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DNA BARCODING OF DIFFERENT BANANA CULTIVARS AND ASSESSMENT OF OIL EXTRACTED FROM THEIR PEELS AS A VALUE-ADDING COMPONENT

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Abstract: Banana (*Musa* sp) peels, usually considered agricultural wastes have been identified as a valuable bioresource for non-food industrial applications. This study combined DNA barcoding and biochemical analysis was combined to assess peel oil from four *Musa* cultivars in Enugu State, Nigeria. Taxonomical identification using *RbcL* gene classified the cultivars as *Musa* ABB group/Ukpoko nsukpo (cooking), *Musa* acuminata AAA/Une (dessert), *Musa* AAB/Une nsukpo (silk banana) and *Musa* AAB/Ukpoko (horned plantain). Oils extracted using Soxhlet method were analyzed for physicochemical properties including fatty acid composition using GC-MS. Oil yields ranged from 7.4% to 14.4%, with the highest yield in *Musa* ABB group/Ukpoko nsukpo. High kinematic viscosity and saponification values shows oil is suitable for soap and biolubricant production, while fatty acid profiles rich in oleic, palmitic, linoleic, and lauric acids support applications in biofuels, cosmetics, and other bio-based products. ANOVA demonstrated cultivar-specific differences in fatty acid content ($p < 0.05$) and phylogenetic analysis confirmed genetic divergence among cultivars. This is the first research in the region applying this integrated approach to provide insights linking genetic identity to biochemical diversity. Results from this study demonstrates the potential of banana peel as a sustainable raw material for industrial valorization, support local economy and agro-waste valorization.

Keywords: Banana peel, Agro-waste, Valorization, Phylogenetics

INTRODUCTION

Bananas are long-shaped edible fruits belonging to the genus *Musa* (Musaceae). They have a green protective peel that turns mostly yellow upon ripening. They are predominantly found in tropical regions or subtropics and their origin traced to Southeast Asia and the surrounding regions (Hikal et al., 2022). According to the statistics compiled by the Food and Agriculture Organization (FAO, 2018), Nigeria is ranked 15th with a yearly production of 3,093,872 tonnes. The most frequently consumed banana species in Nigeria are *Musa* x *paradisica* (cooking banana) and *Musa* *acuminata* (desert banana) (Maduforo et al., 2020). As economically and nutritionally valuable as they are, a significant amount of banana peel waste is generated following consumption, which is usually discarded without valorization. Typically, discarded banana peels serve as an extra forage to meet nutritional needs of animals in region where banana is mostly cultivated. These by-products in excess amount are good sources of raw materials for different industries, thus highlighting agricultural waste recycling. Banana peel boasts

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of an abundant content of essential nutrients, including free fatty acids, with a notable presence of linoleic acid, including omega-3 and omega-6 fatty acids (Azarudeen and Nithya, 2021).

Taxonomic characterization of organisms holds paramount significance in identification of species, gauging biodiversity, conservation of new and already known species, and conducting ecological assessments. Conventional taxonomic classification has mostly leaned on morphological traits for identification purposes. Molecular research has uncovered many biological species with genetic variations without showing physical differences, making them unidentifiable through conventional morphological variation in species criteria. DNA barcoding as a tool for taxonomic study requires short gene markers to determine the species or specific organism to which a DNA sample belongs (Letchuman, 2018). RuBisCo Large subunit (RbcL) gene and maturase K (matK) are the DNA barcodes sourced from chloroplast genes widely accepted for taxonomical identification of plants. Considering the large annual production of Musa species in Nigeria, it is clear that a large amount of waste is generated thus posing environmental challenges. The disposal of the fruit residues is typically done through methods like burying in landfills, burning, or composting. However, these methods are becoming less practical due to environmental concerns (Sial et al., 2019). Banana peel (BP) oil is derived from peels of banana fruit. This oil primarily contains fatty acids like linoleic and α -linolenic acid, which are beneficial for various purposes such as, cosmetics, and as an alternate lubricant (Zaini et al., 2022). The oil composition in banana peels can vary between different varieties of bananas, with some having slightly greater oil content than others. Different methods can be explored in extraction of this valuable oil from banana peels including sohxlet extraction which employs solvents with low boiling points such as hexane, methanol or ethanol to dissolve the essential oil from the peels, resulting in 90%–98% oil recovery (Tsegayefekadu et al., 2020). GC-MS analysis of *Musa sapientum* peel, as conducted by Waghmare and Kurhade (2014) identified several bioactive compounds. These include estragole, hexadecanoic acid ethyl ester, Oleic acid and other components. Fatty acids are effective agent in formulation of dental hygiene products, especially toothpaste. Dayrit (2015), in an exploring relevance of lauric acid in coconut oil, revealed that lauric acid, in conjunction with other fatty acids like linoleic and oleic acids found in *Musa* sp peel oil, has been observed to reduce plaque formation and inhibit hydroxyapatite (HA) dissolution. Senthilkumar et al. (2023) study on application of banana peel oil as biolubricant in machine operations concluded that the physicochemical characteristics of banana peel, are compatible with those of traditional cutting fluids (such as lathe oil) and could serve well as a lubricant.

