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Research Article

Comparative study of chemical composition and evaluation of the *In-Vitro* antioxidant capacity of unripe and ripe banana species (*Musa Sapientum*) biowastes

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Abstract

The effort of developing countries in addressing zero hunger (Goal 2) with good health and well-being (Goal 3) of Sustainable Development Agenda requires a multi-disciplinary analytical approach to waste materials capable of decomposing through aerobic and anaerobic conditions. Hence a research on ripe and unripe banana peels as potential sources of nutrients, essential minerals and antioxidants was carried out using standard analytical techniques. The study revealed that the percentage moisture of the unripe and ripe banana peels ranged from 4.60 – 17.8; crude protein 1.94 – 2.73; fat 1.76 – 3.25; ash content 11.3 – 14.7; crude fibre 14.2 – 15.5; and carbohydrates 48.4 – 52.7. Mineral content showed significantly high levels of Na, K, Ca, Zn, Fe in unripe peels while that of ripe exhibited higher levels of Mn and P. Na/K for both ripe and unripe banana peels is less than 1 while Ca/P ranged from 1.63 - 2.64. The antioxidant capacity using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay ranged from 3.75 - 13.6 mg TE/g and total phenolic content in unripe and ripe banana peels ranged from 8.42 - 15.8 mg GAE/g with higher value in unripe peels. The results indicate that the peels can be utilized as sources of fibre, carbohydrate and essential minerals in fortification of animal feeds.

Introduction

Consumption of fruits has a crucial role in human diet and demand for such important food commodities has increased very significantly as a result of growing world population and the changing dietary habits (Russel & Gould, 1999). Fruits and vegetables are the most utilized commodities among all horticultural crops [1]. They are eaten raw, minimally processed with some undergoing natural processing such as ripening. High production and growth, and lack of proper handling methods and storage infrastructure, have led to the generation of huge biowastes which are usually discarded. The United Nations Food and Agricultural Organization (FAO) has estimated that at least one third of the food produced globally (estimated as 1.3 billion metric tons) is lost and wasted every year [2] and biowastes are the highest among all types of foods, reaching up to 60 % [3]. Banana is one of the most important crops of the tropical plants. It belongs to the family Musaceae and the genus *Musa sapientum*. It is a climacteric fruit, consumed mostly when the fruit is ripe; ripe and unripe

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banana are also processed into snacks and juice. Banana is very perishable and subject to fast deterioration after harvesting. For this reason, high quantities of wastes are being generated prior to consumption and also high quantities of fruits are lost during their commercialization due to poor post harvesting handling. Banana biowastes include the pseudostem, leaves, inflorescence, fruit stalk, rhizome and peels. Documented literature highlighted the valorization of the wastes including banana peels, which constitute a measurable percent of the banana fruit, proven to be a rich source of crude fibre, carbohydrates, crude protein and essential minerals [4]. This shows its potential of serving as basal materials or components of animal feed. Furthermore, banana peels are used as adsorbent in water purification Chaparadza & Hossenlopp [5] like other natural materials such as pecan shells, rubber seed coat, jute fiber, olive stones, pine wood, Indian rosewood sawdust and clay minerals that are usually applied for this purpose [6-13]. It is also used for ethanol production [14] and also in composting) [15].

This work focuses on assessing the chemical composition and evaluating the total antioxidant capacity of banana varieties commonly consumed *M. sapientum* species (cavendish, red and baby/nino banana) in southwest Nigeria in an *in vitro* system. The study set out for comparative analytical evaluation of the proximate composition, mineral content and antioxidant capacity of peels of selected unripe and ripe banana species.

Methodology

Sample and sample preparation

Three varieties of healthy unripe banana (*M. sapientum*) were obtained from a local market in Iworoko Ekiti (with geographical coordinates 7° 44' N, 5° 16' E), Ekiti State, Nigeria. The botanical authentication of the fruit was carried out at the Department of Plant Science, Ekiti State University, Ado Ekiti, Ekiti State. The unripe banana was divided into two portions with one portion subjected to ripening condition while the peel of the other portion was removed in an unripe stage, cut into smaller pieces for easy oven drying at 45 °C for 34 hours. After ripening, the ripened portion was also peeled, chopped and dried. The dried peels of both ripe and unripe were separately pulverized by an electric blender. The two samples were stored in different airtight sterile sample bottles prior to analysis Figure 1.

