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Analysis of fatty acids of the oil from unripe fruit of *Musa paradisiaca* Linn.

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ABSTRACT

The unripe fruits of Musa paradisiaca were de-oiled with n-hexane and the resulting oil was analyzed for its physico-chemical properties. The oil content was estimated as 5.01 g/Kg. Fatty acid composition of the fruit oil was evaluated in this study. The acid value (9.83 ± 0.06) and saponification value (58.70 ± 0.12) were estimated to assess the quality of the oil. Twenty fatty acids including one oxo-fatty acid were identified by Gas-liquid chromatography followed by GC-MS. Saturated fatty acids were present in greater amounts than unsaturated fatty acids. Most predominating saturated and unsaturated fatty acids were Palmitic acid (62.87 ± 0.12%) and Oleic acid (12.38 ± 0.03%) respectively.

Key words: *Musa paradisiaca*, fruit oil composition, GC-MS, palmitic acid, oleic acid

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INTRODUCTION

Endeavour has been taken as a matter of studies on the oils from the seeds and fruits of various plants during the last few decades for their nutritional, industrial and pharmaceutical importances[1-6]. The viability of the fruit/seed oil even the seeds or fruits from which oils were extracted for nutritional purpose can be determined to a great extent by its fatty acid composition. The oil from unripe fruit of a well known plant *Musa paradisiaca* belonging to a small family 'Musaceae', cultivated throughout India has been chosen for this study. The unripe fruit of this plant is extremely used as kitchen vegetables almost in every house of West Bengal, India and also used as a home remedy for intestinal disorders, uremia, nephritis and many other vascular diseases[7]. The unripe fruits are also useful in diabetes[8,9].

In this communication, this is the first time to determine the fatty acid composition of the oil of unripe fruits of *M. paradisiaca* and also to determine whether the composition is greatly responsible for the nutritional capacity of the unripe fruit oil as well as the fruit itself.

EXPERIMENTAL SECTION

Plant material and reagents:
Fresh unripe fruits of *Musa paradisiaca* were collected from the local market at Burdwan, West Bengal, India in December '2014' and authenticated by Prof. A. Mukherjee, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India. A voucher specimen (Debabrata 204) has been deposited at the herbarium of the Botany Department under the University of Burdwan bearing acronym BURD.

Standard methyl esters of fatty acids (FAME) – a mixture of 37 components FAME was purchased from Supelco., USA. All other reagents were of analytical grades and purchased from Sigma Chemical Co. (USA), except n-hexane, chloroform and ethyl acetate which were procured from Merck (India).

Isolation of oil from the unripe fruits of M. paradisiaca:
Fresh unripe fruits of *M. paradisiaca* were chopped into small pieces, dried in air and then crushed into powdered form by manual crusher. The oil was extracted with n-hexane in a soxhlet for 72 hours and after complete evaporation of the solvent under vacuum, fruit oil was obtained. Color and state of the oil were noted visually. The Chemical analysis of the oil of fruits (including acid value and saponification value) was performed according to the methods of Association of Analytical Chemists (1995)[10]. Density and specific gravity were measured at room temperature. All the measurements were made in triplicate and placed in Table-1.

Preparation of FAME:
Fatty acids extraction from the oil of the unripe fruits of *M. paradisiaca* was carried out according to the method described by G.H. Wilkfors et.al[11]. Infrared spectral analysis of the mixture of fatty acids thus obtained was performed on a Perkin-Elmer FT-IR spectrometer (Model No. Spectrum RX1, Holland) using solid KBr (Merck, India) to confirm the isolated product as fatty acids.

The mixture of fatty acids of the fruit oil was then methylated with 12.5% boron trifluoride (BF_3) in methanol[11]. Methyl esters of fatty acid mixture of the oil was purified by thin-layer chromatography using Hexane : Ethylacetate (1:1) as chromatographic solvent and FAME band was eluted with chloroform A.R. (Merck, India) and stored in a refrigerator for further analysis.

Gas-liquid Chromatographic analysis:
FAME-analysis by capillary gas chromatograph (GC) was carried out on a Shimadzu Gas Chromatograph (Model: GC-2010; Shimadzu, Japan) with Flame ionization detector (FID) on a split injector. A SP-2560 capillary column (100 m long × 0.25 mm ID) was used for FAME analysis. The temperatures of injection and detector ports were set at 260^{o}C. The oven temperature programmed was initially at 140^{o}C for 5 minutes, then rose at 4^{o}C/ minute to 240^{o}C and finally held at 20 minutes at 240^{o}C. Nitrogen gas, the carrier gas with a flow rate 33.9 ml/minute; volume injected 1µl; split ratio was 1: 30. Peaks were identified by comparison of their retention times with Supelco 37 component FAME standard mixture (Catalogue No. 18919 – 1 AMP) of Supelco, USA. The percentage composition of the sample was computed from GC peak area.