The fact that banana peel contains varying chemical components depending on the species of banana or origin of the peels is another major challenge. Although there has been research on banana peel composition and oil extraction procedures, limited attention, and especially at the molecular level, has been given to the influence of cultivar identity on oil quality and yield. This is particularly acute in Enugu, where local banana cultivars are genetically and biochemically mostly uncharacterized. DNA barcoding is a rapid and precise method of species identification and phylogenetic analysis, especially in morphologically similar or hybrid-dense plant taxa like *Musa* spp. However, no research has hitherto applied barcoding on banana cultivars cultivated in Enugu state. In addition, no earlier study has also molecularly correlated local cultivars to variation in peel oil characteristics that may possess industrial and nutritional value in Enugu state. This study addresses these gaps by; molecular identification of local banana cultivars from Udi LGA, Enugu State using the *rbcL* gene; Isolation and

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characterization of peel oil from the identified cultivars; Alignment of genetic identity with oil yield and fatty acid profile using GC-MS and statistics; combination of DNA barcoding and biochemical profiling, to offer a pathway for the valorization of banana peel waste.

The findings could inform the selection of specific banana varieties for particular applications in biolubricant manufacturing, natural products development, and agricultural bio-economy planning in West Africa.

2. MATERIAL AND METHODS

Sohxlet extraction apparatus (Accumax, India), thermal cycler, micropipette tips and micro-centrifuge tubes (Eppendorf USA), Bio-Rad Thermal cycler (Serial no. 621BR56569, Singapore), gas chromatography system (Buck Scientific, United Kingdom), Accuris UV transilluminator (Model: E3000-E, China), Bio-rad electrophoresis powerpack (041BR301000, Singapore). Analytical grade reagents were used for physicochemical analysis and molecular grade reagents manufactured by Fisher Bio reagents, USA for molecular studies.

2.1 Sample collection

Four (4) fresh *Musa* sp fruits and leaf (Ukpoko nsukpo, Une, Une nsukpo and Ukpoko) commonly cultivated in umabi village, Udi LGA, Enugu State were obtained from selected homestead farms. The leaves were labelled and set aside for DNA barcoding while peels are separated from the pulp and washed in distilled water to remove dust

2.2 Sample Identification

2.2.1 Polymerase chain reaction (PCR)

Polymerase chain reaction was carried out as described by Estevez et al., (2015) using: 23µl rbcI primer mix + ready-go-to PCR beads+ 2ul of genomic DNA template, to make up a 25µl reaction. The primer pairs rbcLF (5'_TGTAACACGACGGCCAGTATGTCACCACAAACA GAG ACT AAA GC_3') and rbcLR (5'_CAGGAAACAGCTATGACGTAAATCAAGTC CACCCG_3') were used for the PCR. The PCR was performed with Bio-Rad Thermal cycler as follows: 94°C for one (1) minute, followed by 34 cycles of 94°C for 15 seconds, 54°C for 15 seconds and 72°C for 30 seconds and elongation step at 72°C for 5 minutes.

2.2.2 Agarose Gel Electrophoresis

2% agarose solution was stained with a drop of Ethidium Bromide (EBr) was used for pCR product electrophoresis (Bakar et al., 2018). 1xTE buffer was poured just enough to cover wells and 5µl (micro-litre) each of amplicon was pipette into the wells and 5µl 100-bp marker (New England Biolab, UK) was added to the gel's far left well. Electrophoresis was run at 100V for 30mins for adequate separation. Remaining 20µl of PCR amplicons were packed at -20°C and sent out for sequencing.