Proximate composition analysis

Proximate compositions of the banana peels samples were determined for moisture, fat and ash, in triplicates. The protein content was determined by a semi micro Kjeldahl method while the crude fiber content was determined using standard methods as described by AOAC, 1986 [16]. The carbohydrate content was determined by difference.

Mineral composition analysis

The concentration of the mineral content of the samples was carried out in accordance to the existing protocol of Pearson [17] with little modification. The procedure involved

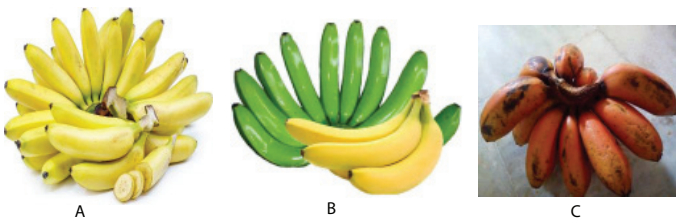

Figure 1: A. Baby banana. B: Cavendish banana. C: Red banana.

dry-ashing of each sample at 550 °C and followed by concentration determination of the minerals in each sample. The concentration determination of each mineral (Mn, Mg, Fe, Zn, Cu, Ca) in each sample was carried out by dissolving 0.5 g of each sample ash in 50 mL distilled water in different beakers; thereafter, 4 mL of mineral acids containing the mixture of HCl and HNO_3 was in the ratio 1:1 was added to each beaker containing the ash solution. Each mixture was thoroughly stirred and heated in a fume cupboard for 15 minutes, cooled and filtered. The resulting concentration of each mineral in each filtrate was analyzed with the aid of Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Instrument Model-200A/2010, Norwalk, Connecticut, 06855), while Na and K was analyzed using Flame Photometer.

Total equivalent antioxidant capacity determination

The radical scavenging potential of the banana peels extract was determined using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay as previously described [18]. 50 mg of each sample was extracted with 20 mL of ethanol and filtered. Working solution of DPPH having absorbance of the range 800 - 1000 at 525 nm was prepared fresh. 0.1 mL of the extract was pipette into a test tube and 9.9 mL of the DPPH solution added and incubated at room temperature for 30 mins (enough time to reach a stable value). This was then transferred into a cuvette and the absorbance was taken at 525 nm. 6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was used as a standard reference to convert the inhibition capacity of each sample to the trolox equivalent antioxidant capacity (TEAC). Standard trolox solution in ethanol were prepared at a concentration of range between 0 – 600 µg/mL. 0.1 mL of each trolox solution was added to 9.9 mL of DPPH radical solution (0 - 24 µM trolox in radical solution). The absorbance was read after 30 mins of incubation at room temperature using Jenway 6705 UV/VIS Spectrophotometer. Results were expressed as mmol trolox equivalents per gram (mM TE/g) extract.

Determination of the total phenolic content

The total phenolic content of the plant extracts was determined using the established Folin Ciocalteu method [19]. A volume of 25 mL of each extract was incubated in a clear 96-well flat bottom plate for 5 min with 125 mL freshly prepared 0.2 N. Folin Ciocalteu's phenol reagent (100 mL 7.5 % sodium carbonate) was added and incubated for 2 hours. The absorbance at 760 nm was measured and the total phenols calculated using the standard gallic acid in 10 % ethanol. Results were expressed as milligram gallic acid equivalents per gram (mg GAE/g) extract.

