Table 2: Fatty acid profile of banana pseudostem and banana flower by gas chromatography-mass spectrometry

Compounds detected[a]	Trivial name of fatty acid	Total percentage composition	Activity[b]
	PB		
Hexadecanoic acid, methyl ester	Palmitic acid[x]	18.93	Lubricant, 5 alpha reductase inhibitor, antiandrogenic and antioxidants
9,12-octadecadienoic acid (Z, Z)-, methyl ester	Linoleic acid[y]	72.85	Antiarthritic, anti-inflammatory, hepatoprotective, hypocholesterol and 5 alpha reductase inhibitor
Octadecanoic acid, methyl ester	Stearic acid[x]	6.80	Cosmetics, lubricant, flavor, hypocholesterol and 5 alpha reductase inhibitor
9-octadecanoic acid (Z)-, methyl ester	Oleic acid[z]	0.47	Cancer-preventive, flavor, hypocholesterol and anti-inflammatory
Eicosanoic acid, methyl ester	Arachidic acid[x]	0.94	**
	FB		
Hexadecanoic acid, methyl ester	Palmitic acid[x]	14.89	Lubricant, 5 alpha reductase inhibitor, antiandrogenic and antioxidants
9,12-octadecadienoic acid (Z, Z)-, methyl ester	Linoleic acid[y]	84.84	Antiarthritic, anti-inflammatory, hepatoprotective, hypocholesterol and 5 alpha reductase inhibitor
11-eicosenoic acid, methyl ester	Eicosenoic acid[x]	0.27	**

[a]Compounds were identified by referring to NIST05 library; [b]Activities were acknowledged by Dr Duke's phytochemical and ethnobotanical databases; **Activity not reported; [x]Saturated fatty acid; [y]Polyunsaturated omega-6 fatty acid; [z]Monounsaturated omega-9 fatty acid. PB: Banana pseudo stem; FB: Banana flower

Table 3: Mineral composition of banana pseudostem and banana flower

	PB	FB
Macroelements (mg/g)		
Sodium (Na)	0.02±0.02[a]	18.34±0.12[b]
Potassium (K)	10.63±0.10[a]	51.29±0.04[b]
Calcium (Ca)	4.01±0.07[a]	10.65±0.05[b]
Magnesium (Mg)	1.55±0.18[a]	23.55±0.21[b]
Phosphorus (P)	2.09±0.04[a]	4.10±0.16[b]
Microelements (ppm)		
Iron (Fe)	30.65±0.16[a]	405.50±0.04[b]
Lithium (Li)	0.012±0.01[a]	0.034±0.01[b]
Boron (B)	39.88±0.04[b]	34.53±0.01[a]
Aluminium (Al)	7.67±0.01[a]	18.43±0.02[b]
Chromium (Cr)	5.04±0.04[b]	3.93±0.02[a]
Manganese (Mn)	27.86±0.09[a]	133.80±0.06[b]
Copper (Cu)	0.02±0.01[a]	0.52±0.03[b]
Nickel (Ni)	0.46±0.04[a]	0.99±0.02[b]
Cobalt (Co)	3.79±0.01[a]	19.44±0.04[b]
Zinc (Zn)	16.60±0.01[a]	207.90±0.10[b]
Lead (Pb)	0.15±0.01[a]	0.42±0.02[b]
Molybdenum (Mo)	0.028±0.01[a]	0.042±0.01[b]
Antimony (Sb)	<0.01	<0.01
Cadmium (Cd)	1.06±0.01[a]	1.50±0.01[b]
Arsenic (As)	0.015±0.01[a]	0.016±0.01[a]
Selenium (Se)	0.010±0.01[a]	0.010±0.01[a]
Phosphoric acid (mg/g)	6.72±0.07[a]	13.12±0.03[b]
Boric acid	66.66±0.02[a]	150.0±0.01[b]
Elemental analysis (%)		
C	35.53±0.07[a]	47.19±0.02[b]
H	6.01±0.02[b]	3.21±0.02[a]
N	1.35±0.01[a]	1.96±0.03[b]
S	0.07±0.01[a]	0.17±0.01[a]
O	52.78±0.06[b]	43.08±0.04[a]

Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P≤0.05) as separated by Duncan multiple range test. SD: Standard deviation; PB: Banana pseudo stem; FB: Banana flower

compared to PB (35.5%) which was contrary to the hydrogen content which was higher in PB (6.01 ± 0.02) as against FB (3.21 ± 0.02). The present findings were on par with the composition of principal elements of banana (*Musa acuminate*) pseudostem by Ketty *et al*.,[44] i. e., (carbon: 36.83%, hydrogen: 5.19%, and nitrogen: 0.93%). The composition of hydrogen was higher in PB than FB and this is due to the high moisture composition of that compared to the FB [Table 1]. The moisture content of the present study was higher in case of PB (13.3%) over FB (8.33%) suggesting the difference in the hydrogen content of both the byproducts. Overall, the moisture content of both PB and FB were lower than commercial wheat flour, which had a value of 12.36%[44] and PB and FB of elakki bale cultivar as reported by Jamuna *et al*.[16]

In support to the above findings, the ash content that is directly proportional to the mineral content was also estimated which suggested the presence of it at high levels. It is clear from our studies that the highest levels of ash content were recorded for *Musa* sp. cv. Nanjangud rasa bale (PB and FB of 4.9 and 6.5%, respectively), and was comparatively higher than those *Musa* sp. cv. elakki bale (0.3 and 0.5%, respectively),[16] banana fruit of 1.1%.[18] Whereas it was comparable with banana (*Musa acuminata* x *balbisiana Colla* cv. Awak) pseudostem flour (3.03%) [15] and lower than banana peel and pulps (6.4%–12.8%).[39]

Amino acids

The quality of EAAs suggests the nutritional value of dietary proteins, and hence the amino acid content in PB and FB were tested. An overall picture of the amino acid content present in PB and FB is given in Table 4 suggest that all the EAAs according to the FAO classification[31] are present in them with FB having a major amino acid content compared to PB. A high glutamic acid content (63.8 and 152.9 mg/g of protein) followed by aspartic acid, leucine, alanine, proline, arginine, cysteine, serine, and lysine was witnessed in both FB and PB, respectively. The importance of glutamine is learnt during critical illness where it acts as a prime carrier of ammonia to the splanchnic area and the immune system. In addition while the sulfur-containing amino acids were above the FAO/WHO,[31] requirement (score ranged from 99 to 240), the other EAAs met FAO/WHO,[31] requirement pattern. Further, the concentration of the amino acids that are lower than the FAO standard protein value is considered as limiting concentration, and in this context, in the present study, lysine was at the limiting concentration and the same has been reported by Thomas *et al*.[39]

Table 4: Amino acid profile of banana pseudostem and banana flower

Amino acids	Content (mg/g protein)		Reference (mg/g protein)[x]	Score (%)	
	PB	FB		PB	FB
Leucine	27.2[a]	63.2[b]	66[c]	41	96
Phenylalanine + tyrosine	20.2[a]	55.6[b]	63[c]	32	88
Lysine	12.5[a]	40.0[b]	58[c]	22	69
Valine	10.4[a]	36.6[c]	35[b]	30	105
Threonine	9.4[a]	33.0[b]	34[b]	28	97
Isoleucine	11.1[a]	28.0[b]	28[b]	40	100
Methionine + cysteine	24.8[b]	60.0[c]	25[a]	99	240
Tryptophan	3.7[a]	12.5[c]	11[b]	34	114
Valine	10.37±0.06[a]	36.64±0.02[b]			
Lysine	12.46±0.05[a]	39.95±0.07[b]			
Leucine	27.19±0.02[a]	63.19±0.08[b]			
Isoleucine	11.05±0.01[a]	28.02±0.02[b]			
Phenylalanine	13.52±0.02[a]	31.03±0.01[b]			
Threonine	9.43±0.01[a]	33.01±0.03[b]			
Histidine	7.51±0.02[a]	16.60±0.02[b]			
Methionine	8.14±0.04[a]	18.11±0.02[b]			
Tryptophan	3.70±0.03[a]	12.48±0.05[b]			
Arginine	14.25±0.04[a]	41.98±0.02[b]			
Proline	19.82±0.02[a]	50.28±0.01[b]			
Aspartic acid	24.19±0.03[a]	78.87±0.03[b]			
Glutamic acid	63.75±0.05[a]	152.92±0.08[b]			
Serine	10.50±0.03[a]	40.65±0.02[b]			
Glycine	14.44±0.06[a]	30.58±0.04[b]			
Alanine	14.05±0.02[a]	51.25±0.07[b]			
Cysteine	16.63±0.04[a]	41.89±0.04[b]			
Tyrosine	6.65±0.02[a]	24.56±0.02[b]			
Total essential amino acids	103.37±0.01[a]	279.03±0.01[b]			
Total nonessential amino acids	184.28±0.02[a]	512.98±0.03[b]			
Ratio (essential/nonessential)	0.56±0.01[a]	0.54±0.02[c]	0.38[b]		

Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P≤0.05) as separated by Duncan multiple range test. [x]Amino acid pattern of preschool children (2–5 years) (FAO/WHO/UNU, 1985). SD: Standard deviation; PB: Banana pseudostem; FB: Banana flower

in fruit peels of the *Musa* Genus: FC, GN, BE, PPT, YKm5, and 039, obtained at three different stages of ripeness, namely, stage 1 (Green), stage 5 (More yellow than green), stage7 (yellow/a few brown spots). The ratio of essential to non-EAAs for PB and FB were 0.56 and 0.54, respectively, which was substantially higher than their requirement in adults (0.38) as recommended by the WHO. In addition, the protein values of PB (7.3%) and FB (19.3%) in the present study were marginally higher than the values reported for *Musa* spp. *Baxijiao* and *Paradisiaca* flowers (1.62%–2.7%), elakki bale cultivar (PB: 2.5 and FB: 12.5%), banana fruit peels (ranged from 8.3%–10.2%), banana (*Musa acuminata* x *balbisiana Colla* cv. Awak) pseudostem flour (0.89%–3.52%), banana peels of yelakki bale (7.7%), pachabale (6.7%) and nendrabale (4.6%) and green banana Cavendish (AAA) flour (4.1%). Proteins being the source for the supplementation of amino acids, it can thus be suggested that PB and FB are potent sources of EAAs.[3,14-16]

Antioxidants

Antioxidant adjuncts have proven beneficiary in many diseases where they play a protective role in the prevention of ROS mediated damage to the cells and tissues. Hence, in the present study, we have evaluated the antioxidant potential of PB and FB and the results thus obtained are tabulated in Table 5a. Studies have suggested both PB[45] and FB[4,16] of banana as potent antioxidants extractable with aqueous and organic solvents. Most antioxidant studies involve its evaluation using the extractable form which creates a lacuna in assessing the nonextractable substance for their antioxidant capacity and hence, we evaluated the GAR according to Pastoriza *et al.*[7] Further, antioxidant activity using a single assay does not give conclusive evidence hence, three common radical scavenging assays namely ABTS which determines the single electron-transfer capabilities, DPPH which evaluates the hydrogen-donating potency and Fe + 3 (FRAP) which reflects the reductive antioxidant power of the antioxidant compounds[9] were carried out to assess *in vitro* antioxidant activity of PB and FB [Figure 5b]. As mentioned previously, along with the method of evaluation, another factor contributing to the antioxidant potential of the samples is the method of extraction and hence, a conventional solvent extraction (with different solvents), a direct measure using the QUENCHER procedure, an *in vitro* gastrointestinal digestion, and the combination of the latter with the application of the QUENCHER procedure[10] to the insoluble fraction [termed henceforth the GAR method] are the methods of extraction employed in the present study. The results provide promising evidence for the need for employing such methods of antioxidant estimation since the chemical extraction method (solvents and aqueous) gave lower results, ranging from 2 to 2.5 times lower in comparison with the Quencher and GAR methods. They are in agreement with the previous reports for 27 fresh and cooked foods, estimated by Pastoriza *et al.*[7] On the other hand, with regard to the chemical extraction method, both PB and FB extracted with the solvent ethanol fared better than methanol and aqueous counterparts. They were also higher than the Quencher method, but lower than GAR method of antioxidant evaluation. The previous phytochemical analysis also reports high amounts of total phenolic content in the ethanolic extract of PB and FB[4,5] which are well-known as the major phytochemicals (phenolic acids and flavonoids) to possess antioxidant activities in fruits and vegetables.

Further, to acquire a detailed phenolic composition of the extracts, HPLC analysis was performed and the results are detailed in Table 5c suggesting the presence of diverse phenolic acids, namely, gallic acid, p-hydroxybenzoic acid, chlorogenic acid, sinapic acid, caffeic acid,

Table 5: Enzymatic antioxidant potential and global antioxidant response of banana pseudostem and banana flower using different methods and distribution of antioxidant activity in soluble and insoluble fractions after *in vitro* digestion (a); yield, total phenolic content and antioxidant activity of banana pseudostem and banana flower sequential solvent extracts (b) and phenolic acids identification (c)

(a)

