



## ***Gas Chromatography Mass Spectrometry Investigation and Minerals, Proximate Components and their Relationship on Hydromethanolic Extract of Unripe Musa sapientum Peel and Pulp***

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### Article info

Received 24 Oct 2020

Revised 25 Nov 2020

Available Online 03 Dec 2020

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### Abstract

*Hydromethanolic extracts of Nigeria variety of unripe Musa sapientum peel and pulp were subjected to phytonutrient gas chromatography-mass spectrometry (GC-MS) investigation and proximate and mineral content analysis. Moreover, this study also aims to propose a new method to differentiate isolated proximate components and minerals. GC-MS characterized eight main physiochemical each and additionally identified 16 and 13 fragments associated with peels and pulps respectively. Our results proved varietal differences in the nutritional values of unripe and ripe peels and pulps ordered: unripe peel > unripe pulp > ripe peel > ripe pulp respectively in descending order with respective concentration: 300.63 > 280.12 > 151.92 > 146.22. Interestingly, harmonic mean analysis postulated also varietal differences in the overall distribution of mineral contents thus: unripe peel > ripe peel > unripe pulp > ripe pulp; while proximate compositions as: ripe peel > unripe pulp > ripe pulp > unripe peel in descending order with respective concentrations 8.45 > 7.59 > 7.58 > 2.48; and 4.24 > 3.57 > 3.23 > 2.63 respectively, which highlighted efficacy and superiority of the different parts and types of the fruit in their food-medicine promoting properties. Our result justifies scientifically the existence of different important compounds in unripe Musa sapientum peels and pulps that may be responsible for medicinal actions against specific major disorders of interest.*

**Keywords:** GC-MS analysis; Musa sapientum peel and pulp; Nutritional value; Harmonic mean; Bioactive compounds; Chemical composition

### Introduction

Banana is scientifically known as *Musa sapientum* [1] and in the field of natural remedy, has been used throughout the world as source of food and income for people [1,2]. Moreover, in recent years, many studies suggest that various parts of banana act as food medicines for treatment of diseases like diabetes [3], hypertension, cancer, ulcers, diarrhea [4], urolithiasis, Alzheimer's, inflammation, body fat reduction, anaemia [5,6]. Its consumption can enhance physical strength, metabolic response, oxidative stress status, lipid profiles, and interleukin-23 [7]. Unripe *Musa*

*sapientum* peel has been implicated in wound healing [7]. Sadly, there is paucity of evidence to support these assertions.

The literature survey on scientific information using gas chromatography-mass spectrometry (GC-MS) analysis of unripe *Musa sapientum* peel and pulp remains rather sparse. Hence the aim of the present study was focused on using GC-MS analysis [9] of hydromethanolic extract [10,11] of unripe *Musa sapientum* to characterize effective biologically active,

phytotherapeutic and nutraceutical molecules in the unripe fruit which serves as novel food medicines. GC-MS is an analytical technique that allows the separation of mixtures of volatile organic compounds and the identification, purity evaluation and quantification of each constituent compound in food science [9]. Due to its sensitivity, speed, versatility, inexpensiveness, and the ability to identify many different compounds in a mixture, therefore, it is a favourite instrumental technique for the analysis of unripe *Musa sapientum* peel and pulp which has not been previously reported.

Moreover, extraction is the most important step in the analysis of bioactive compounds in phyto-medicine studies. The strength of solvent plays a key role in this process; hydromethanol has been showed to give a better response as far as extraction potency of medicinal plants are concerned [10,11]. Beside the conventional generalization method of presenting results of analysis, the aim of this new study was to use harmonic mean comparative differential approach to present scientific information from which our understanding of the mechanistic-link in isolated proximate and mineral constituents in unripe and ripe *Musa sapientum* peels and pulps in food medicine may emerge.

