Trends in Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Trends in Applied Sciences Research

ISSN 1819-3579
DOI: 10.3923/tasr.2020.115.124

Research Article
Enhancement of Bioactive Compounds During Fermentation of Unripe *Musa paradisiaca*

[1]Atunnise Adeleke Kazeem, [1]Salau Tioluwani Bamidele, [2]Odufuwa Kuburat Temiptope, [1]Olukanni David Olumide, [2]Adeyanju Moronke Muinat and [1]Salau Bamidele Adewale

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Osun State, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ogun State, Nigeria

Abstract

Background and Objective: Plantain is known as a rich source of several bioactive components yet there are several local claims on how those bioactive components could be promoted by employing fermentation. This study was set to investigate the effect of fermentation on the phytochemical constituents; exploring Fourier Transformer Infrared (FTIR) spectroscopy and Ultraviolet-visible (UV) spectroscopy for possible detection of structural modifications on phytochemicals as well as to evaluate the impact of fermentation on the antioxidants potentials on unripe plantain fruit extract. **Materials and Methods:** Plantain fruit was sourced at a local market in Ede, Osun state, Nigeria, peeled, washed, homogenized with distilled water; the proximate, phytochemical, FTIR, UV and antioxidants analyses were investigated. **Results:** Fermentation significantly increased protein content while crude and soluble fibre fraction reduced significantly. All the selected phytochemical components increased significantly except for saponin and tannin that remain unaffected. The FTIR and UV analyses showed that the fermentation process resulted in the modification of some bioactive compounds (quercetin to quercetagetin, flavones and 2"-O-Xywsylvitexin) and formation of a class of phenolics (p-coumaric acid). **Conclusion:** This study showed the antioxidant potential of unripe *M. paradisiaca* could be greatly amplified through fermentation; hence the formation of a new compound could be the key behind these potentials.

Key words: Antioxidants, fermentation, fourier transformer infrared spectroscopy, *Musa paradisiaca*, bioactive compounds

Citation: Atunnise Adeleke Kazeem, Salau Tioluwani Bamidele, Odufuwa Kuburat Temiptope, Olukanni David Olumide, Adeyanju Moronke Muinat and Salau Bamidele Adewale, 2020. Enhancement of bioactive compounds during fermentation of unripe *Musa paradisiaca*. Trends Applied Sci. Res., 15: 115-124.

Corresponding Author: Atunnise Adeleke Kazeem, Department of Biochemistry, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Osun State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Africans' beliefs in herbs and other functional foods are as old as the origin of Africa. Despite the integration and popularity of western conventional medicine across the world, Africans strongly acknowledge the potency of herbs and other functional food (Nutraceutics) as the major source of remedy that poses no side effect[1].

Natural products of fruits and vegetable origin have shown as promising pathways in the prophylaxis and therapeutic of both acute, chronic disease and ailments, including terminal diseases like cancer[2,3]. The potentials of these herbs and functional foods are essentially linked to the antioxidant properties as well as other bioactive components[4,5] which are undeniably safe; pose almost no side effects and readily available[6]. A few of these plant products include but not limited to citrus, lettuce, tomatoes, mints, banana and plantain.

Plantain (*Musa paradisiaca*) is one of the most consumed fruits in the West African sub-region, rich in vitamins A, B6, C, minerals and dietary fibre[6]. *Musa paradisiaca* contains several bioactive compounds, like phenolics, carotenoids, lycopene and phytosterols, which are highly needed in the diet for optimum functioning of physiological processes and remediation of cellular assaults in both human and animals. Bioactive molecules present in plantain fruit have shown notable antioxidant activities against various forms of Reactive Oxygen Species (ROS)[7], either endogenous or from other sources. Although there is a large volume of articles on *M. paradisiaca* potentials against various ailments including the terminal ones, however, there is dearth of information to ascertain the possible bioactive compounds responsible for this claim.

This inadequate scientific investigation of the biomedical potential of fermented plantain fruit extracts motivates this study. Hence, this work was set to determine the effect of varying fermentation period on unripe plantain fruit extract with regards to broad-spectrum ROS scavenging potential and evaluate structural modifications by exploring UV-Vis and FTIR (Fourier Transformer Infrared) spectroscopy.

MATERIALS AND METHODS

Sample collection and preparation: Unripe plantain fruit was sourced from a local market in Sekona, Osun state, Nigeria. This study was done between January-September, 2019. The plantain was peeled, washed with distilled water, chopped into smaller sizes then homogenized using electric blending with distilled water (ratio 1: 9 w/v) and divided into three equal portions. The fresh portion was immediately covered and refrigerated at -20°C. The second and third portions (48 and 96 h fermented) were exposed to air for about 30 min to encourage adequate microbial inoculation by natural means (chance inoculation). Afterward, the container was covered with air-tight cork and allowed to stand for 48 and 96 h. After 48 h of fermentation, the 48 h portion was refrigerated at -20°C to terminate the fermentation process while the 96 h was left for another 48 h. The unfermented (0 h) and fermented (48 and 96 h) samples were filtered using muslin cloth into a new sterile bottle and refrigerated at -20°C for further analyses.

Microbial screening for potential pathogens: The microbial screening was done using standard laboratory procedures at the Microbiology Laboratory of the Department of Microbiology, College of Medicine, University of Lagos, Nigeria.

