Evaluation of Nutritional and Bioactive Components and Antioxidant Potential of Bamboo Rice (*Bambusa arundinacea* Willd.) with Pharma-Nutritional Role

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Sebastian *et al*.: Evaluation of Nutritional Bioactive Components and Antioxidant Potential of *Bambusa arundinacea*

Bamboo rice, an underutilised grain obtained as seeds of *Bambusa arundinacea* Willd., is consumed by many Indian population for its nutritional and other health benefits. Many of these health benefits are linked to the presence of bioactive phytonutrients. However, comprehensive quantification of different bioactive components has not been reported in bamboo rice. Thus, this study was intended to quantify its fatty acid profile, amino acid composition, phytosterols, polyphenols and measure the antioxidant potentials. Bamboo rice samples collected from six different regions of the Western ghats of India were analysed independently and compared with commonly consumed brown and polished rice. The total saturated, monounsaturated and polyunsaturated fatty acids in bamboo rice were 36.97 %±6.01 %, 28.59 %±2.01 % and 34.44 %±6.22 % fatty acid methyl esters, respectively. In bamboo rice, the total amino acid content was (96.76/100) g protein while the total essential amino acid content was (38.67/100) g with lysine being the limiting amino acid. The grain had higher concentrations of phytosterols, especially ergosterol and beta-sitosterol, when compared to brown and polished rice. The relative distribution of the polyphenols was in the order of catechinin>protocatechuic acid>sinapic acid>4-coumaric acid>myricetin>ferulic acid>2-coumaric acid>luteolin 7-O-glucoside>4-hydroxy benzoic acid>gallic acid>caffeic acid>ellagic acid. Ferric reducing antioxidant power and 2,2-diphenyl-1-picrylhydrazyl assay expressed more activity in the methanolic extract while the aqueous extract showed better activity for 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid assay. This study has clearly shown that bamboo rice is rich in bioactive phytonutrients when compared to brown and polished rice. It also highlighted the need for comprehensive research on this grain to explore its pharmacological and therapeutic effects.

Key words: Bamboo rice, phytosterols, fatty acids, amino acids, polyphenols, antioxidant activity, biotherapeutics

Bioactive Compounds (BACs) are specific compounds in foods known to be biologically and physiologically active, that can be of immense benefit to human health besides meeting the body's basic nutritional needs. These compounds can interact with constituents of living tissue and exhibit various potential effects[1]. Major elements, including vitamins and phytochemicals, which are obtained naturally during food or plant processing, are considered BACs[2]. Moreover, food components like proteins, peptides, amino acids, flavonoids and other phenolics are vital in improving the body's natural resistance to oxidative damage[3]. These compounds can provide protection against high amounts of free radicals and reactive oxygen species which can induce cell damage by interacting with other molecules[4]. Reportedly, serum cholesterol levels are significantly reduced in populations consuming diets that are particularly rich in natural phytosterols. Phytosterols such as sterols and stanols are also known to be beneficial in preventing colon cancer[5]. Among all the vital phytochemicals, phenolic compounds are considered key phytoconstituents having strong antimicrobial, hepatoprotective, anti-inflammatory, anticancer and antioxidant potential[6]. These biological activities exhibited by plant polyphenols increase their potential to treat diseases,

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including diabetes, cancer and Alzheimer's disease[7]. Studies have also shown that polyphenols, mainly flavonoids are potential inhibitors of severe acute respiratory syndrome coronavirus-2 infection[8]. Applying proper packaging techniques to foods rich in these BACs can aid in retaining their phenolic content and antioxidant activity[9].

Rice bran contains several nutraceutical compounds, including γ-oryzanol, tocopherols, tocotrienols and phenolics. Brans of pigmented rice contains higher nutraceutical components when compared to non-pigmented rice bran[10]. Generally, some nutraceutical components exist in bound forms in rice bran. They are usually bound chemically to the cell wall constituents like pectin, cellulose, hemicellulose and arabinoxylans[11]. These BACs in cereal-based foods aid in reducing chronic diseases related to ageing, including heart diseases and various cancers[12]. Since the toxicity and possible carcinogenicity of synthetic antioxidants have raised concerns regarding their safety, food manufacturers prefer to attain antioxidative effects from natural sources, like extracts of plant materials, including cereal grains[13].

