufacture of plasticizers and in cosmetic formulations.
Eicosanoic Acid, Methyl Ester (also known as Methyl Behenate):	used as a plasticizer in the production of flexible PVC and other polymeric materials. It enhances the flexibility, durability, and workability of plastics.
Hexadecanoic Acid, 2-hydroxy-1,3-propanediyl Ester (also known as Methyl 2-hydroxyhexadecanoate):	Used in the production of biodiesel, as well as in the formulation of certain pharmaceuticals, nutritional supplements. Raw material in the cosmetic industry.
Cyclopropaneoctanoic Acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, Methyl Ester:	Utilized in the manufacture of surfactants, waxes, and as an ingredient in various personal care and cosmetic products. It is also used in the production of certain types of biodiesels.
UNDECANAL (also known as Hendecanal):	Used in the formulation of personal care products, particularly in cosmetic creams and lotions. It also finds applications in the production of specialty surfactants and as a lubricant.
Octadecanoic Acid, Methyl Ester (also known as Methyl Stearate):	is likely used in specialized chemical synthesis applications, particularly in the development of high-performance materials and pharmaceuticals due to its unique cyclopropane structure.

Conclusion: Seed kernels oil of *Annona squamosa* Linn. (custard apple) was extracted using Soxhlet's method, and the presence of fatty acid methyl esters (FAME) was qualitatively analyzed using gas chromatography-mass spectrometry (GC/MS). The extraction yielded crude oil ranging from 17.5% to 23.2% on a dry weight basis for local varieties. GC/MS analysis identified eighteen peaks corresponding to fourteen fatty acids and their derivatives, along with phenolic acids, collectively constituting 100% of the oil. Major components included oleic acid (44.4%), linoleic acid (24.4%), alpha-linolenic acid (1.0%), and palmitic acid (18.12%). The retention times (RT) of these compounds were 16.127, 17.849, 14.136, and 24.057, respectively. Literature reviews and AI tool searches confirm that these fatty acids and by-products from custard apple seeds have potential industrial applications.

The custard apple seed oil contains significant amounts of oleic, linoleic, and palmitic acids, making it a valuable source of fatty acids with potential industrial uses, particularly in sectors requiring high-quality oils and derivatives. these can be used in industrial, cosmetic, or research contexts rather than direct therapeutic applications.

References:

1. Chen Y, Chen Y, Shi Y, Ma C, Wang X, Li Y, *et al*. Antitumor activity of *Annona squamosa* seed oil. *J Ethnopharmacol*,2016:4(12):193:362-367.
2. Dalia Eshra H, Attia R Shehata, Abdel-Nabey A. Ahmed, Jehan I. Saber. Physicochemical Properties of the Seed Kernels and the Oil of Custard Apple (*Annona squamosa* L.). *International Journal of Food Science and Biotechnology*,2019:4(4):87-93.
3. Sledzinski T, Mika A, Stepnowski P. Monika Proczko-Markuszewska, Lukasz Kaska, Tomasz Stefaniak & Julian Swierczynski. Identification of Cyclopropaneoctanoic Acid 2-Hexyl in Human Adipose *Tissue and Serum. Lipids*,2013:48:839–848
4. Ozusaglam MA, *et al*.: J. Pure App. Mircro,2013:7:1567
5. Virendra S Rana. Fatty Oil and Fatty Acid Composition of *Annona squamosa* Linn. Seed Kernels, *International Journal of Fruit Science*,2015:15(1):79-84