	Methods	PB	FB
Enzymatic antioxidants[w]	Superoxide dismutase	14.56±0.70[a]	19.08±1.66[b]
	Catalase	3.68±0.54[a]	7.86±1.01[b]
	Ascorbate peroxidase	0.32±0.44[a]	0.49±1.89[b]
	glutathione reductase	0.76±2.61[a]	1.53±0.47[b]
GARABTS[v]	Total	54.07±0.25[a]	70.15±0.55[b]
	Soluble	37.58±1.88[a]	45.62±1.38[b]
	Insoluble	15.41±3.33[a]	22.99±2.96[b]
	Quencherx	17.86±0.69[a]	24.05±1.98[b]
GARDPPH[v]	Total	0.78±0.60[a]	1.37±2.09[b]
	Soluble	0.49±1.60[a]	0.99±1.23[b]
	Insoluble	0.21±0.75[a]	0.27±3.08[b]
	Quencherx	0.33±0.38[a]	0.42±1.08[b]
GARFRAP[v]	Total	3.47±1.65[a]	6.53±1.34[b]
	Soluble	2.02±1.03[a]	4.99±1.77[b]
	Insoluble	1.26±0.50[a]	1.44±1.04[b]
	Quencherx	1.59±0.54[a]	2.78±0.82[b]

(b)

	Extracts	Yield (g/kg)	TPC[y]	ABTS[v]	DPPH[v]	FRAP[v]
PB	Methanol	25.45±0.46[a]	98.98±0.58[a]	11.98±1.87[a]	0.22±0.17[b]	1.23±1.01[b]
	Ethanol	91.20±0.48[c]	211.43±1.98[c]	21.87±0.40[b]	0.43±0.34[c]	3.33±0.33[c]
	Water	89.09±0.58[b]	122.34±0.41[b]	10.64±1.50[a]	0.20±0.67[a]	1.06±0.46[a]
FB	Methanol	61.37±0.55[a]	121.59±0.58[b]	18.80±1.31[a]	0.25±1.24[a]	1.58±1.79[b]
	Ethanol	126.87±1.74[c]	228.87±2.05[c]	24.03±1.00[b]	0.73±0.27[b]	4.83±0.51[c]
	Water	101.84±0.54[b]	105.78±0.48[a]	18.08±0.98[a]	0.24±2.50[a]	1.44±2.00[a]

(c)

Phenolic acid	PB (µg/mg) extract			FB (µg/mg) extract		
	Methanol	Ethanol	Water	Methanol	Ethanol	Water
Gallic acid	5.82	31.13	15.88	73.44	61.20	73.76
p-hydroxybenzoic acid	11.48	62.68	32.78	61.65	94.97	19.43
Chlorogenic acid	6.09	11.87	8.61	14.08	13.76	14.42
Sinapic acid	14.91	37.06	3.19	2.02	3.22	9.81
Caffeic acid	25.33	19.11	4.06	1.59	1.07	-
Vanillin	14.80	7.17	2.69	1.95	7.62	3.38
p-coumaric acid	7.58	2.09	4.29	0.25	1.45	-
Epicatechin	3.07	0.72	5.37	0.52	0.75	-
Catechin	9.34	4.12	1.76	0.91	2.63	-
Quercetin	4.39	6.06	1.19	0.32	1.67	-

[w]Units/min/mg of protein; [v]Mmol equivalents of trolox/kg sample; [x]Direct procedure without extraction of PB and FB and expressed as in GAR (mmol equivalents of trolox/kg sample); [y]Mg equivalents of gallic acid/g. Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P≤0.05) as separated by Duncan multiple range test. SD: Standard deviation; PB: Banana pseudostem; FB: Banana flower; GAR: global antioxidant response; TPC: Total phenolic content; ABTS: 2,2'-azino-bis, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power

vanillin, p-coumaric acid, quercetin, catechin, and epicatechin at different concentrations. Ethanol extracts in both PB and FB were found to contain high concentrations of phenolic acids in comparison to methanol and aqueous extract. p-hydroxybenzoic acid was the most predominant phenolic acid recorded in PB and FB (62.7 µg/mg and 95 µg/mg), followed by gallic acid (31.1 and 61.3 µg/mg, respectively) with varying concentrations. Caffeic acid was predominant in the methanol extract of PB (23.3 µg/mg), whereas gallic acid was predominant in that of FB (73.4 µg/mg). Although methanol and aqueous extract had phenolic acids, the concentration was lesser than the ethanol extract [Table 5c]. However, under physiological conditions, these results cannot be reproduced by administering the extracted antioxidants directly. Irrespective of the extraction method, some amount of the sample always remains insoluble in one or the other solvent and hence Arda *et al*.[46] developed a direct procedure (QUENCHER) to evaluate the TAC of foods without an extraction step. Since, this method cannot differentiate between the physiologically active fraction and the insoluble one, a combination of enzymatic digestion step for the soluble fraction and the Quencher method for the insoluble fraction thus furnish an optimal antioxidant potential of the given sample.