GC-Mass Spectrographic analysis:
Methyl esters of fatty acids were analyzed by a Gas Chromatography – Mass spectrometry on a Shimadzu GCMS – QP 2010 Plus (Shimadzu, Japan) fitted with a SP –2560 capillary column (100 m × 0.25 mm i.d). The temperatures of injection and detector ports were set at 260^{o}C. The oven temperature programmed was initially at 140^{o}C for 5 minutes, then rose at 4^{o}C/minute to 240^{o}C and finally held at 240^{o}C for 5 minutes. The carrier gas was nitrogen with a total flow rate 16.3 ml/minute. MS Condition: Ionization voltage was 70 eV; ion source temperature was 270^{o}C and mass range was 30–700 mass units. The individual peaks were identified by comparison of their retention indices (Figure-1) with standard chromatogram as well as by comparing their mass spectra with NIST/Wiley library of mass spectral database.

RESULTS AND DISCUSSION

Extracted oil from the unripe fruits of *M. paradisiaca* with n-hexane had color like mustard oil and liquid in nature at our laboratory temperature (28^{o}C). Yield was 5.01 g/Kg (5.01 ± 0.01g). Density and specific gravity of the fruit oil were 0.776 ± 0.002 and 0.779 ± 0.003 respectively. The chemical characteristics of the oil were evaluated from acid value and saponification value determinations and these values were found to be 9.83 ± 0.06 and 58.70 ± 2.12 respectively (Table-1).

Acid value of an oil is an intrinsic factor for assessing its nutritional and industrial value of the oil[12] and indicates the free fatty acids present in the oil. Low acid value and saponification value may be the indications of its suitability for nutritional use.

The infrared spectral analysis of the mixture of fatty acids obtained after saponification of the oil was done and showed bands at 3408.22 cm^{-1} for – OH stretching of carboxyl group; 2922.16 cm^{-1}, 2852 cm^{-1} for C–H stretching of – CH_2 group; 1735.93 cm^{-1} for –C=O stretching; 1465.90 cm^{-1} for probable C=C stretching (unsaturation) and 1170.79 cm^{-1} for –C–O stretching of carboxyl group. The aforesaid data confirm the presence of fatty acids in the mixture thus also in oil of the unripe fruit of *M. paradisiaca* (Figure-2).

Table 1: Some Physical and Chemical Characteristics of the oil extracted from the unripe fruits of *Musa paradisiaca*

Parameters	Unripe fruit oil of *M.paradisiaca*
Physical state at room temperature	Liquid
Color	Mustard Color
Total oil Content (g/Kg)	5.01 ± 0.01
Density (g/ml)	0.776 ± 0.002
Specific gravity	0.779 ± 0.003
Acid Value (mg KOH/g)	9.83 ± 0.06
Saponification Value (mg KOH)	58.70 ± 2.12

Table 2: FAME analysis of unripe fruit oil of *Musa paradisiaca Linn*

Peak	Retention time (in minute)	Name of the Fatty acid methyl ester	Relative Percentage*
1	11.440	Unidentified	0.05 ± 0.01
2	12.422	Decanoic acid(Caproic acid) (C_{10}: 0)	0.06 ± 0.02
3	14.871	Dodecanoic acid (Lauric acid) (C_{12}: 0)	0.28 ± 0.03
4	17.880	Tetradecanoic acid (Myristic acid) (C_{14}: 0)	1.08 ± 0.01
5	19.503	Pentadecanoic acid (C_{15}:0)	1.17 ± 0.03
6	21.337	Hexadecanoic acid (Palmitic acid) (C_{16}:0)	62.87 ± 0.12
7	22.320	9- Hexadecenoic acid (Palmetoleic acid) (C_{16}:1)	0.56 ± 0.04
8	22.847	Heptadecanoic acid (C_{17}: 0)	0.50 ± 0.01
9	23.388	Unidentified	0.52 ± 0.03
10	24.491	Octadecanoic acid (Stearic acid) (C_{18}:0)	3.48 ± 0.08
11	25.086	Nonadecanoic acid (C_{19}: 0)	4.12 ± 0.03
12	25.400	9- Octadecenoic acid (Oleic acid) (C_{18}:1)	12.38 ± 0.03
13	26.785	10,13 –Octadecadienoic acid (C_{18}: 2)	0.98 ± 0.03
14	27.785	Unidentified	1.68 ± 0.05
15	28.786	9,12 – Octadecadienoic acid(z,z) (Linoleic acid) (C_{18}: 2)	0.87 ± 0.03
16	29.457	9,11 - Octadecadienoic acid(E,E) (C_{18}: 2)	1.42 ± 0.03
17	30.492	Docosanoic acid (Behenic acid) (C_{22}: 0)	1.25 ± 0.04
18	31.807	Tricosanoic acid (C_{23}: 0)	0.99 ± 0.03
19	33.148	Tetracosanoic acid (C_{24} : 0)	2.95 ± 0.06
20	34.584	Pentacosanoic acid (C_{25} : 0)	1.23 ± 0.03
21	36.091	Hexacosanoic acid (C_{26}: 0)	1.26 ± 0.03
22	37.749	Tricontanoic acid (C_{30}: 0)	0.16 ± 0.01
23	38.620	Heptadecanoic acid, 8 –oxo-	0.14 ± 0.02
		Identified unsaturated fatty acids	16.21
		Identified saturated fatty acids	77.28
		Unidentified	6.37
		Oxo-fatty acids	0.14
		Ratio of Identified Unsaturated and Saturated fatty acids	1: 4.77