## Materials and Methods

### Plant, Chemicals and Reagents

Following ethical approval, in November 2019, unripe banana fruit was obtained from a farm in Anambra State, south-eastern geo-political region of Nigeria. The banana fruit was identified and authenticated at the herbarium unit, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and voucher specimens deposited for future references. Some were ripened. Peels were separated from pulps and both parts were dipped in 0.5% citric acid to prevent enzymatic degradation [12,13]. Both parts were shade dried for 96 hours. Dried peels and pulps were ground to paste. All chemicals and reagents used are branded by Sigma-Aldrich.

### Preparation of unripe and ripe banana extract

The ground unripe and ripe banana peels and pulps were extracted with hydromethanolic solvent (1:4 v/v) [10,11]. The extraction was carried out in sealed test tubes placed in water bath for 120 min at 25°C. The extracts were centrifuged and then evaporated to dryness in a vacuum evaporator at 40°C. The final residues of unripe peels and pulps obtained were subjected to GC-MS analysis while nutritional value evaluations (proximate and mineral analysis) in ripe and

unripe pulp and peel was carried out using standard procedures.

### Proximate analysis

Proximate analysis was performed using standard analytic methods [6,14].

### Moisture content

Moisture content was measured using air-oven following official methods of Association of Official Analytical Chemists. A material test chamber M720 (Binder GmbH, Tuttlingen, Germany) was used to dry the samples till constant weight. The percentage of moisture content was calculated as:

$$\% \text{ moisture} = (1 - \text{weight dry sample} / \text{weight wet sample}) \times 100$$

### Lipid content

Determination of lipid content was performed following Soxhlet method previously described by Nouredini and Byun, using a Soxhlet™ 2050 automated analyzer (FOSS Analytical, Hillerød, Denmark). Petroleum ether was used for the extraction, whereas percentage of lipid was obtained following equation below:

$$\% \text{ lipid} = \frac{\text{Weight (extraction cup + residue)} - \text{weight (extraction cup)}}{\text{weight sample}} \times 100$$

### Protein content

The Kjeltac™ 2200 Auto Distillation Unit (FOSS Tecator, Höganäs, Sweden) was used. A nitrogen-to-protein conversion factor of 4.4 was used for the determination of protein present in the samples.

### Ash content

A dry ash method was used to determine the ash content. The samples were incinerated in a furnace (Furnace 62700, Barnstead/Thermolyne, and Dubuque, IA, USA) at 550 °C. The remaining inorganic material was cooled, weighed and further used for the determination of mineral contents. An ash solution was prepared by dissolving the ash in 100 mL of 1 M HCl.

### Carbohydrate content

The total carbohydrate content (%) in the samples was calculated by difference method [1,2]. Briefly, Percentage of carbohydrate was given by:  $100 - (\text{Percentage of ash} + \text{percentage Moisture} + \text{percentage fat} + \text{percentage protein})$ .

## Nutritive value

Nutritive value was finally determined by the relationship: Nutritive value =  $4 \times$  percentage of protein +  $9 \times$  percentage of fat +  $4 \times$  percentage carbohydrate.

## Mineral Analysis

A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements of zinc, calcium, iron, Magnesium and Manganese respectively. The results for the mineral contents were expressed as mg/100 g dry weight (DW).

## Gas Chromatography Mass Spectrophotometry

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out on a GC 7890 (Agilent) comprising automatic liquid sampler and gas chromatograph interfaced to mass spectrophotometer (GC-MS). Helium was used as a carrier gas and the injector temperature was kept at 3500C. The oven temperature was programmed from 1000C held for 5 min to 3750C at 200C/min. The hydromethanolic extracts [10,11] of Nigerian unripe *Musa sapientum* peels and pulps were subjected to soxhlet extraction methods and concentrated at 40°C using hot air oven. The concentrated extracts were subjected to phytochemical gas chromatography-mass spectrometry analysis. The compounds obtained as a result of GC-MS

screening were identified on the basis of their retention time, peak area and by interpretation of mass spectra; and the name, molecular weight and structure of the components ascertained.