Proximate analysis

Moisture content: The moisture content was determined by using a dry-oven method. Clean and dry Petri-dishes were weighed by using meter balance and their respective weights were recorded (W1). Five gram of the sample was weighed into pre-weighed dried dishes (W2) spreading as much as possible. The dishes containing the sample were transferred into an oven maintained at 105±2°C and dried for 3 h. After 3 h they were transferred to the desiccators to cool and then weighed. This process was continued until a constant weight (W3) was observed as the percentage of moisture content:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight due to drying (W2-W3)}}{\text{Weight of sample taken (W2-W1)}} \times \frac{100}{1}$$

Protein content determination: Lowry protein method was slightly modified according to the method described by Hartree[8]. The assay was carried out by diluting the samples to 1 mL with water and adding 0.9 mL of solution A (2 g L^{-1} potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) and 100 g L^{-1} sodium carbonate (Na_2CO_3) in 0.5 M NaOH) before incubation for 10 min at 50°C. Following this, the samples were cooled down to room temperature, added 1 mL of solution B (0.2 g L^{-1} $KNaC_4H_4O_6{\cdot}4H_2O$ and 0.1 g L^{-1} copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) in 0.1 M NaOH) and left for 10 min. Finally, 3 mL of solution C (Folin-Ciocalteu phenol reagent in H_2O (1:16 v/v)) was added before incubation for 10 min at 50°C. A standard curve was made of bovine serum albumin (BSA; 0-1 mg L^{-1}) and absorbance was read at 650 nm.

Fibre content determination: Crude fibre estimation was done as follows: 2 g (W1) of the sample was weighed into conical flask (1 L) containing 200 mL of boiling and 1.25% of H_2SO_4 which was added and boiled gently for 30 min. The mixture was filtered through muslin cloth and the residue washed thoroughly with hot distilled water and then rinsed once with 10% HCl twice with ethanol and rinsed to drain dry, then the residue was scrapped into a crucible and allowed to dry in the oven at 105±2°C, cooled in a desiccators and weighed (W2). The residue was ashed at 55°C, for 90 min in a muffle furnace, cooled and weighed again (W3)[9]:

$$\text{Fibre (\%)} = \frac{W2 - W3}{W1} \times \frac{100}{1}$$

Plant secondary metabolites estimation

Determination of saponin: Saponin quantitative determination was carried out using the method priorly reported[10]. In brief, the method requires 20% aqueous ethanol added to sample, heat in water bath at 55°C for 4 h. Saponin content was calculated in percentage using gravimetry method.

Determination of tannins: The estimation of tannins was carried out by a colorimetric assay[11] while different concentrations of tannic acid (6.25-50 mg) were prepared by serial dilution from the stock solution (50 mg/100 mL of 70% acetone). The absorbance was measured at 725 nm after the addition of 0.5 mL of folin phenol reagent and 2.5 mL of Na_2CO_3.

Estimation of total carotenoids and lycopene: The method of Zakaria *et al.*[11] was used. Total carotenoids and lycopene were extracted from the plantain juice samples (A and B) using petroleum ether and estimated by spectrophotometer at 450 and 503 nm, respectively.

Estimation of total phenols: The method is based on the oxidation of molecules containing -OH groups. In brief, 1 mL of each extract in three replicate was used. This test is done by using Tannic acid as standard. The total phenolic content was expressed as mg tannic acid equivalents per 1 g dry of the sample[12].

UV-vis and FTIR spectra analysis

FTIR analysis: Plantain aqueous samples were used for FTIR analysis. Ten microliter was encapsulated in 10 mg of KBr pellet, to prepare translucent sample discs. The encapsulated sample of each portion was loaded in FTIR spectroscope, with a Scan range from 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. FTIR spectra data table used was IR Spectrum Table and Chart | Sigma-Aldrich Inc[13].

UV-vis analysis: Plantain extracts were centrifuged at 5000 rpm for 5 min. The supernatant was collected and used for UV-Vis analysis. The UV lambda range of 190-400 nm was selected on the UV-Vis spectrophotometer[14].

Antioxidants potentials analysis

Ferric thiocyanate (FTC) method: The standard method as described by Kikuzaki and Nakatani[15] was used. A mixture of 4.0 mg sample in 4 mL absolute ethanol, 4.1 mL of 2.5% linolenic acid in absolute ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0) and 3.9 mL of water was placed in a vial with a screw cap and then placed in an oven at 40°C in the dark. To 0.1 mL of this solution was added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Three minutes after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance was read at 500 nm every 24 h until the absorbance of control reached the maximum. Butylated hydroxytoluene (BHT) and α-tocopherol were used as positive controls while the mixture without sample was used as the negative control.

Ferric Reducing Antioxidant Power (FRAP) method: The ferric reducing potential of the sample was analyzed by taking 1 mL of different dilutions of standard solutions of gallic acid (10-100 μg mL^{-1}). The samples that had been adjusted to fall in the linearity range, 500 μg mL^{-1} were taken in 10 mL volumetric flasks and mixed with 2.5 mL of potassium buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide[16]. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture to stop the reaction. To 2.5 mL of the above solution, 2.5 mL of distilled water was added and then 0.5 mL of 0.1% $FeCl_3$ was added and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance read was converted to gallic acid equivalent as milligrams per gram of dry material (GAE g^{-1}) using a gallic acid standard curve.

Nitric oxide scavenging assay: Nitric oxide radical inhibition was estimated using Griess Illosvory reaction[17,18]. Griess Illosvory reagent was generally modified by using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%) and the concentration of nitrite was assayed at 546 nm and calculated with the control absorbance of the standard nitrite solution:

$$\text{Scavenging activity } (\%) = \frac{A_{control}\text{-}A_{test} \text{ or } A_{std}}{A_{control}} \times 100$$

Hydrogen peroxide scavenging assay: Hydrogen peroxide scavenging potential was determined with a little modification as described by Gulcin *et al.*[19]. The percentage inhibition activity was calculated from the formula:

$$\text{Inhibition activity } (\%) = \frac{A_0\text{-}A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as gallic acid (10-100 μg mL^{-1}).