The results of the antioxidant activity using Quencher method [Table 5a] for PB and FB (ABTS: 17.8 and 24; DPPH: 0.33 and 0.42; FRAP: 1.59 and 2.78 mmol equivalents of the standard Trolox per kg of sample, respectively) were in accordance with the results obtained by Arda *et al*.[46] for different cereal products. The order of magnitude was same as the GAR method for PB and FB samples despite a 2–3 times reduction in most parts of the results. Such a reduction could be attributed to the absence of the enzymatic digestion step which could otherwise result in different compounds obtained after the enzymatic reactions. Overall, the best results were obtained by the GAR method which exhibited highest antioxidant activity with FB faring better than PB. In particular, the insoluble fraction exhibited about 40%–50% of the total antioxidant

activity and since this fraction is excluded during the extraction process, this is the most recommended method for the measurement of TAC. Although the antioxidant role of the insoluble fraction is questioned since they are not extractable, they are expected to exert their effect by the surface reaction phenomenon. Furthermore, some part of the insoluble fraction may undergo digestion by the intestinal microflora thus releasing some substances which can also exert antioxidant properties and considering these; it would be essential to measure the antioxidant capacity of even the insoluble fraction of the digested food.[47]

Further, with respect to the antioxidant assays, the different affinities of the radicals to scavenge various antioxidant groups present in different samples suggest the need to use more than a single assay to determine the antioxidant potential of a particular sample. In this regard, in the present study, the TAC as measured with two radical scavenging assays (ABTS and DPPH) fared differently for both the byproducts. In support of these results, Roger *et al.*[48] demonstrated that the macromolecules are seldom attacked by the hydrophobic radicals, which could be the reason for the lower activity in DPPH as compared to the ABTS assay wherein DPPH is a hydrophobic radical while ABTS is more of a hydrophilic probe. Furthermore, DPPH being more selective in the reaction with H-donors, it could also be the reason for its lower TAC values in this assay. Further, the FRAP activity which is based on the reduction of the Fe^{+3}–TPTZ complex in the ferrous form at low pH, exhibited 6.5 mmol Trolox Eq./Kg for FB and for PB with a statistically significant difference in the values ($P > 0.05$). The results, however, in comparison with ABTS were lower, but better than the DPPH assay.[49]

In addition, enzymatic (SOD, CAT, APX, and GR) antioxidant potential has been evaluated for the FB and PB. As evident from Table 5a, FB showed maximum activity of SOD (19.1 U/min/mg protein) followed by catalase (7.9 U/min/mg protein), GR (1.5 U/min/mg protein) and APX (0.49 U/min/mg protein). On the other hand, PB also exhibited enzymatic activities for SOD (14.6 U/min/mg protein) followed by catalase (3.7 U/min/mg protein), GR (0.76 U/min/mg protein), APX (0.32 U/min/mg protein) and found was to be lower in comparison to FB. Higher SOD, APX, and GR enzymatic antioxidant activities in PB and FB clearly indicates their greater ability to detoxify ROS such as superoxide, hydroxyl, and peroxide radicals formed in human cell by endogenous and exogenous factors which in turn could lead to geriatric degenerative conditions, cancer and a wide range of other human diseases.

CONCLUSION

In summary, the present study manifests that both PB and FB possess rich nutraceutical properties because of the presence of various bioactive ingredients with numerous benefits. It provides evidence that the two banana byproducts are rich in proximate nutrient composition, minerals, fatty acids, and antioxidants (both enzymatic and nonenzymatic) and hence could be used in the human diet. The beneficiary properties are mainly derived from their minerals, carbohydrates, dietary fibers and proteins together with the low content of fat and calories. Furthermore, as a rich source of phytochemicals, minerals and vitamins reside in PB and FB they can be further evaluated for use as a key ingredient for valuable drugs. To add to these, the high total dietary fiber content and a balanced ratio between insoluble dietary fiber and soluble dietary fiber in both PB and FB are attractive targets for the food industry. These could be used in the development of a nutritional supplement because of their health-related properties of dietary fiber and associated bioactive compounds.

In addition to the strong basis provided by the nutritional aspects of PB and FB, their potential as antioxidants are also confirmed by a series of studies which included different methods of extraction as well as different assays to determine their antioxidant potential. It is demonstrated that the GAR method exhibited antioxidant activity higher than that reported with traditional procedures, which asserts the role of both insoluble as well as soluble fractions of the digested food to possess antioxidant properties. To summarize on the whole, this paper reinforces the concept that PB and FB are potent sources of several biologically active ingredients and also possess rich antioxidant property.

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Conflicts of interest

There are no conflicts of interest.

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