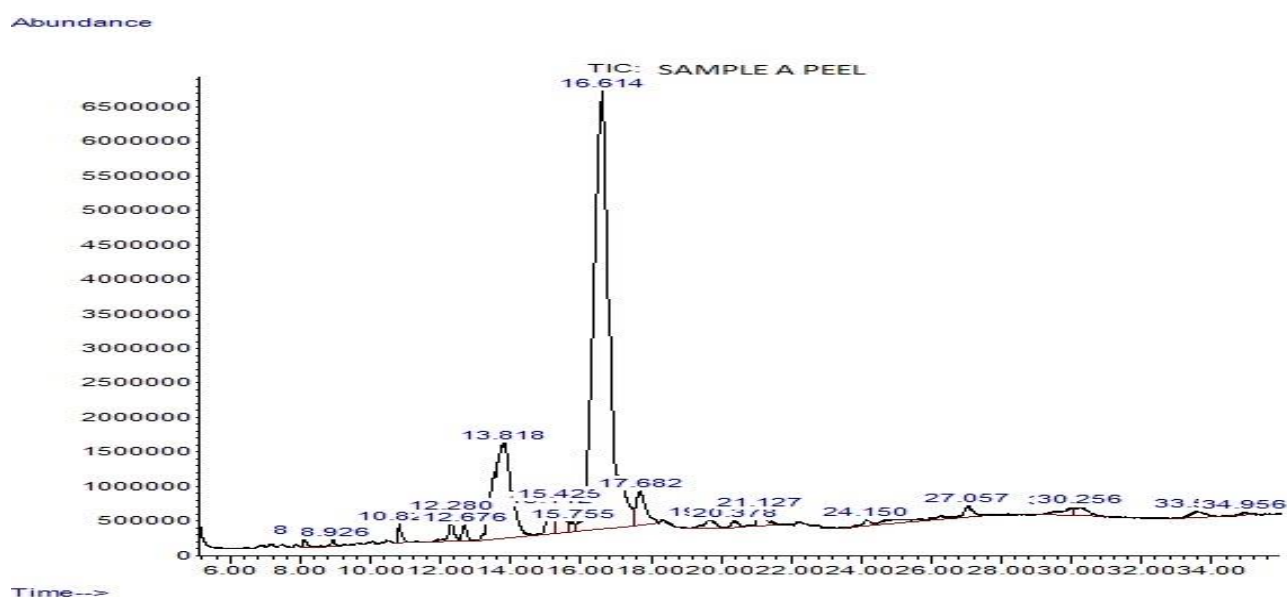
## Statistical Analysis

Microsoft Excel 2010 was used for basic statistical illustrations. Percentage difference was calculated using standard formula according to [15,16].