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Results and discussion

The proximate composition of unripe and ripe peels of local *M. Sapientum varieties*

Table 1 of this report contained the proximate composition of both ripe and unripe peels of *M. sapientum.* The proximate composition of unripe and ripe peels of local *M. sapientum* varieties, as presented in Table 1, reported that peels contain a high percent of moisture, with unripe peels of red banana having a higher percent (17.3 ± 0.12) while unripe cavendish banana has the lowest moisture composition (4.60 ± 0.08). The comparatively low moisture composition in unripe cavendish banana peel could be as a result of de-moisturization resulting from exposure to sun. The content of protein and lipids ranged within 1.94 ± 0.04 – 2.73 ± 0.03 and 1.76 ± 0.03 – 3.03 ± 0.04 respectively. The values reported in comparison to that reported for the fruit showed that the peels are more reliable source of protein and lipids [20,21]. The peels demonstrated a high percent of ash from 11.3 ± 0.16 - 14.7 ± 0.04. Higher percent was observed in unripe and ripe cavendish banana peel (13.5 ± 0.07 and 14.7 ± 0.04 respectively) and that could suggest that peels of cavendish banana as rich source of minerals. It was also observed that the calculated metabolizable energy, crude fibre and carbohydrate content of the peels decreased diversely as a result of ripening (Wills, et al. 1984), who reported a decrease in the carbohydrate and crude fibre content in banana pulps due to ripening. Wills, et al. (1984) postulated that the utilization of sugar in respiration could result to a decrease in carbohydrate content while the hydrolysis of hemicelluloses and breakdown of pectic substances resulted to the decrease in fibre content as ripening takes place. The result in turn suggests that utilization of unripe peels of the banana in diets or as supplements could aid growth and proper digestion.

Desirable flavour, quality and texture are usually attained in fruits by ripening processes. Ripening process, in turn is usually accompanied by a change in the chemical composition of fruits. In Table 2, we have the differences in the mean proximate composition of ripe and unripe peels of *M. sapientum.* Table 2 shows the progressive increase or decrease in the chemical composition of banana peels due to ripening. From the result,

Table 1: Proximate Composition.

Parameters	A	B	C	D	E	F	Mean ± SD	CV (%)
Moisture	4.60 ± 0.08	15.5 ± 0.20	14.5 ± 0.12	16.4 ± 0.11	17.3 ± 0.12	17.8 ± 0.02	14.4 ± 4.93	34.4
Protein	2.41 ± 0.01	2.73 ± 0.03	1.94 ± 0.04	2.63 ± 0.03	2.23 ± 0.02	2.18 ± 0.04	2.35 ± 0.30	12.8
Fat	2.94 ± 0.04	3.25 ± 0.03	2.63 ± 0.07	1.76 ± 0.03	3.05 ± 0.04	2.43 ± 0.03	2.68 ± 0.54	20.2
Ash	13.5 ± 0.07	14.7 ± 0.04	13.3 ± 0.11	11.6 ± 0.05	11.9 ± 0.04	11.3 ± 0.16	12.7 ± 1.34	10.5
Crude fiber	15.2 ± 0.04	15.5 ± 0.13	15.3 ± 0.01	14.9 ± 0.03	14.6 ± 0.06	14.2 ± 0.03	15.0 ± 0.48	3.21
[b]Carbohydrate	51.3 ± 0.03	48.4 ± 0.01	52.3 ± 0.01	51.9 ± 0.04	50.9 ± 0.01	49.7 ± 0.13	51.3 ± 1.55	3.02
[c]Fatty acid	2.35	2.60	2.10	1.41	2.41	1.94	2.14 ± 0.42	19.6
[d]Energy (kg/100 g)	1025.9	990	1019.7	1005.7	1015.9	1012.3	1011.6 ± 12.6	1.24

A= Unripe Cavendish banana peel
B= Ripe Cavendish banana peel
C= Unripe baby/Nino banana peel
D= Ripe Baby/Nino banana peel
E= Unripe red banana peel
F= Ripe red banana peel
S.D: Standard deviation
C.V: Coefficient of Variation
[a]Each value represents the mean ± standard deviation values of three replicate determinations
[b]Carbohydrate percentage was calculated as (100-total of other values)
[c]Calculated fatty acids (0.8 x crude fat)
[d]Calculated metabolizable energy (kJ/100g) (protein x 17 + fat x 37 + carbohydrate x 17)

Table 2: Differences in the Mean Proximate Composition (%).