Traditional rice varieties possess unique medicinal and nutritional properties. However, these old rice landraces are nearing extinction because they are deemed less economical than the newly introduced short-duration rice varieties[14]. Modern generations have a limited understanding of traditional food systems and their significance. In rural areas, people often rely on ethnomedical practitioners, although they are quite scarce. The poor nutritional status of vulnerable groups is often linked to the loss of local traditions and knowledge systems. Therefore, documenting information about indigenous foods, particularly those with nutritional and medicinal benefits, is essential. This study focuses on the bioactive components of bamboo rice. Bamboo rice, part of the cereal family Gramineae, closely resembles rice grains and is widely consumed by many tribal populations in Southern India[15,16]. Previous studies have quantified a few nutrients in bamboo rice[17-19]. Recent research by Sebastian *et al.*[20] revealed prominent protein molecules in bamboo rice and confirmed a superior nutrient profile, including protein, dietary fiber, vitamins B2 and B5, zinc, calcium, copper and iron, with lower phytic acid content compared to brown and polished rice[20]. Manohari *et al.*[21] conducted a preliminary phytochemical analysis of bamboo rice from Tamil Nadu, identifying phytochemicals such as cardiac glycosides, reducing sugars, tannins, phlobatannins, phenols and flavonoids, while saponins, alkaloids, terpenoids, and anthraquinones were absent[21].

So far, there has been very limited quantitative analytical literature on the concentrations of the vital components such as fatty acids, phytochemicals and associated antioxidant activity in bamboo rice. Therefore, this study focuses on the analysis of key nutrients, including amino acids, fatty acids, phytosterols and polyphenols, in bamboo rice and evaluation of its *in vitro* free radical scavenging potential. The current study aims to promote the wider utilization of this underutilized grain in functional food development by food industries and by nutritionists for estimation of dietary nutrient intakes. This study enriches the current understanding and records of indigenous foods and traditional food systems, thereby aiding in the preservation and promotion of local culture and biodiversity.

MATERIALS AND METHODS

Sample collection:

De-husked bamboo rice (*Bambusa arundinacea* Willd.) samples (Barcode: BSID0001202, Botanical Survey of India, Indian Virtual Herbarium) were procured from six different regions of South Western Ghats of India. Samples were collected from the indigenous population of Berambadi State Forest (BRB) [11°46'23"N 76°30'04"E] and Kodagu (BRK) [12°22'58"N 75°46'38"E] belonging to the state of Karnataka; Vythiri (BRV) [11°32'59"N 76°03'01"E], Sulthanbathery (BRS) [11°37'56"N 76°15'23"E] and a local market (BRM) located in the Wayand district of Kerala [11°42'54"N 76°06'28"E]; Gudalur [11°29'12"N 76°29'16"E] from Nilgiri district (BRG) located in Tamil Nadu. Bamboo rice samples were collected from 3-4 different sources in each region and combined to create a composite sample. All the bamboo rice samples were hand-pounded, where the husk was removed while retaining the bran, ensuring consistency with commercial brown rice. The collected grains were cleaned thoroughly after separating foreign matter like stones, straw and dirt. A widely consumed rice variety, sona masoori, was sourced from a local market in Hyderabad in the forms of brown and polished (10 % Degree of Milling (DoM)) rice and analysed for comparison with bamboo rice. All the rice samples were finely powdered using a commercial cyclone mill (UDY Corporation, United States of America (USA)), and stored in an airtight container at -20° until further

analysis.