1, 1-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric assay: DPPH solution of 0.1 mM in alcohol was prepared and protected from light influence by maintaining the dark condition and was folded with an aluminum foil and 3 mL of this solution was added to 1 mL various concentrations of (100-2000 μg mL^{-1}) extracts or standard solution of 10-100 μg mL^{-1}. Absorbance was taken after 30 min at 517 nm. The percentage inhibition activity was calculated using the formula:

$$\text{Inhibition activity } (\%) = \frac{A_0\text{-}A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as ascorbic acid[20,21].

Total antioxidants activity: The extracts (750 μL) of each sample were mixed with an equal amount of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (a source of ferric ions). The mixture was incubated at 50°C for 20 min followed by addition of an equal amount of trichloroacetic acid (10%) to stop the reaction and was then centrifuged at 3000 rpm for 10 min. The upper layer (1.5 mL) was separated and mixed with an equal amount of distilled water and 0.1 mL $FeCl_3$ solution (0.1%). A blank was also prepared by using the same procedure and the absorbance was measured at 700 nm as the reducing power[22].

Statistical analysis: Means of three replicates, as well as their standard error of mean (SEM) were determined. The test of significance between the treatments was done using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) to compare between the groups via SPSS version 23.00 software.

RESULTS

The results revealed that no pathogenic organism was present in the fermented sample. The dominant fermenters include yeast, lactobacillus and aero-tolerant propionic acid bacteria as shown in Table 1. Table 2 showed significant ($p<0.05$) increases in protein and moisture contents. The increase was proportional to fermentation time. Crude fibre and soluble contents were significantly ($p<0.05$) reduced while the insoluble fibre fraction increased significant ($p<0.05$) during fermentation. In Table 3, total phenolics, lycopene and

Table 1: Dominant fermenters plate counts

Media	1/10 dilution	1/100 dilution	Remark
Tryptone soya agar	TNTC	TNTC	Too numerous
Eosin methylene blue agar	TNTC	4×10^4	Lactose fermenters not *E. coli*, not *Klebsiella* sp.
Salmonella shigella agar	4.0×10^2	4.2×10^3	Lactose fermenters
MacConkey agar	TNTC	TNTC	Too numerous lactose fermenters
Mannitol salt agar	TNTC	1.2×10^4	Non staphylococcal organisms
Thiosulphate citrate bile salt sucrose agar	No growth	No growth	No *Vibrio cholera*
Sabouraud dextrose agar	TNTC	TNTC	Fermentative yeasts turning plates alcoholic
Cetrimide agar	No growth	No growth	No *Pseudomonas aeruginosa*
Nutrient agar for *Proteus* sp.	No swirming	No swirming	No *Proteus* sp., organism has morphology of yeast
Anaerobic tryptone soya agar	9.2×10^2	9.0×10^2	Mixed culture of yeast and *Bacillus*

TNTC: Too numerous to count

Table 2: Total protein, fibre fractions and moisture contents of fermented plantain

Fermentation period (h)	Total protein (mg/100 g dry weight)	Crude fibre (%)	Soluble fibre (%)	Insoluble fibre (%)	Moisture (%)
0	7.88 ± 0.02^a	10.19 ± 0.00^c	8.65 ± 0.00^b	1.53 ± 0.00^a	36.33 ± 0.00^a
48	64.12 ± 0.31^b	8.85 ± 0.03^b	6.51 ± 0.03^a	2.33 ± 0.03^b	79.84 ± 0.00^b
96	131.34 ± 0.58^c	7.87 ± 0.04^a	5.24 ± 0.08^a	2.63 ± 0.03^b	99.90 ± 0.00^c

Values were represented as Mean±SEM of three parallel data, Values with different superscript in the same column are significantly different ($p<0.05$)

Table 3: Tannin, saponin, carotenoids, lycopene and total phenolics of plantain at different fermentation period

Fermentation period (h)	Tannin (mg/100 g dry weight)	Saponin (mg/100 g dry weight)	Carotenoids (10^2 µg/100 g dry weight)	Lycopene (10^2 µg/100 g dry weight)	Total phenolics (mg/100 g dry weight)
0	6.17±0.35	1.27±0.00	2.17±0.00[a]	2.75±0.02[a]	37.05±0.00[a]
48	5.14±0.02	0.66±0.03	4.17±0.98[b]	6.68±0.31[b]	101.22±0.40[b]
96	5.08±0.15	1.01±0.04	8.07±1.42[c]	10.11±0.58[c]	105.37±2.61[b]

Values were represented as Mean±SEM of three parallel data, Values with different superscript in the same column are significantly different ($p<0.05$)

Table 4: Fourier transformer spectra table interpretation

Range of peak	Fermentation period (h)	Peak value	Functional group	Functional group name	*Vibration
3400	0	3394.83	O-H	Alcohol/phenol	Stretch
	48	3387.11	O-H	Alcohol/phenol	Stretch
	96	3421.83	2°N-H	Secondary amine	Stretch
3000	0	2929.97	C-H	Aldehyde	Stretch
	48	2929.97	C-H	Aldehyde	Stretch
	96	2929.11	-	-	
2300	0	2360.95	S-H	Thiol	Stretch
	48	2332.02	S-H	Thiol	Stretch
	96	2360.95	S-H	Thiol	Stretch
1600	0	1635.69	C=C	Mono alkene	Bending
	48	1639.55	C=C	Mono alkene	Bending
	96	1618.33	C=C	Ring alkene	Bending
1200	0	1153.47	C-O	Alkoxy	Bending
	48	1153.47	C-O	Alkoxy	Bending
	96	1055.38	-	-	
1000	0	1022.31	C-O	Alkoxy	
	48	1022.31	C-O	Alkoxy	
	96	1020.38			
900-500	0	576.74	Sp^2 C-H	Cis bend	Bend
	48	578.66	Sp^2 C-H	Cis bend	Bend
	96	785.74	Sp^2 C-H	Ring bend	Bend