**Values are mean ± S.D, n=3*

Twenty fatty acids including one oxo-fatty acid were identified and quantified from GC-analysis followed by GC-MS analysis (Table-2) and that represented 93.63% of the total components. It appeared that the unripe fruit oil contained higher amount of saturated fatty acids (77.28%) than unsaturated ones (16.21%) in the ratio of 1:4.77.

Most abundant saturated fatty acid was Palmitic acid (62.87 ± 0.12%). Other saturated fatty acids, Nonadecanoic acid (4.12 ± 0.03%), Stearic acid (3.48 ± 0.08%), Tetracosanoic acid (2.95±0.06%, Hexacosanoic acid (1.26 ± 0.03%), Behenic acid ((1.25 ± 0.04%), Pentacosanoic acid (1.23 ± 0.03%), Pentadecanoic acid (1.17 ± 0.03%) and Myristic acid(1.08 ± 0.01%) and Tricosanoic acid(0.99 ± 0.03%) were found in good amounts. The oil also contained insignificant amount of some other saturated fatty acids like Caproic acid, Lauric acid, Heptadecanoic acid and tricontanoic acid (Table-2). The most predominating unsaturated fatty acid was oleic acid (12.38 ± 0.03%),

followed by 9,11(E,E)–Octadecadienoic acid (1.42 ± 0.03%). Three other unsaturated fatty acids were Linoleic acid, 10,13–Octadecadienoic acid and Palmetoleic acid, but found in lesser amounts (Table-2).