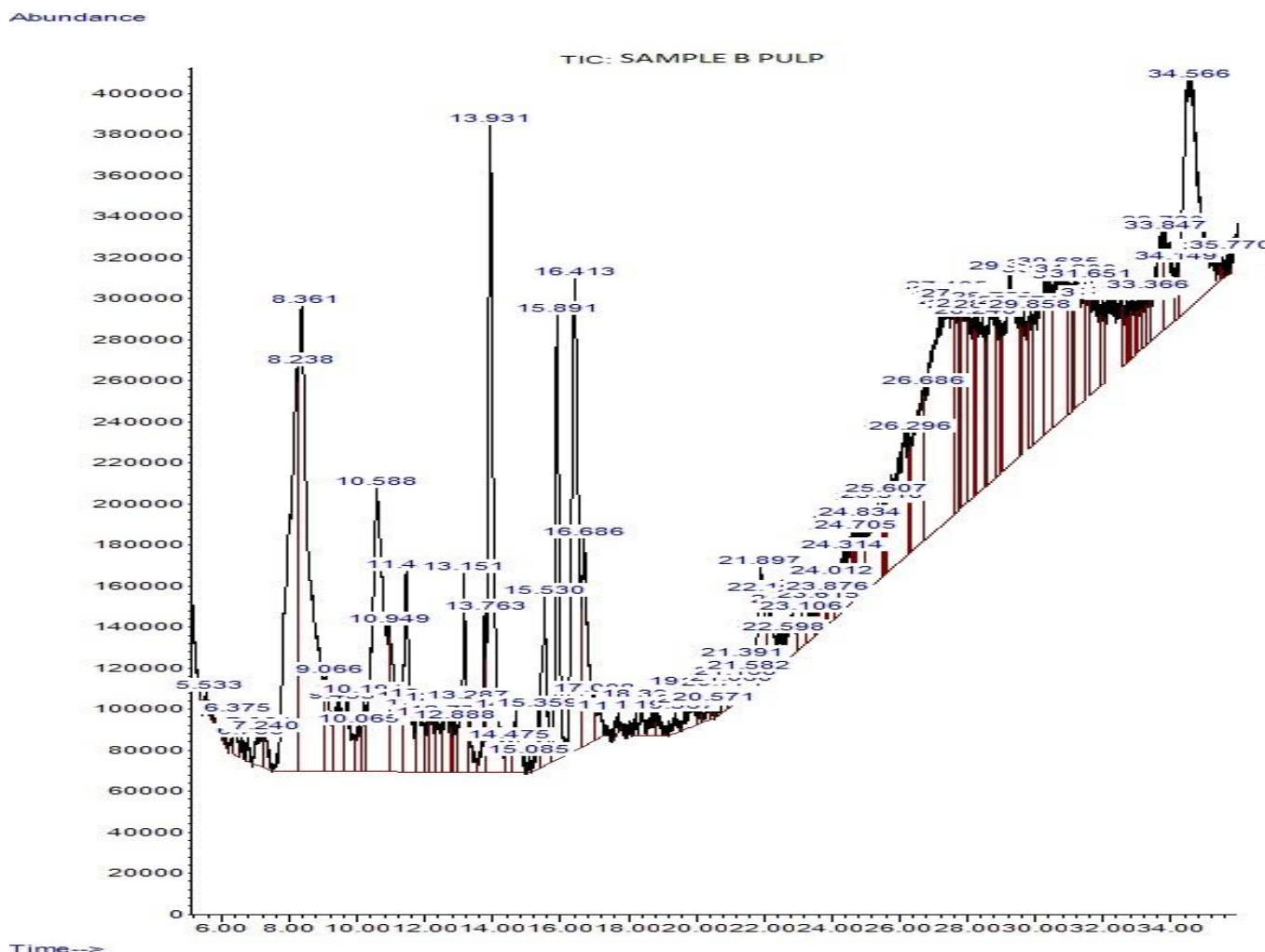
## Result and Discussion

GC-MS chromatogram of the hydromethanolic [10,11] extract of unripe *Musa sapientum* peels and pulps are depicted in Figures 1 and 2; and identified compounds with their retention time and peak area percent together with mass-to-charge ratio (m/z, g/mole) and molecular weight for the different fragments are discussed in Tables 1 to 4 respectively.

The results of GC-MS analysis allowed to the elucidation of eight main bioactive constituents each with their retention time and peak area percent; and additionally, identified sixteen and thirteen fragments associated with unripe *Musa sapientum* peels and pulps respectively, consistent with observations that banana peel is rich in phytochemical compounds than its pulp [14,17-19].



**Figure 1:** GC-MS chromatogram of the hydromethanol extract of unripe *Musa sapientum* peel.



**Figure 2:** GC-MS chromatogram of the hydromethanol extract of unripe *Musa sapientum* pulp.

**Table 1:** GC-MS analysis of bioactive components in unripe *Musa sapientum* peel.

S.No	Physiochemical Compounds	Retention Time	Peak Area Percent
1	trans-13-Octadecenoic acid cis-13-Octadecenoic acid 9-Octadecenoic acid	16.614	63.97
2	n-Hexadecanoic acid	13.818	15.95
3	9,17-Octadecadienoic acid 9,12-Octadecadienoic acid Linoelaidic acid	17.682	3.12
4	9-Octadecenoic acid, methyl ester 6-Octadecenoic acid, methyl ester 11-Octadecenoic acid, methyl ester	15.425	2.74