Referred in Fig. 1, *Source: Sigma-Aldrich Inc.,[13]

Table 5: UV spectra (between 190 and 380 nm) peak values of fermented *M. paradisiaca* fruit extract

Period of fermentation (h)	Band II	Band I	*Peak value	#Probable compounds
0		349.60	1	Quercetin
48		363.20, 316.20	2, 9	Quercetagetin
		354.2	5	Quercetin
		338.6	6	Quercetagetin
		361.20, 316.20, 345.20	3, 9, 5	3-hydroxyflavone
96		363.4	11, 1	Quercetin
	260.4	357.40, 344.20	11, 2, 3	3-hydroxyflavone
		342.2	11, 4	2"-O-xywsylvitexin
		339.2	11,5	Quercetagetin
		328.20, 324.20	11, 6, 7	Acacetin
		318.20, 316.20	11, 8, 9	Flavone
	Not applicable	308.6	10	p-coumaric acid

*Referred in Fig. 2, #Source: Mabry *et al.*[14]

carotenoids content increased significant ($p<0.05$) as the fermentation period extend up to 96 h while Tannin and Saponin levels were not altered significantly ($p>0.05$).

Table 4 shows the comparison in the notable peaks of the FTIR spectra of Fig. 1. Hydroxyl O-H stretch (3400 cm^{-1} peak) noted in 0 and 48 h fermentation samples were modified to 2°N-H (anime) stretch at 96 h. Also, mono alkene C=C stretch (1635.69 cm^{-1} peak), as well as cis bend C-H (576.74 cm^{-1} peak) in 0 and 48 h samples were modified to C=C and C-H ring bends, respectively in 96 h fermented sample. Also, the aldehyde (C-H) stretch at 2929.97 cm^{-1} peak and alkoxy C-O bend present in both 0 and 48 h samples are absent in 96 h fermented sample. The thiol (S-H) stretch at 2360.95 peak region present in the unfermented sample (0 h) remains unchanged in both 48 and 96 h fermentation samples.

The UV spectrum chart in Fig. 2 was interpreted in Table 5. The peak values range between 312-318 nm indicates the presence of a conjugated ring structure of flavonoids in both fermented and unfermented samples. Although, flavonoids usually form two Bands (Band I and II) the Band II range between 240-260 nm while Band I (usually 300-380 nm) is more specific because it is determined by several factors including functional group and localization of attached groups around the A ring of the parent compound. Hence,

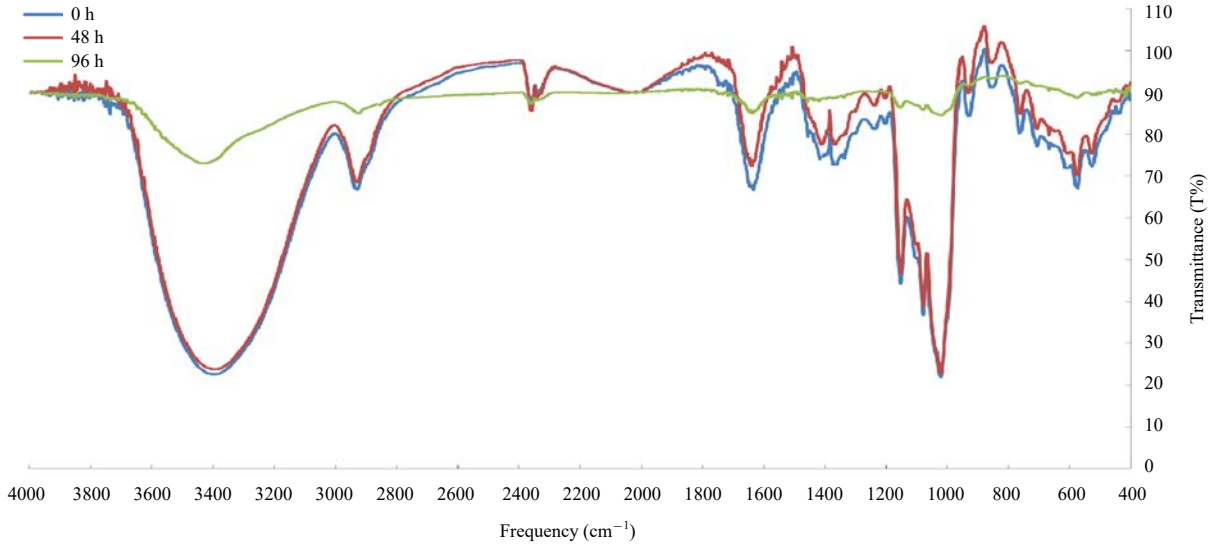

Fig. 1: FTIR spectra of unfermented and fermented *M. paradisiaca* fruit extracts