Parameter	A-B	C-D	E-F	Mean ± SD	CV (%)
Moisture	-11.00(-236.00%)	-1.95(13.47%)	-0.52(-3.01%)	-4.49 ± 5.68	-126.50
Protein	-0.32(-13.28%)	-0.69(-35.57%)	0.05(2.24%)	-0.32 ± 0.37	-115.63
Fat	-0.31(-10.54%)	0.87(33.08%)	0.62(20.33%)	-0.19 ± 0.62	-326.32
Ash	-1.19(-8.81%)	1.74(13.05%)	1.74(13.05%)	0.76 ± 1.69	2.22
Crude fiber	-0.27(-1.77%)	0.40(2.61%)	0.37(2.53%)	0.17 ± 0.38	223.53
[b]Carbohydrate	2.88(5.61%)	0.38(0.73%)	1.19(2.34%)	1.48 ± 2.15	488.64
[c]Fatty Acids	-0.20(8.33%)	0.70(33.33%)	0.50(20.83%)	0.33 ± 0.47	142.42
[d]Energy	35.85(3.49%)	14.00(1.37%)	3.56(0.35%)	17.79 ± 16.48	92.64

A= Unripe Cavendish banana peel
B= Ripe Cavendish banana peel
C= Unripe Baby/Nino banana peel
D= Ripe Baby/Nino banana peel
E= Unripe Red banana peel
F= Ripe Red banana peel
S.D: Standard deviation
C.V: Coefficient of variation
-ve value denotes an enhancement due to ripening
+ve value denotes a reduction due to ripening

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ripening seems to be a depleting factor. It was observed that the moisture and protein content of the peels increased due to ripening. The result shows that ripe peels exhibits a low shelf life of peels and this suggests that utilization of banana peels should be encouraged before ripening takes place or perhaps efforts to preserve the ripe peels need to be engaged. The ash, crude fibre, carbohydrates, fatty acids and metabolizable energy content also decreases as ripening takes place. The result shows a suitability of unripe banana peels as animal feeds and food supplements compared to ripe peels.

Mineral composition of ripe and unripe peels of *M. Sapientum varieties*

The result of mineral content (Table 3) shows the presence of high concentration of potassium (K) in the peels. The concentration (mg/100g) ranged within 4565 ± 5 and 4870.0 ±20.0. The peels appreciable high potassium content indicates its potential to regulation of body fluids and maintains normal blood pressure. Soetan, et al. [22] stressed the importance of calcium as an important constituent of bones and a component actively involved in the regulation of nerve and muscle functions. The study revealed that the calcium content of the peels ranged from 279.0 ± 1.0 - 360.0 ± 32.0, with the highest concentration found in unripe red banana peel while ripe baby banana has the lowest concentration. Iron known for oxygen transport is of low concentration in the peels although the low value has also been reported for the fruit [21]. The low concentration suggests that banana peel will be an idyllic source of iron since its excess could result to abnormal functioning of the immune system, cell growth and the heart [23]. Higher Zinc (5.55 ± 0.15 mg/100g) and potassium level (171.3 ± 0.75 mg/100g) was observed for unripe cavendish banana peels suggest that the peel could play an important role in body defense such as reduction of blood pressure, wound healing and breaking down of carbohydrates. The peel's potential to aid formation of skeletal and cartilage was revealed, as high percentage of manganese was found to be high, ranging from 64.6 ± 0.01 - 74.3 ± 0.25 mg/100g. The result also agrees with that reported by Anhwange [24]. The ratio of sodium and potassium in food samples are of great importance as the sodium level often influence the increase of blood pressure while potassium aids the maintenance of the blood pressure. It could be suggested that the peels are safe for consumption as the potassium to sodium content is high while the sodium to potassium content is very low. The differences in the mean mineral content of unripe and ripe peels of *Musa sapientum* presented in Table 4 showed that the sodium and manganese level of the peels increased as ripening takes place in cavandish and red banana which is not so for baby banana peels.