**Citation:** Ilochi ON, Chuemere AN. Gas Chromatography Mass Spectrometry Investigation and Minerals, Proximate Components and their Relationship on Hydromethanolic Extract of Unripe *Musa sapientum* Peel and Pulp. *Int J Biomed Investig* 2020; 3: 126. doi:[10.31531/2581-4745.1000126](https://doi.org/10.31531/2581-4745.1000126)

5	Erucicacid z-2-Octadecen-1-ol cis-Methyl-11-eicosenoate	27.057	2.54
6	2-Heptadecanone 2-Pentadecanone	12.28	1.36
7	Cyclopropaneoctanal Docosenoic acid 13-Octadecadienol	21.127	1.27
8	Oleic acid	30.256	1.06

**Table 2:** GC-MS analysis of bioactive components in unripe *Musa sapientum* pulp.

S.No	Physiochemical Compounds	Retention Time	Peat Area Percent
1	5-Hydroxy-4-methyl-3-heptanone 6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide Carbonicacid, Prop-1-en-2-yl tridecyl ester	8.361	7.35
2	Oleic acid n-Hexadecanoic acid	27.405	7.02
3	Stigmasterol Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	34.566	5.32
4	n-Hexadecanoic acid Tridecanoic acid	13.931	4.9
5	Dodecane 1-fluoro-Oxalic acid CyclobutylTetradecyl ester 1-Heptadecanamine	10.588	4.83
6	trans-13-Octadecenoic acid cis-13-Octadecenoic acid	16.413	4.34
7	Ethyl 2-acetamido-3,3,3-trifluoro-2-(4-fluoranilino) propionate Acetamide 2-chloro-N-(3-cyano-4,6-dihydro-4,4,6,6- teramethylthieno [2,3-c] furan-2-yl)-Diethyl 3-chloro-2- hydroxypropylmalonate	29.263	3.36
8	Thymol TMS derivatives Silanol 7-Chlorocinchoninic Acid	30.685	2.52



**Table 3:** Mass spectra molecular weight of the detected sixteen fragments of the peel.

m/z value of molecular ion detected (g/mole)	Name of the molecular ion detected	Molecular formula for the detected molecular ion
320	9 – Octadecenoic acid, methyl ester (E)-	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>
298	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>
296	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
284	trans-13-Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
282	Erucic acid	C <sub>19</sub> H <sub>39</sub> O <sub>2</sub>
280	2- methyl – Z, Z- 3, 13 - Octadecadienol	C <sub>19</sub> H <sub>36</sub> O
280	9, 12 – Octadecenoic acid (Z, Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
270	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
268	Z – 2 – Octadecen – 1 - ol	C <sub>18</sub> H <sub>36</sub> O
266	9 – octadecenal, (Z)-	C <sub>18</sub> H <sub>34</sub> O
264	9, 17 – Octadecadienal, (Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
256	n- Hexaecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
254	2-Heptadecanone	C <sub>17</sub> H <sub>34</sub> O
242	Methyl tetradecanoate	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>
226	Hexadecane	C <sub>16</sub> H <sub>34</sub>
212	Tetradecanal(Myristicaldehyde)	C <sub>14</sub> H <sub>28</sub> O

Similarly, GC-MS ethanol extract also characterized eight compounds in banana peel [12], but, however, without the observed fragments in hydro-alcohol extract. More importantly, the finding validates hydro-alcohol as a better solvent for extraction of medicinal plants of physio-pharmacological interest [10,11]. Furthermore, the present study depicted that the maximum peak area for the peel was shown for trans-13-Octadecenoic acid (63.97%) and n-Hexadecanoic acids (15.95%) with retention time of 16.614 and 13.818, meanwhile for the pulp was 5-Hydroxy-4-methyl-3-heptanone (7.35%) and n-Hexadecanoic acids

(7.02%) with retention times of 8.361 and 27.405 respectively. One other result of the study revealed that the mass-to-charge ratio (m/z) of the mass spectra of the fragments (g/mole) in peels was lower (212-338) as against the pulps (296-522). In comparison, n-Hexadecanoic acids, trans-13-Octadecenoic acids and oleic acids were found for both peel and pulp. These bioactive constituents evidently has been found to be compounds with known biological and medicinal activities which therefore was strongly indicative that unripe peel and pulp possess a wide range of different health-promoting properties yet to be explored.