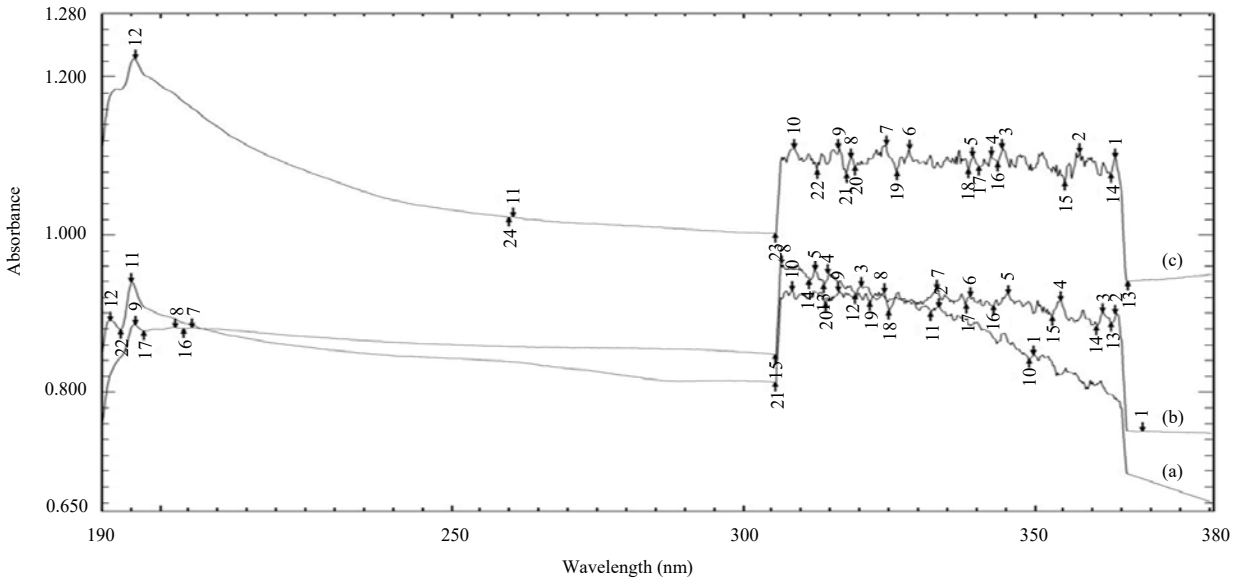

Fig. 2: UV spectra of unfermented, unripe plantain fruit extract
(a) 0 h, (b) 48 h and (c) 96 h

the focus of this study will be based on the lambda range of Band I. From the UV spectra, quercetin is present in both unfermented and fermented *M. paradisiaca* extract. In addition to the quercetin. Quercetagetin and 3-hydroxyflavones were detected in 48 and 96 h fermented *M. paradisiaca* extract, but not in the unfermented sample. Other than the modified flavonoids molecules mentioned, two other flavonoids derivatives were detected and p-coumaric acid.

Figure 3 depicts the free-radicals scavenging profile of *M. paradisiaca* fruit extract in fermented and unfermented forms. Significant ($p<0.05$) percentage increase in free-radical scavenging potentials in was observed in FRAP, DPPH, H_2O_2 and FTC as the fermentation period continued to 96 h.

Figure 4 showed the percentage inhibition potential of peroxidase, nitric acid, Thiobarbituric acid (TBA) and total antioxidants. Significant ($p<0.05$) percentage increase in

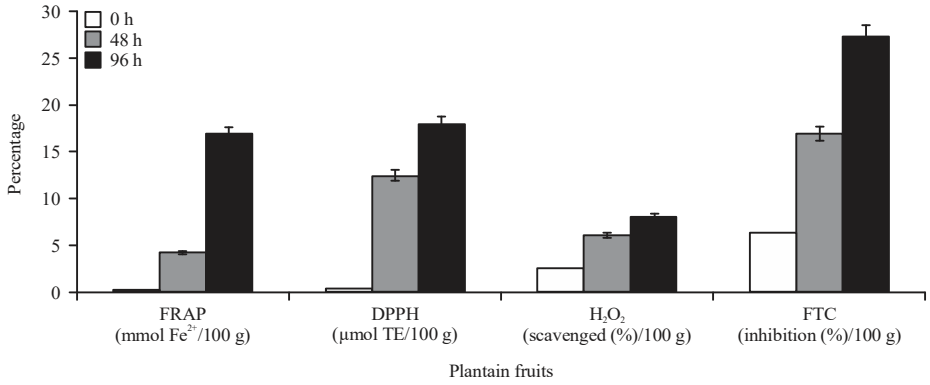

Fig. 3: FRAP, DPPH, H_2O_2 and FTC level in plantain fruit

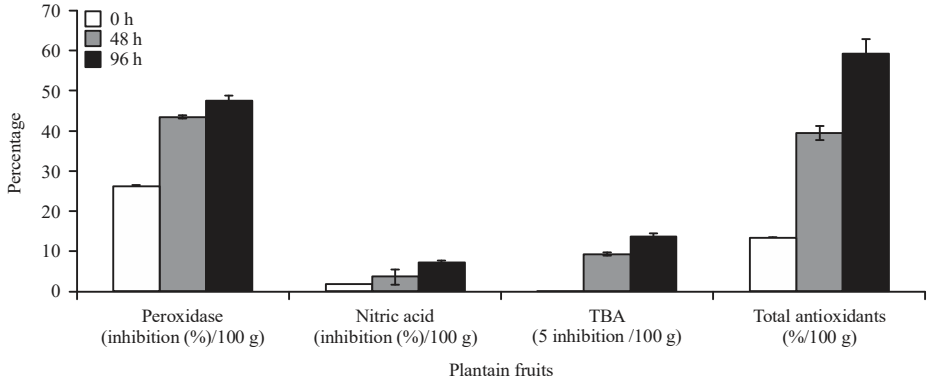

Fig. 4: Peroxidase scavenging power, nitric acid, TBA and total antioxidants of plantain fruits

free-radical scavenging potentials was observed across the board. The order of increase is unfermented >48 h fermented >96 h fermented sample.

DISCUSSION

The results of this study showed that the dominant organisms involved in the fermentation of unripe *M. paradisiaca* fruit are non-pathogenic Propionibacterium, Lactobacillus and Yeast. These identified organisms reported as producers of short-chain fatty acids, phenethylamine, lactic acids and few other beneficial components of fermented food products. Propionic acid is a potent broad-spectrum antibiotic against molds and aerobic spore-producing microbes and may as well kept fermented *M. paradisiaca* extract safe for consumption[23].