Iron and zinc contents of the peels were observed to decrease randomly in all as a result of ripening. The calcium and calcium to potassium ratio level only increase due to ripening in Cavendish banana peels (44.00(-15.77%) and -1.01(-61.96%) respectively. Potassium to sodium level and phosphorus increased in baby banana and red banana peels. This could suggest that the potential of the peels as mineral source is highly affected by ripening.

Banana peels have been reported to have a high capacity to scavenge DPPH free radicals and good lipid peroxidation inhibitors [25]. Many studies has also shown that banana peel extract possess a stronger antioxidant activity than pulps [26,27]. The result, as presented in Table 5 shows that the antioxidant properties of banana peels reduced with ripening with values ranging from 3.75 ± 0.16 mg TE/g to 13.64 ± 0.30 mg TE/g. The potential of phenolic compounds as antioxidant is attributed to its ability of inactivating free radicals. The phenolic content of the peel extract ranged from 8.42-15.79mg GAE/g. A comparatively higher value has been reported for rhizomes of various bananas by kandasamy and aradhya [28]. The result presented in Table 5 revealed the presence of higher phenolic content in unripe cavendish banana peels (14.80±0.04) while the lowest percent was found in ripe cavendish banana peels (8.42±0.37). It was also that the phenolic content value varied in the samples due to ripening, with unripe peels having a higher content compared to ripe peels, except in the case of red banana peels [29-31].

Table 3: Mineral composition (mg/100g)[a] of ripe and unripe peels of *M. sapientum* varieties.

	A	B	C	D	E	F	Mean ± SD	CV (%)
Na	148.9 ± 0.65	148.9 ± 0.6	157.0 ± 1.00	143.8 ± 1.25	163.3 ± 0.75	169.0 ± 1.00	155.1 ± 9.68	6.24
K	487.0 ± 20.0	475.6 ± 6.25	481.8 ± 2.50	467.3 ± 2.50	456.5 ± 5.00	476.5 ± 15.0	474.1 ±108.6	2.29
Mn	67.3 ± 0.25	69.0 ± 0.00	64.7 ± 0.05	64.6 ± 0.01	72.3 ± 0.30	74.3 ± 0.25	68. 7 ± 3.98	5.79
P	171.3 ± 0.75	122.5 ±1.50	157.2 ± 1.35	167.3 ± 1.25	138.3 ± 0.3	143.8 ± 1.25	150.1 ± 18.6	12.4
Ca	279.0 ± 1.00	323.0 ± 1.00	343.5 ± 3.50	246.0 ± 2.00	360.0 ± 32.0	292.5 ± 2.50	307.3 ± 42.7	13.9
Zn	5.55 ± 0.15	4.55 ± 0.05	4.45 ± 0.15	4.20 ± 0.10	4.25 ± 0.05	3.85 ± 0.05	4.48 ± 0.58	13.0
Fe	0.17 ± 0.01	0.40 ± 0.00	1.40 ± 0.00	1.22 ± 0.02	1.39 ± 0.01	0.70 ± 0.02	0.88 ± 0.53	60.2
Ca/P	1.63	2.64	2.19	1.47	2.60	2.03	2.09 ± 0.48	23.0
Na/K	0.03	0.03	0.03	0.03	0.04	0.04	0.20 ± 0.01	5.00
K/Na	32.72	31.94	30.68	32.49	27.96	28.20	30.67 ± 2.12	6.91

A= Unripe Cavendish banana peel
B= Ripe Cavendish banana peel
C= Unripe Baby/Nino banana peel
D= Ripe Baby/Nino banana peel
E= Unripe Red banana peel
F= Ripe Red banana peel
S.D: Standard deviation
C.V: Coefficient of Variation
[a]Each value represents the mean ± standard deviation values of three replicate determinations

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Table 4: Differences in the mean mineral content (mg/100g of unripe and ripe banana (*Musa sapientum*) peels.