**Table 4:** Mass spectra molecular weight of the detected eleven fragments of the pulp.

m/z value of molecular ion detected (g/mole)	Name of the molecular ion detected	Molecular formula for the detected molecular ion
522	Bisnorandrostande, 3, 12 –diacetox – 17 – (1 – methyl -4 –oxo-5-phenylpentyl	C <sub>33</sub> H <sub>46</sub> O <sub>5</sub>
489	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, - tetradecamethyl-	C <sub>13</sub> H <sub>40</sub> O <sub>6</sub> Si <sub>7</sub>
412	Stigmasterol	C <sub>29</sub> H <sub>48</sub> O

382	Oleic acid	$C_{18}H_{34}O_2$
366	Carbonic acid, but -2 - yn -1 - yloctaecyl ester	$C_{23}H_{48}O_3$
356	Ethyl 2 -(2 -Chloroacetamido) -3,3,3 -trifluoro -2 -(3 -fluoroanilino) propionate	$C_{13}H_{13}ClF_4N_2O_3$
356	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$
355	1H- Indole - 2 - carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropyl	$C_{21}H_{25}NO_4$
330	Succinic acid, di(2-methoxyphenyl) ester	$C_{18}H_{18}O_6$
322	Ethyl 2 - acetamido -3,3,3 -trifluoro - 2 -(2 -fluoroanilino) propionate	$C_{13}H_{14}F_4N_2O_3$
324	n-propyl 11-Octadecenoate	$C_{21}H_{40}O_2$
320	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$
296	Trans - 13 - Otadecenoic acid, methyl ester	$C_{19}H_{36}O_2$

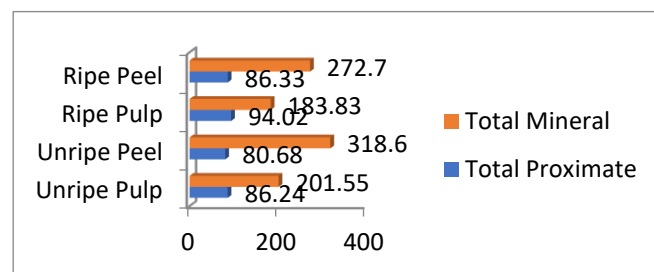
Results for the estimation and determination of proximate and mineral nutritional compositions and quality for the unripe

and ripe peels and pulps, nutritive values (Kcal), harmonic mean and overall mean are summarized in table 5 and total proximate and mineral contents, relationship between total proximate and moisture contents and nutritive values are shown in Figures 3, 4 and 5 respectively.

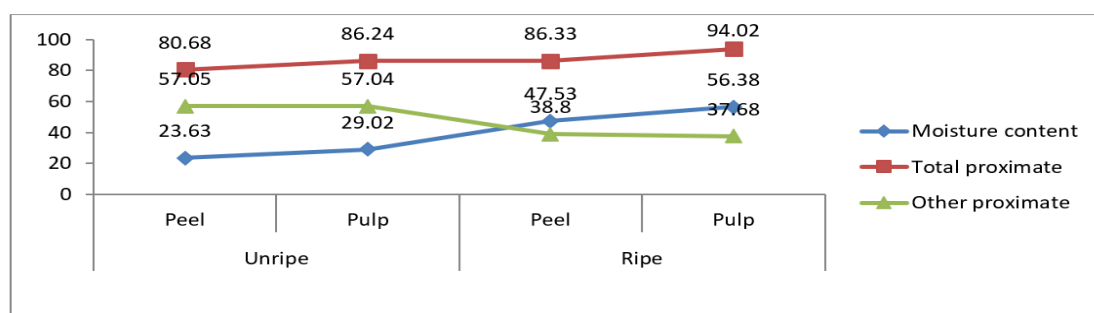
The observation that proximate components of unripe and ripe *Musa sapientum* peels and pulps was rich and additionally, showed marked varietal differences in moisture, carbohydrates, lipids, proteins, ash, crude fibre, as well as in mineral contents depicted on the above table, was in consonance with previously research reports [6,13,14,20-23].