This investigation showed that fermentation increased total protein, insoluble fibre and moisture contents. Increased protein content recorded may be ascribed to both catalytic enzymes and other functional proteins secreted by the fermenters[24]. The rate of protein content increase recorded was relatively high within the first 48 h afterward, the rate decreased relatively through another 48 h. This may be one of the indices which substantiate the fact that fermenters could be the major producers of protein within the first few periods of fermentation process[25] while the subsequent increase is due to proteolytic action vis-a-vis hydrolysis of conjugated peptides in plantain into oligopeptides or short-chain peptides such as; angiotensin-I converting enzyme (ACE-I) inhibitor[26]. This further suggests that protein produced by the fermentation process may be much more than just nutritional benefits. An investigation by Faipoux *et al.*[26] revealed yeast protein plays the role of signal inducer in regulating dietary patterns by enhancing satiety. Hence, fermented plantain extract may play an important role in ameliorating life-style induced metabolic syndrome and as well as reduce ROS generation. FitzGerald *et al.*[27] proposed that a group of peptides can lower the blood pressure in hypertensive patients.

Dietary fibre is a collective term for indigestible polysaccharides but can be hydrolyzed by fermentation. It includes insoluble form and the more highly fermentable soluble type[28]. This may be responsible for the reduction in dietary and soluble fibre fraction reported in this work.

Phenolics and carotenoids consumption is associated with a reduced risk of cardiovascular and neurological dysfunctions[29]. The effect of fermentation on tannin, saponin, carotenoids, lycopene and phenolics contents in the unripe *M. paradisica* sample in Table 3 showed that tannin and saponin contents tend to be resistant to fermentation both at 48 and 96 h period. A similar situation was report when *Manihot esculanta* was subjected to fermentation[30]. Within the period of 48 h fermentation polyphenols content could be four folds higher than the native phenols content and this rate was sustained for another 48 h. A large number of microbes including Saccharomyces cerevisiae have been reported to be responsible for phenolics as well as carotenoids and lycopene production during fermentation. Shinohara *et al.*[31] reported that the Saccharomyces species produces phenolics during wine production by decomposition of ferulic acid. Also, studies have shown that certain fermenters could synthesize carotenoids and lycopene[32] up to about 60%. Dharmaraj *et al.*[33] identified Streptomyces strain as one of the organisms responsible for carotenoids production during fermentation. According to Kiplamai *et al.*[34] fermentation may propagate bioavailability of carotenoids through bio-transformation and modification to produce more absorbable ones including fatty acids free and retinol. Furthermore, this increase may be ascribed to the availability of hydrolyzed polysaccharides which stimulate microbes to synthesize carotenoids metabolites[35].

FTIR analysis in this study showed that fermentation significantly modified certain functional groups with respect to the fermentation time. At 48 h, the modification seems insignificant, however, at 96 h the FTIR spectra reveal more structural modification and possible transformation and production of new bioactive molecules. For example, the hydroxyl stretch (3394.83 cm^{-1}) in 0 and 48 h substituted with secondary amine (NH_2), while the C=C (mono alkene) bending (0 h 1635.69; 48 h 1639.55) was transformed into the ring alkene bending (1618.33) in 96 h fermented sample. This structural index may in a way emphasize the formation of new oligopeptides that are probably of great benefit. Lactobacillus has been reported as one the major player in bioactive oligopeptides production during fermentation[36]. Bioactive oligopeptides has been reported to display distinctive biological functions like Angiotensin Converting Enzyme (ACE) inhibition, mineral chelation, satiating, immunomodulating, antioxidant as well as antimicrobial activities[37].

According to Rio *et al.*[38] less than 10% of native polyphenols are absorbed while the remainders are metabolized in the colon by the fermenters (microbiota) to produce a more absorbable form called Polyphenol Microbial Metabolites (PMMs). Thus, it promotes polyphenols bioavailability, distribution in the body as well as free-radicals scavenging potential[39].

The UV-Vis analysis of the fermented *M. paradisiaca* fruit extracts was analyzed between 200-380 nm. Table 2 shows that absorption peaks of the fermented sample compared to unfermented extract. The UV analysis recorded quercetin in both unfermented and fermented *M. paradisiaca* fruit extracts. Quercetagetin, a derivative of quercetin revealed in 48 and 96 h sample could be attributed to the oxidative effect of fermenters on quercetin to form a more potent antioxidant molecule. Also, the fermentation process seems to have converted some of the flavonoids to flavones in fermented samples. Ninety-six hour fermentation process seems to have a distinctive impact on unripe *M. paradisiaca* fruit as a new bioactive molecule (p-coumaric acid) was recorded at lambda max 308.60. It is noteworthy to state that p-coumaric acid is a precursor of apigenin. Liu *et al.*[40] reported that the p-coumaric level was increased during the fermentation of Jogi fruit.

Oxidative stress occurs when free radicals overwhelm the endogenous antioxidant system, in turn, results in cellular damage amongst other complications[41]. The selected array of free radicals scavenging potentials of the fermented *M. paradisiaca* fruit extract in Fig. 1 and 2 showed that both 48 and 96 h fermentation periods significantly scavenged all of the free radicals when compared with the unfermented extract. The increased antioxidant capacity may be attributed to the increased phenolics reported in this study[42]. The structural modification recorded in the FTIR spectrum (Fig. 1), as well as the formation of new phenolics compounds shown in the UV spectrum (Fig. 2), may also have enhanced overall antioxidant potential. Also, the ratio of OH (hydroxyl) group to the size of the phenolics compound may have propelled the antioxidant potential of the fermented samples both at 48 and 96 h. According to a comparative study carried out on quercetin and apigenin, apigenin showed high potency over quercetin[43] and it was also documented that apigenin has higher bio-absorbability as over 51% of it could easily be excreted via urine[44] hence; its toxicity potential could be lower than quercetin which is present in the unfermented extract. The overall increase antioxidant power recorded in the fermented unripe plantain suggests that there is a strong correlation between increase phenolics compound and antioxidant potential recorded[40].