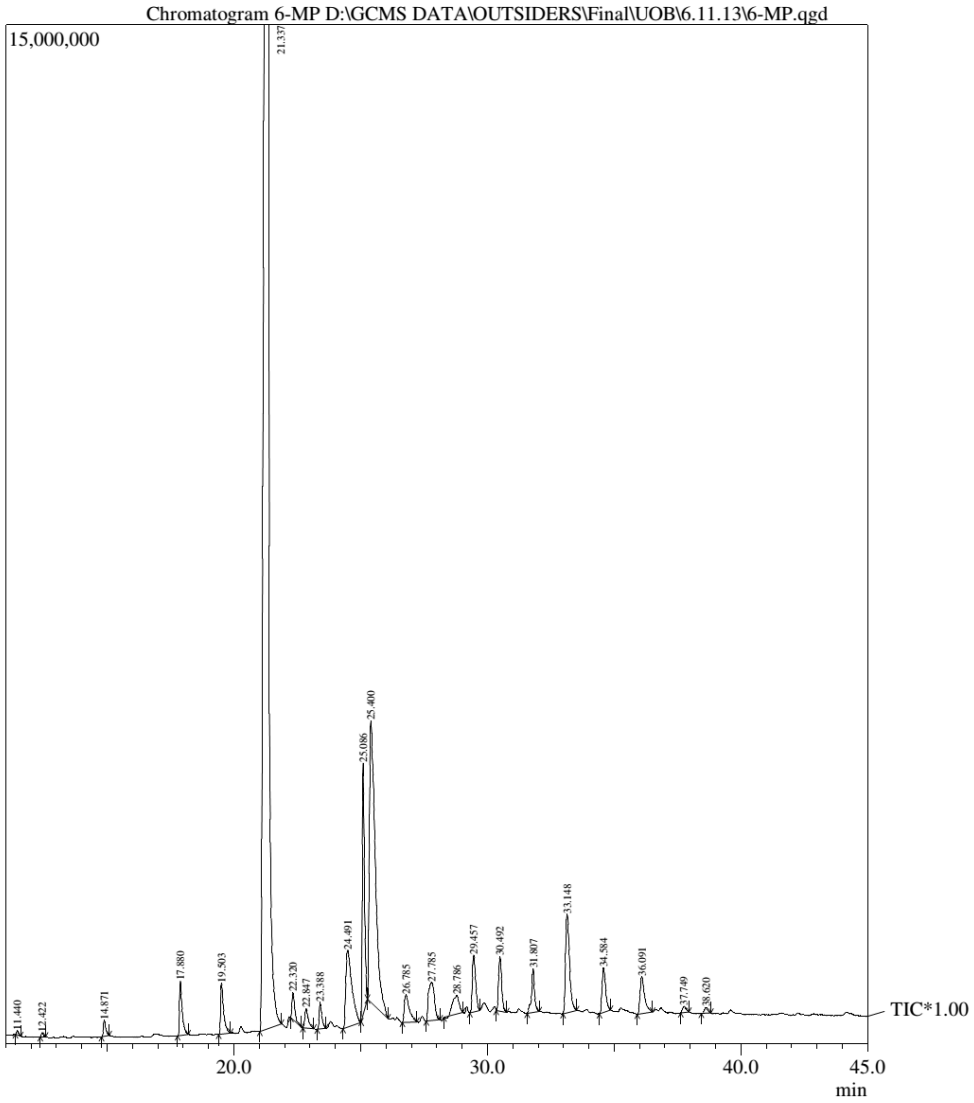

Figure-1: GC-Chromatogram of Fatty acid methyl ester of the oil of *M. paradisiaca*

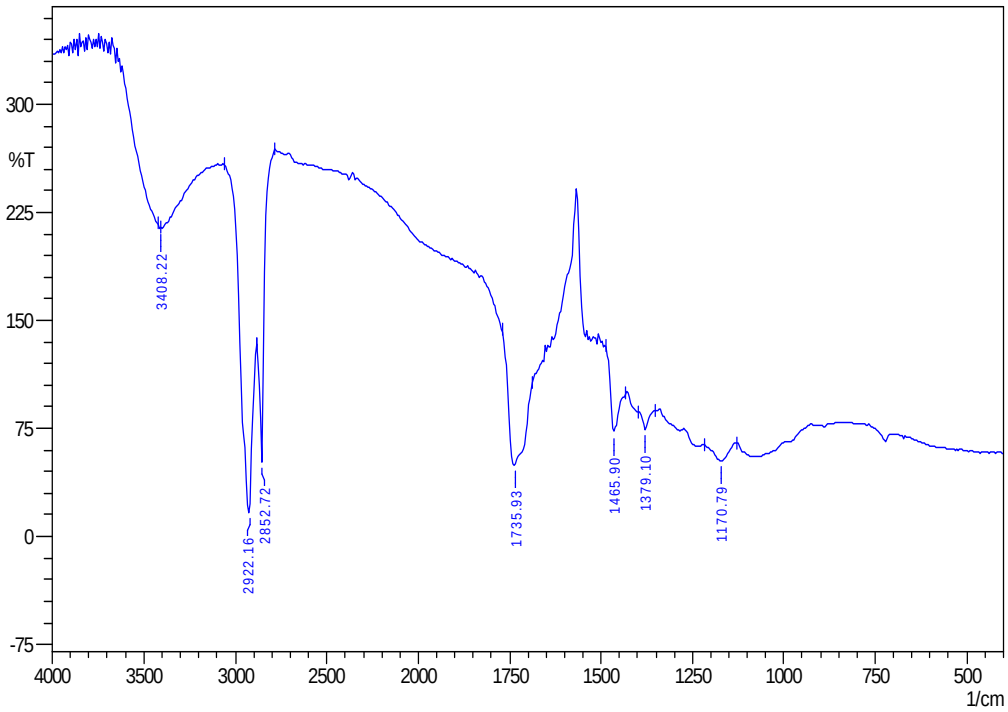

Fig – 2: IR-Spectra of oil from *Musa paradisiaca* fruit

CONCLUSION

From the aforementioned results and discussion, the unripe fruit oil of *M. paradisiaca* may be nutritionally viable. That means the fatty acid composition of the oil supports in favor of the viability of the use of unripe fruits of *M. paradisiaca* as nutritional purpose.

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