2.2.3 Sequence Analysis

DNA sequence of P1(Ukpoko nsukpo), P2 (Une), P3 (Une nsukpo), and P4 (Ukpoko) was trimmed and aligned using DNA subway and Phylogeny tree constructed using MEGA 11. Similarity thresholds of 99% were used to assign specimens to species level. Pairwise genetic distances within and among species were calculated under the Kimura 2-parameter (K2P) model (Kimura, 1980) performed in MEGA software. The same software was used to cluster closely related taxa into a Neighbour-Joining (NJ) phylogeny, employing 1000 bootstrap replicates.

2.3 Oil extraction (sohxlet method)

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Oil extraction was done on a 500 g capacity soxhlet apparatuses described by Redfern et al. (2014). 100g each of the prepared sample was added to the thimble. 300 ml n-hexane was measured into a round bottom flask for the extraction. The oil obtained was dried in water bath at 90C for 2 hours to remove residual solvents, cooled and stored in air tight container. Oil yield was calculated thus

$$\text{Oil yield (\%)} = \frac{\text{Weight of oil}}{\text{Sample weight}} (100)$$

2.4 Oil characterization

2.4.1 Physicochemical Analysis

The obtained oil was characterized for moisture, density, saponification, free fatty acid, refractive index, acid value, moisture, peroxide value, boiling point, smoking point, kinematic viscosity and molecular weight.

2.4.1.1 Density

Density was determined as described in ASTM Standard Test Method D5002 for determination of density

2.4.1.2 Kinematic viscosity

A Poulten Selfe and Lee Ltd. glass u-tube viscometer (PSL ASTM-IP 350) was used to measure the kinematic viscosity. As described by Knothe and Rason (2017), the amount of time in seconds that the sample took to flow from the bulb's top meniscus to its lower meniscus was determined as the sample flow time.

2.4.1.3 Moisture content

The dry oven technique was used to determine the moisture content as described by Wick (1985). 5g of each sample oil was weighed into a weighed, clean, dry petri dish. The petri dish with the sample was introduced into the laboratory hot air oven set at 105 °C. After an hour, the sample removed from the oven and placed in a desiccator. After cooling, the final weight is taken.

$$\% \text{ moisture} = \left(\frac{\text{Weight loss}}{\text{Sample weight}} \right) 100$$

2.4.1.4 Refractive index

Hanner Instruments' HI96800 tabletop refractometer was used to calculate the refractive index.

2.4.1.5 Kinematic viscosity

A Poulten Selfe and Lee Ltd. glass u-tube viscometer (PSL ASTM-IP 350) was used to measure the kinematic viscosity. As described by Knothe and Rason (2017).

$$\text{Kinematic viscosity} = ct(\text{mm}^2\text{s}^{-1})$$

Where c is viscosity constant 0.4891

2.4.1.6 Acid value

Acid value was determined according to the method of specified in ASTM, 2004.

$$\text{Acid value} = \frac{(\text{Titre value})(\text{conc. of KOH}) (56.1)}{\text{Sample weight}}$$

2.4.1.7 Peroxide value

The peroxide value was calculated using the Boerlage and Broeze (1990) technique. A 250g conical flask containing 0.5g of sample oil was filled with a mixture of 25ml glacial acetic acid and chloroform in a 2:1 ratio. One millilitre of 10% potassium iodide was added, quickly shook, covered, and left in the dark for a full minute.

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35 ml of starch indicator was added and the mixture titrated with 0.02N sodium thiosulphate solution until it became white instead of pale black. Titration was performed on a blank.

$$\text{Peroxide value} = \frac{(100)(V1 - V2)(\text{Conc. of titrant})}{\text{Sample weight}}$$

Where V1 is titre value of sample and V2 is titre value of blank

2.4.2 Gas chromatography – mass spectrometry (GC-MS) analysis

This technique or technology takes advantage of variance in elution characteristics samples (oil) to determine fatty acids and methyl ester composition of same samples (Kaluvarachchi et al., 2017). For this reason, free fatty acid and methyl ester profile of oil was determined using GC-MS.

3. RESULTS AND DISCUSSIONS

3.1. DNA Barcoding

3.1.1 Gel electrophoresis

Plate 1 revealed DNA isolated from all four (4) were of high quality (shown by the presence of a specific DNA band) and RbcL gene was amplified in all samples. The amplicon sizes for rbcL genes for all samples was 600bp. Electrophoregram of the PCR-amplified rbcL products is shown thus.