CONCLUSION

This study showed that the antioxidant potential of unripe *M. paradisiaca* could be greatly amplified through fermentation; hence the formation of a new compound could be the key behind these potentials. Aside from the antioxidants properties, fermented unripe plantain could be a potential source of antimicrobial agents as it could not tolerate the growth of some pathogenic organisms. In addition, oligopeptides formation through fermentation of unripe plantain suggests a novel tool for the biosynthesis of biologically active signaling compounds for remediation of chronic diseases including the terminal ones like cancer.

SIGNIFICANCE STATEMENT

The study revealed the potential capacity of fermentation in the enhancement of bioactive compounds with concomitant formation of new compounds majorly nutraceutical. This could be the reason for medicinal and nutritional values of fermented unripe plantain used in the treatment unripe plantain used in the treatment of degenerative oxidative stress diseases. Thus, this study will be of immense importance to scientific world, especially food and nutrition scientist; food, pharmaceutical and nutritional industries and academia at large.

REFERENCES

1. Busia, K., 2005. Medical provision in Africa-past and present. Phytother. Res., 19: 919-923.
2. Rai, P.K., D. Jaiswal, N.K. Rai, S. Pandhija, A.K. Rai and G. Watal, 2009. Role of glycemic elements of *Cynodon dactylon* and *Musa paradisiaca* in diabetes management. Lasers Med. Sci., Vol. 24. 10.1007/s10103-008-0637-0.
3. Pham-Huy, L.A., H. He and C. Pham-Huy, 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 4: 89-96.
4. Faponle, A.S., A. Atunnise, B.O. Adegbesan, O.O. Ogunlabi, K.T. Odufuwa and E.O. Ajani, 2015. Separate and co-administration of *Amaranthus spinosus* and vitamin C modulates cardiovascular disease risk in high fat diet-fed experimental rats. J. Pharmacogn. Phytother., 7: 27-34.
5. Liu, Q., G.Y. Tang, C.N. Zhao, R.Y. Gan and H.B. Li, 2019. Antioxidant activities, phenolic profiles and organic acid contents of fruit vinegars. Antioxidants, Vol. 8. 10.3390/antiox8040078.
6. Kouadio, N., B. Goualie, H. Ouattara, S.K.A. Kra and S. Niamke, 2016. Microorganisms associated with traditional plantain-based food "dockounou" during spontaneous fermentation. Food Environ. Saf. J., 13: 276-282.
7. Akula, R., P. Giridhar and G.A. Ravishankar, 2011. Phytoserotonin: A review. Plant Signaling Behav., 6: 800-809.
8. Hartree, E.F., 1972. Determination of protein: A modification of the lowry method that gives a linear photometric response. Anal. Biochem., 48: 422-427.
9. Madhu, C., K.M. Krishna, K.R. Reddy, P.J. Lakshmi and E.K. Kelari, 2017. Estimation of crude fibre content from natural food stuffs and its laxative activity induced in rats. Int. J. Pharm. Res. Health Sci., 5: 1703-1706.
10. Odufuwa, K.T., A. Atunnise, H.J. Kinnah, P.O. Adeniji and B.A. Salau, 2013. Changes in saponins content of some selected Nigerian vegetables during blanching and juicing. IOSR J. Environ. Sci. Toxicol. Food Technol., 3: 38-42.
11. Zakaria, M., K. Simpson, P.R. Brown and A. Krstulovic, 1979. Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes. J. Chromatogr. A, 176: 109-117.
12. Cetkovic, G., B.J. Canadanovic, S. Djilas, S. Savatovic, A. Mandic and V. Tumbas, 2008. Assessment of polyphenolic content and *in vitro* antiradical characteristics of apple pomace. Food Chem., 109: 340-347.
13. Sigma-Aldrich Inc., 2019. IR spectrum table and chart. https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html
14. Mabry, T.J., K.R. Markham and M.B. 1970. The Ultraviolet Spectra of Flavones and Flavonols. In: The Systematic Identification of Flavonoids, Mabry, T.J., K.R. Markham and M.B. Thomas (Eds.)., Springer, Berlin, Heidelberg, ISBN: 978-3-642-88460-3, pp: 41-164.
15. Kikuzaki, H. and N. Nakatani, 1993. Antioxidant effects of some ginger constituents. J. Food Sci., 58: 1407-1410.
16. Lim, Y.Y. and J. Murtijaya, 2007. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Sci. Technol., 40: 1664-1669.
17. Chakraborthy, G.S., 2009. Free radical scavenging activity of *Costus speciosus* leaves. Indian J. Pharm. Educ. Res., 43: 96-98.
18. Lee, H.S., 1992. Antioxidative activity of browning reaction products isolated from storage-aged orange juice. J. Agric. Food Chem., 40: 550-552.
19. Gulcin, I., H.A. Alici and M. Cesur, 2005. Determination of *in vitro* antioxidant and radical scavenging activities of propofol. Chem. Pharm. Bull., 53: 281-285.
20. Sreejayan, N. and M.N. Rao, 1996. Free radical scavenging activity of curcuminoids. Drug Res., 46: 169-171.
21. Zhang, K.Z., K. Deng, H.B. Luo, J. Zhou, Z.Y. Wu and W.X. Zhang, 2013. Antioxidant properties and phenolic profiles of four Chinese Za wines produced from hull less barley or maize. J. Inst. Brew., 119: 182-190.
22. Gulfraz, M., M. Imran, S. Khadam, D. Ahmed and M.J. Asad *et al*., 2014. A comparative study of antimicrobial and antioxidant activities of garlic (*Allium sativum* L.) extracts in various localities of Pakistan. Afr. J. Plant Sci., 8: 298-306.