	A-B	C-D	E-F	Mean ± SD	CV
Na	-0.05(-0.03%)	13.20(8.41%)	-5.75(-3.52%)	2.47 ± 9.72	6.24
K	11.40(2.34%)	14.50(3.01%)	-20.00(-4.38%)	1.97 ± 1.98	2.29
Mn	-1.75(-2.60%)	0.05(0.07%)	-1.95(-2.70%)	-1.22 ± 1.10	5.79
P	48.80(28.49%)	-10.10(-6.4%)	-5.50(-3.98%)	11.07 ± 32.79	12.42
Ca	-44.00(-15.77%)	97.50(28.38%)	67.5(18.75%)	40.33 ± 74.56	13.89
Zn	1.00(18.02%)	0.25(5.62%)	0.40(9.41%)	0.55 ± 0.40	12.95
Fe	0.23(135.29%)	0.18(12.86%)	0.69(49.64%)	0.37 ± 0.96	60.23
Ca/P	-1.01(-61.96%)	0.72(32.88%)	0.57(21.92%)	0.09 ± 0.28	22.97
Na/K	0.00(0.00%)	0.00(0.00%)	0.00(0.00%)	[a]---- ± -----	-----
K/Na	0.78(2.38%)	-1.81(-5.90%)	-0.24(-0.86%)	-0.42 ± 1.30	6.91

A= Unripe Cavendish banana peel
B= Ripe Cavendish banana peel
C= Unripe baby banana peel
D= Ripe baby banana peel
E= Unripe red banana peel
F= Ripe red banana peel
S.D: Standard deviation
C.V: Coefficient of Variation
[a]Not applicable
Numbers in parentheses are percentage increase for the mean difference of the mineral content
-ve value denotes an enhancement due to ripening
+ve value denotes a deflation due to ripening

Table 5: Relationship between the Antioxidant Capacity and Total Phenolic Content of Unripe and Ripe Musa Sapientum Peels.

Samples	Total phenolic content	Antioxidant capacity	[B]pac
A	[a]8.42 ± 0.04	8.03 ± 0.23	0.95
B	14.8 ± 0.37	3.75 ± 0.16	0.25
C	12.0 ± 0.76	13.6 ± 0.30	1.18
D	15.8 ± 0.14	4.45 ± 1.72	0.28
E	11.2 ± 0.54	6.68 ± 0.16	0.60
F	8.95 ± 0.03	4.89 ± 0.19	0.55

A= Unripe Cavendish banana peel
B= Ripe Cavendish banana peel
C= Unripe baby banana peel
D= Ripe baby banana peel
E= Unripe red banana peel
F= Ripe red banana peel
[a]Mean± Standard deviation of the total phenolic content and the antioxidant capacity of unripe and ripe *M. sapientum* peels
[b]PAC- Phenol antioxidant coefficient calculated as ratio of TEAC(mg TE/g)/ total phenolic content (mg GAE/g)

In order to obtain more reliable comparison and relationship between the total phenolic content and antioxidant capacity among the samples, an additional parameter ([b]PAC- Phenol antioxidant coefficient calculated as the ratio between total phenolic content-TPC and antioxidant capacity) was introduced. The PAC results showed non-correlation between increasing TPC and increasing antioxidant capacity of the samples; hence, a higher value of TPC does not imply a higher value of antioxidant capacity of the samples. This could potentially be because of the presence of other antioxidants in varying concentrations which were not determined in the samples.

Conclusion

The study shows that banana peels are reliable source of minerals and bioactive substances in *Musa sapientum* peels and therefore suggests that the peels possess valuable medicinal potential yet to be exploited. Also, ripening which is often characterized with chemical changes appeared to deplete the chemical composition and the antioxidant properties of the peels. In that case, the study suggest that banana peels, usually discarded as wastes should be utilized as food adjuncts and animal feeds which will boost food security, improve zero hunger and reduce unemployment rate. Furthermore, for the effective utilization of banana peels, unripe banana should be reduced or prevented from ripening as ripening depletes its chemical composition.

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