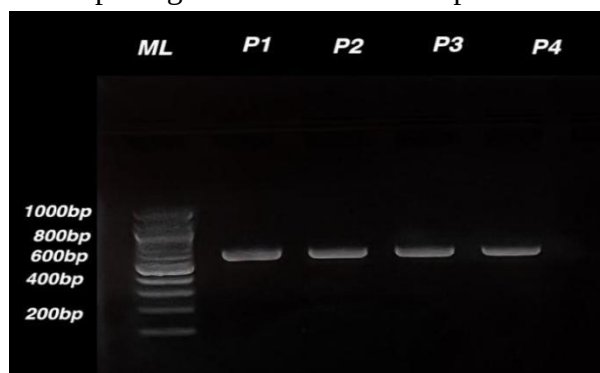


Plate 1. Electrophoregram of PCR rbcL products of Musa spp

3.1.2. BLAST Result

Table 1 shows BLAST result from NCBI identifying samples P1, P2, P3 and P4 are as Musa ABB group isolate Bhut manohar (Ukpoko nsukpo) i.e cooking banana, Musa acuminata AAA group (Une) i.e Cavendish, Musa AAB group cultivar Nanjuanagud Rasbale (Une nsukpo) i.e Silk banana and Musa AAB group cultivar Dhirmoni (Ukpoko) i.e Horned plantain respectively.

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Table 1. BLAST Result Identifying Four (4) Banana Varieties

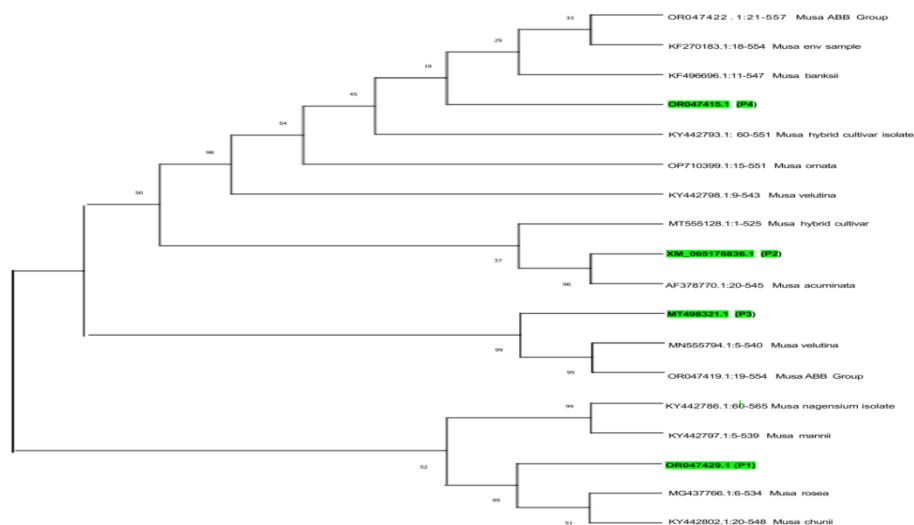
Sample ID	Local Name	PCR	Query cover (%)	Per. Ident. (%)	Significant Alignment	Acces. Num.	e-value
P1	Ukpoko nsukpo	1	100	100	Musa Bhut manohar (ABB Group cultivar)	OR047429.1	0
P2	Une	1	100	99.8	Musa acuminata (AAA group)	XM_065176.836.1	0
P3	Une nsukpo	1	99	100	Musa Nanjuanagud Rasbale (AAB group)	MT498321.1	0
P4	Ukpoko	1	100	99	Musa Dhirmoni (AAB group)	OR047415.1	0

1= yes

2= No

3.1.3 Phylogenetic analysis

Figure 2 represents evolutionary relationship of banana varieties as determined using Neighbour joining method. This infers evolutionary history to display the ideal tree. The proportion of duplicate trees or how many times closely related taxa clustered together in the bootstrap test (1000 times) is displayed next to the branches. Among the codon locations were 1st, 2nd, 3rd and noncoding. There were 537 sites in final collection. Sample P1(Ukpoko nsukpo) shows a low bootstrap value of 19% to Musa banksii, sample P2(Une) shows high bootstrap value of 98% to Musa acuminata, P3 (Une nsukpo) has a bootstrap value of 99% with Musa mannii and P4 (Ukpoko) has a bootstrap value of 99% Musa velutina and Musa ABB group. The phylogenetic trees built using genetic data from both similar cultivars and gives correctly accurate visual trace of the lineage and evolutionary heritage in samples, revealing how different varieties have diverged from common ancestors over time.