23. El-Adawy, M., M. Abd El-Aziz, K. El-Ahazly, N.G. Ali and M. Abu El-Magd, 2018. Dietary propionic acid enhances antibacterial and immunomodulatory effects of oxytetracycline on Nile tilapia, Oreochromis niloticus. Environ. Sci. Pollut. Res., 25: 34200-34211.
24. Amirdivani, S. and A.S. Baba, 2011. Changes in yogurt fermentation characteristics and antioxidant potential and *in vitro* inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint, dill and basil. LWT-Food Sci. Technol., 44: 1458-1464.
25. Leng, R.A. and J.V. Nolan, 1984. Nitrogen metabolism in the rumen. Symposium: Protein nutrition of the lactating dairy cow. J. Dairy Sci., 67: 1072-1089.
26. Faipoux, R., D. Tomé, A. Bensaid, C. Morens, E. Oriol, L.M. Bonnano and G. Fromentin, 2006. Yeast proteins enhance satiety in rats. J. Nutr., 136: 2350-2356.
27. FitzGerald, R.J., B.A. Murray and D.J. Walsh, 2004. Hypotensive peptides from milk proteins. J. Nutr., 134: 980S-988S.
28. Adam, C.L., P.A. Williams, M.J. Dalby, K. Garden and L.M. Thomson *et al*., 2014. Different types of soluble fermentable dietary fibre decrease food intake, body weight gain and adiposity in young adult male rats. Nutr. Metab., Vol. 11. 10.1186/1743-7075-11-36.
29. Du, X. and A.D. Myracle, 2018. Fermentation alters the bioaccessible phenolic compounds and increases the alpha-glucosidase inhibitory effects of aronia juice in a dairy matrix following in vitro digestion. Food Funct., 9: 2998-3007.
30. Oboh, G. and A.A. Akindahunsi, 2003. Biochemical changes in cassava products (flour and gari) subjected to *Saccharomyces cerevisae* solid media fermentation. Food Chem., 82: 599-602.
31. Shinohara, T., S. Kubodera and F. Yanagida, 2000. Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. J. Biosci. Bioeng., 90: 90-97.
32. Tsubokura, A., H. Yoneda, M. Takaki and T. Kiyota, 1999. Bacteria for production of carotenoids. U.S. Patent No. 5858761. U.S. Patent and Trademark Office, Washington, DC.
33. Dharmaraj, S., B. Ashokkumar and K. Dhevendaran, 2009. Fermentative production of carotenoids from marine actinomycetes. Iran. J. Microbiol., 1: 36-41.
34. Kiplamai, F.K., P.J. Tuitoek and L. Ethangatta, 2010. Effects of fermentation on the total carotenoids, fat, free fatty acids and minerals in soybean and sweetpotato flour blends. J. Food Process. Technol., Vol. 6. 10.4172/2157-7110.1000507.
35. Cialer, A., M. Arnold and R.F. Anderson, 1959. Microbiological production of carotenoids. IV. Effect of various grains on production of beta-carotene by mated strains of *Blakeslea trispora*. Applied Microbiol., 7: 94-98.
36. Beganović, J., B. Kos, A.L. Pavunc, K. Uroić, P. Džidara and J. Šušković, 2013. Proteolytic activity of probiotic strain Lactobacillus helveticus M92. Anaerobe, 20: 58-64.
37. Raveschot, C., B. Cudennec, F. Coutte, C. Flahaut, M. Fremont, D. Drider and P. Dhulster, 2018. Production of bioactive peptides by *Lactobacillus* species: From gene to application. Front. Microbiol., Vol. 9. 10.3389/fmicb.2018.02354.
38. Rio, D.D., G. Borges and A. Crozier, 2010. Berry flavonoids and phenolics: Bioavailability and evidence of protective effects. Br. J. Nutr., 104: S67-S90.
39. Vanzo, A., M. Scholz, M. Gasperotti, F. Tramer, S. Passamonti, U. Vrhovsek and F. Mattivi, 2013. Metabonomic investigation of rat tissues following intravenous administration of cyanidin 3-glucoside at a physiologically relevant dose. Metabolomics, 9: 88-100.
40. Liu, Y., H. Cheng, H. Liu, R. Ma, J. Ma and H. Fang, 2019. Fermentation by multiple bacterial strains improves the production of bioactive compounds and antioxidant activity of goji juice. Molecules, Vol. 24. 10.3390/molecules24193519.
41. Boots, A.W., G.R.M.M. Haenen and A. Bast, 2008. Health effects of quercetin: From antioxidant to nutraceutical. Eur. J. Pharmacol., 585: 325-337.
42. Venkatesan, T., Y.W. Choi and Y.K. Kim, 2019. Impact of different extraction solvents on phenolic content and antioxidant potential of *Pinus densiflora* bark extract. BioMed. Res. Int., Vol. 2019. 10.1155/2019/3520675.
43. Adeoye, A.O., J.O. Olanlokun, H. Tijani, S.O. Lawal, C.O. Babarinde, M.T. Akinwole and C.O. Bewaji, 2019. Molecular docking analysis of apigenin and quercetin from ethylacetate fraction of Adansonia digitata with malaria-associated calcium transport protein: An in silico approach. Heliyon, Vol. 5. 10.1016/j.heliyon.2019.e02248.
44. Barnes, S., J. Prasain, T. D'Alessandro, A. Arabshahi and N. Botting *et al*., 2011. The metabolism and analysis of isoflavones and other dietary polyphenols in foods and biological systems. Food Funct., 2: 235-244.