Aside from the generalization of varietal differences in the nutritional compositions, the present study depicted that the overall highest proximate content (%) was ripe

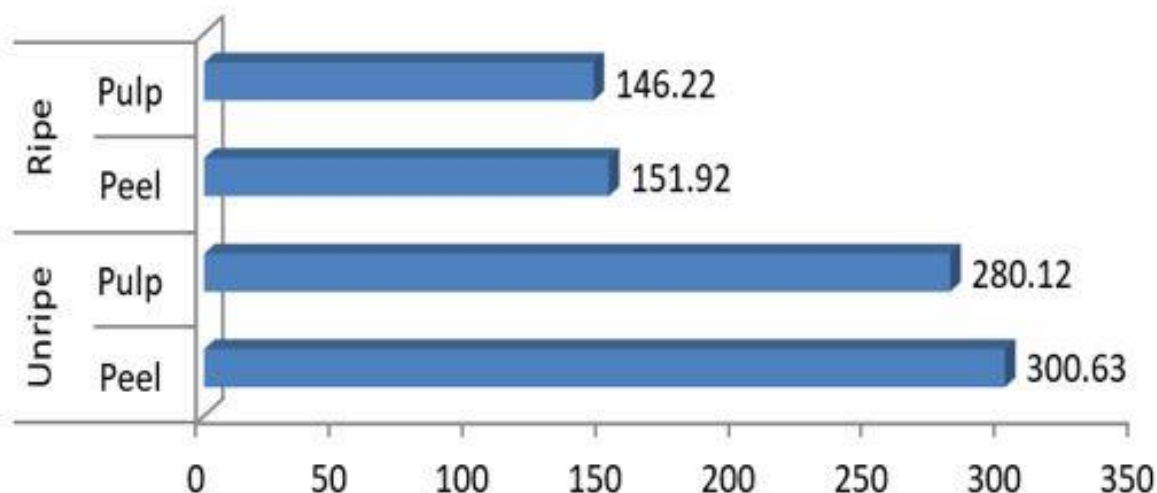
pulp (94.02) followed by ripe pulp (86.33) then unripe pulp (86.24) and least unripe peel (80.66). Unripe pulp has comparatively high protein, fat content also carbohydrate in sufficient amount with low fibre and



**Figure 3:** The relationship between proximate and mineral nutritional compositions.



**Figure 4:** The relationship between total proximate and moisture content.



**Figure 5:** Nutritive Value.

**Table 5:** Analysis of nutritional composition of proximate (%) and mineral (mg/g) contents, nutritive values, harmonic mean of unripe and ripe *Musa sapientum* peels and pulps

Parameters	Unripe peel	Unripe pulp	% difference	Ripe peel	Ripe pulp	% difference
Proximate Moisture content	23.63	29.2	26.12	47.53	56.38	51.57
Ash content	1	1.3	1.13	5.3	2.7	3.57
Fat content	5	5.5	5.23	1.7	1.6	1.64
Crude fibre	1.8	2.2	1.98	2.81	2.56	2.67
Protein content	2.15	4.62	2.93	4.59	2.41	3.16
Carbohydrate content	47.1	43.42	45.18	24.4	28.37	26.23
Overall Total	80.68	86.24	85.57	86.33	94.02	88.84
Harmonic Mean	2.62	3.57	3.02	4.24	3.23	3.66

The moisture content was found to increase on ripening, the ripe pulps having the highest moisture contents (56.38%) followed by ripe peel (47.53%) then unripe pulp (29.20%) and least unripe peel (23.63%) concurring with some observations [6,20]. Interestingly, increases in moisture content almost parallel increases in overall proximate content ordered: unripe peel < unripe pulp < ripe peel < ripe pulp. The result showed there was an inversely proportional relationship between moisture content and the other bio-proximate constituents (Figure 4). It could be plausibly that higher moisture content might modulate micro-

nutrient imbalances with associated undefined health-risks evident by much lower overall proximate content for ripe (38.24%) than unripe (57.05%) (Figure 4). Notwithstanding, high moisture contents has been associated with moisture gains from atmospheric or from microbial activities during the ripening periods [23].