**Figure 2.** Phylogenetic tree inferred by Neighbour-joining method

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3.2 Soxhlet Extraction

3.2.1 Oil Yield

Table 2 shows that sample Ukpoko nsukpo (P1) has the highest oil yield, while sample Une (P2) has the lowest. The average oil yield among the varieties is 11.202%, with a range from 7.408% to 14.39%. The data reflects some variability as indicated by the standard deviation of approximately 2.94%.

Sample	Ukpoko nsukpo (P1)	Une (P2)	Une nsukpo (P3)	Ukpoko (P4)
Oil yield(%)	14.4	7.4	12.3	10.7

Table 2: Percentage Oil Yield Extracted from Different Varieties of Banana Peels

3.3 Oil Characterization

3.3.1 Physicochemical Analysis

Table 3 shows the physicochemical analysis of oil extracted from Musa sp peel. The resulting oils show high level of kinematic viscosity with Ukpoko nsukpo (P1) having highest value at 93.8(mm²s⁻¹). Saponification value of oils is record at 248.19, 193.28, 215.22 and 267.05 for Ukpoko nsukpo (P1), Une (P2), Une nsukpo (P3) and Ukpoko (P4) respectively. This suggests that peel oils from Ukpoko nsukpo, Une nsukpo, and Ukpoko are not edible but suitable to be applied in soap making. Une (P2) peel oil records the highest boiling point of 118°C and smoking point value of 95°C. Ukpoko nsukpo (P1) has the highest density, viscosity, acid value, and peroxide value, suggesting it has a higher risk of oxidation and rancidity while Une nsukpo (P3) shows balanced properties with the lowest peroxide value. Low boiling, low smoking point, high acid value and free fatty acid which contributes to off-flavours and potential health concerns suggests that banana peel oil are not suitable as cooking oils for deep frying(i.e frying) but are suitable in soap making as it records significant saponification value. Oils with viscosity values falling within the range of 32 to 100 centistokes (cSt) at the operating temperature are considered suitable for lubrication purposes (Zahir et al., 2017). The peels oils extracted conform to the standard for lubrication purposes and can be produced as biolubricants owing to high level of kinematic viscosity with highest value at 93.8(m²s⁻¹) in Ukpoko nsukpo (P1). This is similar to the report by Senthilkumar et al., (2022) that the prepared bio-oil from banana peel powder could be used as a lubricant for machine operation. The physicochemical properties quantified shows density as the only parameter with statistically significant differences (p = 0.04). Ukpoko and Ukpoko nsukpo showed more elevated levels of stearic and linoleic acid esters, whereas Une nsukpo showed unique fatty acids like lauric and myrist-oleic esters. Other properties, such as acid value, peroxide value, and kinematic viscosity, were not significantly different but still revealed practical implications especially for soap making and lubrication.

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Table 3. Physicochemical Characterization of Oil From Different Musa Sp Peel

Parameters	Samples				p-value
	P1	P2	P3	P4	
Density	0.944	0.926	0.921	0.941	0.047
Kinematic Viscosity (mm ² s ⁻¹)	93.9	61.44	66.13	87.21	0.383
Refractive index @ 29°C	1.492	1.488	1.488	1.4879	0.500
Acid value (mgKOH/kg)	26.22	14.74	17.91	23.16	0.186
Free fatty acid (mgKOH/kg)	13.11	7.37	8.96	11.58	0.292
Moisture (%)	0.05	0.07	0.09	0.08	0.306
Peroxide (meqKOH/kg)	8.63	3.95	3.77	7.21	0.523
Saponification (mgKOH/kg)	248.19	193.28	215.22	267.05	0.213
Boiling point (°C)	103	118	114	108	0.241
Smoking point (°C)	83	95	92	79	0.136
Molecular weight (g/mol)	758.21	942.65	852.87	690.07	0.137

0.05 = *, 0.005 = **, 0.001 = ***, Not significant= ns

ANOVA Analysis shows The parameter density shows a statistically significant difference among the samples (P = 0.05). For the other parameters, the P > 0.05, indicating that there are no statistically significant differences among the samples for these parameters.