Fatty acid composition:

The samples were analysed for their fatty acid profile as per the Fallon et al.[22] method with slight modification. 0.5 g of the sample was weighed into the Pyrex culture tube and 0.5 ml of heptadecanoic acid (C17) (1 mg/ml) was added and dried under a stream of Nitrogen (N_2). Then 0.7 ml of 10 N Potassium hydroxide (KOH) and 5.3 ml of methanol (with 0.05 % butylated hydroxytoluene) were added and vortexed for 30 s. The tubes were placed in water bath at 55° for 1.5 h and were allowed to shake vigorously for 5-20 s every 20 min. After cooling the tubes to room temperature, 0.58 ml of 24 N Sulfuric acid (H_2SO_4) was added. The tubes were vortexed and incubated at 55° for 1.5 h with vigorous shaking at 20 min intervals. After proper vortexing, 3 ml of n-hexane was added to it and centrifuged for 5 min at 2000 revolutions per minute (rpm) (~600 g). The hexane layer was collected in 5 ml test tubes containing 0.1 g of Sodium sulfate (Na_2SO_4), vortexed, and allowed to settle. It was then dried under a stream of N_2 and 1.5 ml of Suprasolv® dichloromethane was added. The dichloromethane layer containing Fatty Acid Methyl Esters (FAME) was transferred into a labelled Gas Chromatography (GC) auto-sampler vial and dried under N_2. Suprasolv® dichloromethane was used to make the final volume up to 1 ml. The samples were then analysed in GC (7890A GC System, Agilent Technologies Inc., USA) with a column (Phenomenex BPX-70 fused silica capillary column) of dimension 30×0.32 mm Internal Diameter (ID). Peaks obtained were identified for individual fatty acids by comparing their retention time with external standards (SupelcoTM37 component FAME Mix-Certified Reference Material (CRM47885)) procured from Sigma Aldrich Co. St. Louis, USA.

Amino acid composition:

The rice sample equivalent to 10 mg protein was taken and hydrolysed with 6 N Hydrochloric acid (HCl) in sealed ampoules[23]. The ampoules were kept in oven (Bio Technics, India) for 22 h at 110°. Continuous flash evaporation was carried out under pressure (Buchi, Switzerland) to remove excess acid and the sample was mixed with citrate buffer (pH 2.2). An automated amino acid analyser (Biochrom-30, Cambridge, UK) was used for the analysis, where an aliquot (20 µl) of the sample was taken. Methionine and cysteine were separately quantified after their conversion by performic acid oxidation into methionine sulfone and cysteic acid[24]. A

standard hydrolysis procedure was then further carried out. Individual amino acids were detected and quantified with authentic standards (Standard Reference Material (SRM) 2389) from the National Institute of Standards and Technology (Gaithersburg, Maryland, USA) and later provided in g in 100 g of protein. The amino acid score was computed as per Food and Agriculture Organization/World Health Organization/United Nations University (1985), where amino acids required for preschool children of age 2 to 5 y were considered.

Phytosterols:

Phytosterols were analysed following the methods described by Sorenson et al.[25] and Toivo et al.[26]. Acid hydrolysis was performed in 3-5 g of sample using absolute ethanol and 6 M HCl. The samples were kept in a water bath at 80° for 60 min with vigorous shaking every 10 min. Further, 5 ml of absolute ethanol and 20 ml of hexane:diethyl ether mixture were added at a ratio of 1:1 (v/v) and vortexed. The organic phase (approximately 15 ml) was collected into another tube after centrifugation for 10 min at 2000 rpm (~600 g). The organic solvent was evaporated using a nitrogen evaporator at 35°. For saponification, 20 ml of 95 % ethanol and 4 ml of 50 % KOH (w/v) were added to the samples and kept in a water bath at 80° for 30 min with vigorous shaking every 3 min. After cooling, 20 ml of cyclohexane and 10 ml of deionised water were added to the tubes and vortexed. The organic phase (approximately 10 ml) was collected and transferred into fresh centrifuge tubes and was evaporated using a nitrogen evaporator at 35°. The samples were derivatised by dissolving the dry extract in 4-5 ml of cyclohexane, transferred into new tubes and evaporated under nitrogen. Chloroform (1 ml), 0.05 ml (50 µl) of hexamethyldisilazane and 0.1 ml (100 µl) of trimethylchlorosilane was added and made to stand for 15 min at room temperature after proper vortexing. 10 ml of Milli-Q water was added to this, vortexed and centrifuged at 2000 rpm (~600 g) for 2 min. The water layer was separated and the chloroform layer was collected into tubes containing 0.5 g of Na_2SO_4, filtered with 0.45 µm syringe and collected into GC vials for analysis in GC (7890A GC system, Agilent Technologies, USA) with a column (Phenomenex BPX-70 fused silica capillary column) of dimension 30 m×0.32 mm ID. Peaks obtained were identified for individual phytosterols by comparing their retention time with external standards, including ergosterol (Chemical Abstracts Service (CAS): 57-87-4), stigmasterol (CAS: 83-48-7), campesterol (CAS: 474-