The research findings also revealed marked nutritive value varietal differences for the different parts and types of the fruits ranked: unripe peel > unripe pulp > ripe peel > ripe pulp respectively in descending order



with respective concentration (k/cal): 300.63>280.12>151.92>146.22; with overall descending concentration ordered: unripe (580.75) > ripe (298.14). Unripe extracts with higher mineral compositions (peel = pulp) and low moisture contents (peel=pulp) exhibited almost twice stronger nutritive values than ripe (Figures 4 and 5).

In the present study, overall highest mineral content (mg/g) was shown for unripe peel (318.6), followed by ripe peel (272.7), then unripe pulp (201.55) and least ripe pulp (183.83). The percent difference in mineral content in descending concentration was ranked: unripe (236.14) > ripe (204.38). Unripe peel almost had the highest value of minerals with calcium the largest among the entire samples consistence with previous studies [14]. Preponderance of the mineral concentration of the unripe and ripe peels was suggestive that peels could be used to fortify pulps as value added foods and to serve as functional mineral food source to boost metabolism.

As found, unripe pulp presented appreciable higher zinc content (12.35 mg/g) than either ripe pulp (4.48 mg/g), unripe peel (3.12 mg/g), or ripe peel (2.90 mg/g), in contrast to the previous Nigerian report [6]. Our findings inferred that unripe pulp could be beneficial in body's defensive (immune) mechanism and other metabolic systems to properly work.

Beside the general conventional approach for the presentation of the nutritional compositions, harmonic mean comparative differential analysis has been postulated for the first time to present scientific information on the mechanistic-link in the isolated proximate and mineral constituents in the different parts and types of the fruits. It aims allowing confirming qualitatively and quantitatively the efficacy of the unripe and ripe peels and pulps as a potential source for folk medicine, the exploration of new compounds plausibly as new tools for health benefits. Subsequently, harmonic mean analysis established the proximate composition ordered as follows: ripe peel > unripe pulp > ripe pulp > unripe peel respectively in descending order with respective concentration: 4.24 > 3.57>3.23 >2.63; and the percent difference as: ripe (3.66) > unripe (3.02). On the other hand, mineral distribution was proposed in descending order: unripe peel > ripe peel > unripe pulp > ripe pulp respectively with respective concentration (mg/g): 8.45> 7.59 >7.58> 2.48; and the percent difference: unripe (7.99) > ripe (3.72); while the harmonic mean percent difference was: unripe (5.5) > ripe (3.69). It showed unripe are good source of mineral nutritional foods than ripe. Overall, the peels were the

most mineral nutrient-rich than the edible parts, justifying as potential source for food of physiological interest and pharmaceutical industry to promote their consumption.

The result of the present study highlights scientifically physio-therapeutic efficacy and superiority of the elucidated bioactive compounds and nutritional compositions in the different parts and types of the fruit, allowing scientific confirmation of the use of unripe and ripe *Musa sapientum* peels and pulps for curative and/or prevention of various human diseases. Nonetheless, it is hereby recommended that research should be carried out to properly exploit and process the important compounds in unripe (peel + pulp) fruit as a diet for the development of a high-quality, efficacious and cheap source in the field of safe plant-base food-medicine formulation against specific major disorders of interest.

### Conflict of interest

The authors declare no potential conflict of interest.

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*This manuscript was peer-reviewed*

*Mode of Review: Single-blinded*

*Academic Editor: Dr. Mohamad Taleuzzaman*