3.3.2 Fatty Acid Composition using GC-MS Analysis.

Table 4 represents the fatty acid compounds found in four cultivars of banana peel oil. Ukpoko nsukpo (P1) shows highest oleic acid content (17.1%) with notably high content of palmitic acid ester (7.81%), palmitic acid (12.09%) and oleic acid ester (13.66%). Unlike other samples, it records presence of unique component like magaric acid ester(0.96%). Une (P2) records lower percentage in palmitic acid ester (5.02%) and palmitic acid (5.4%) with moderate levels of oleic acid (9.11%) and oleic acid ester (9.48%). Une nsupko (P3) shows very low palmitic ester (1.43%), lower oleic acid (3.55%) and oleic acid ester (2.4%) presence and lauric ester (13.74%) uniquely present. Ukpoko (P4) has the highest palmitic acid ester concentration (22.64%) among all, low percentage but special presence of palmit-oleic acid (0.68%). Oleic acid is lowest at 2.63% however oleic acid ester is significantly present at 14.67%. Ukpoko (P4) shows a notable presence of stearic ester (16.49%) and linoleic acid ester (14.41%) compared to other cultivars. Une (P2) displays a balanced presence of various fatty acids but lacks unique components found in other cultivars while Une nsukpo (P3) has a diverse profile with lower fatty acid contents but includes fatty acid compounds like capric ester and myrist-oleic which are found in other cultivars.

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Table 4. Fatty acid compounds present in for samples of banana peel oil

Cultivar (Group)	Fatty Acid	Percentage Area (%)
Ukpoko nsukpo (P1)	Palmitic acid ester	7.81
	Palmitic acid	12.09
	Magaric acid ester	0.96
	Oleic Acid	17.1
	Oleic acid ester	13.66
	Stearic acid ester	7.32
(P2) Musa acuminata AAA Une	Linoleic	12.35
	Palmitic acid ester	5.02
	Palmitic acid	5.4
	Myristic	2.58
	Oleic acid	9.11
	Oleic acid ester	9.48
(P3) Musa AAB Une nsukpo	Myristic ester	0.45
	Stearic	0.42
	Palmitic ester	1.43
	Oleic acid	3.55
	Oleic acid ester	2.4
	Stearate	4.42
	Capric ester	0.13
	α -linolenic	0.67
	Linoleic	7.25
	Lauric ester	13.74
	Myrist-oleic	1.62
	Palmit-oleic acid	0.68
P4 Musa AAB Ukpoko	Palmitic acid ester	22.64
	Oleic Acid	2.63
	Eicosatrienoic acid	2.97
	Linoleic acid ester	14.41
	Oleic acid ester	14.67
	Stearic ester	16.49
	Linolenic acid	3.43

Statistical

Analysis

Table 4 shows the ANOVA results show that the variance explained by the model (between groups) is greater than the variance within the groups, which is consistent with the significant F-statistic and p-value. F-statistic of

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4.586 indicates that the variability between the group means is approximately 4.586 times greater than the variability within the groups. the p-value is 0.001, which is less than the typical alpha level of 0.05, indicating statistically significant differences between the group mean.

Table 4. ANOVA summary for fatty acids present in the four (4) banana cultivars

source	DF	SS	MF	F-Stat	P-vauue
Between groups	4	826.81	206.7	4.59	0.001
within groups	15	676.16	45.08		
total	19	1502.97			

P = 0.001, indicating statistically significant difference

4. CONCLUSION

DNA Barcoding is an effective method of identifying and studying phylogenetic variation of different varieties of Musa sp plant. Musa sp plant have complex plant groups with hybrids and polyploids, using a one universal barcode marker for taxonomic identification might not be very effective. This inefficacy arises due to variations in substitution rates, which include both low rates of variation between species (interspecific rates) and high rates of variation within species (intraspecific rates). The variety or cultivar of Musa sp is an important factor that affects oil yield from banana peels and free fatty acid composition of the extracted oil. Despite the limited quantity, the inclusion of palmitic, linoleic, and oleic acid in Musa sp fruit peel oil makes it suitable for dietary formulation purposes including livestock feed. Presence of lauric acid makes it a potent antimicrobial agent suitable for application in various industries. Limited or no research has been conducted to accurately identify the four major banana varieties found in Udi L.G.A, Enugu state (Ukpoko nsukpo, Une, Une nsukpo and Ukpoko) using DNA barcoding and assess oil extracted from the fruit peel. Knowledge from this study makes it easy to decide and identify which banana variety is more suitable for a particular industrial application.

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Author's Contribution: All authors read and approved the final manuscript.

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APPENDIX.