62-4) and Beta (β)-sitosterol (CAS: 83-46- 5) procured from Sigma Aldrich Co. St. Louis, USA. A standard stock solution was prepared separately for stigmasterol (5 mg/ml), β-sitosterol (5 mg/ml), campesterol (1 mg/ml) and ergosterol (10 mg/ml). A standard mixture with all the individual phytosterols at 0.5 mg/ml concentration was prepared for the analysis.

Estimation of Total Phenolic Content (TPC):

The TPC was determined following the method described by Tachakittirungrod *et al.*[27]. 1 g of sample was weighed into two 15 ml centrifuge tubes and 10 ml of water was added into one tube and 10 ml of methanol into the other. The solution was vortexed for 2 min and centrifuged at 3000 rpm for 10 min. 5 ml of Folin-Ciocalteu reagent was mixed with one ml of the sample extract. The mixture was thoroughly vortexed, and 4 ml of Na_2CO_3 solution was added and kept for incubation for 60 min at room temperature. Absorbance was measured at 765 nm in a spectrophotometer (SPECORD S 600, Germany). TPC was expressed as Gallic Acid Equivalents (GAE) in g/100 g of sample. A stock solution of gallic acid was prepared. 1 ml of the stock solution was made up to 5 ml with water for the working standard. From this, different concentrations of gallic acid standards were prepared at 20, 40, 60, 80 and 100 μg/ml, respectively. The standard graph was plotted with concentration on the X-axis and absorbance on the Y-axis. The equation (y=mx+c) was obtained from the plot. The concentration of samples was calculated through the above equation observed from the graph by substituting the values[27].

Quantification of individual polyphenols:

Individual polyphenols were quantified following the method described by Giusti *et al.*[28]. High-Performance Liquid Chromatography (HPLC) grade reference standards for gallic acid (CAS: 149-91-7), protocatechuic acid (CAS: 99-50-3), 4-hydroxy benzoic acid (CAS: 99-96-7), catechin (CAS: 154-23-4), caffeic acid (CAS: 331-39-5), sinapic acid (CAS: 530-59-6), ferulic acid (CAS: 537-98-4), 4-coumaric acid (CAS: 501-98-4),ellagic acid (CAS:476-66-4), 2-coumaric acid (CAS: 614-60-8), luteolin-7-O-glucoside (CAS: 5373-11-5), myricetin (CAS: 529-44-2), resveratrol (CAS: 501-36-0), daidzein (CAS: 486-66-8), quercetin (CAS: 522-12-3), luteolin (CAS: 491-70-3), naringenin (CAS: 67604-48-2), apigenin (CAS: 520-36-5), kaempferol (CAS: 520-18-3), hesperetin (CAS: 69097-99-0) and flavone (CAS: 525-82-6) were purchased from Extrasynthese, Genay, France. A stock was prepared from each polyphenol standard (1 mg/ml) and a working standard mix was prepared from the stock solution (25 ng/μl) for analysis. The samples were extracted by 10 ml of 90 % methanol with 0.5 % acetic acid. The samples (0.5 g) were vortexed for 5 min and the supernatants were separated by centrifugation at 4000 rpm for 15 min. After three extractions, the extracts were dried with a rotavapour (Buchi). The residue was dissolved in 3.0 ml of methanol. A 0.45 μm syringe filter (Whatman) was used to filter the samples, which were then analysed using Ultra HPLC (UHPLC) with Chromeleon software (Dionex UltiMate 3000 Rapid Separation Liquid Chromatography (RSLC) USA). The compounds were quantified using gradient elution with a mobile phase containing solvents A and B (consisting of 0.1 % formic acid in water v/v and 0.1 % formic acid in acetonitrile, respectively). Luna C_{18} column (150×2 mm ID; 3 μm particle size) was used for the separation. A column temperature of 40° was maintained. An injection volume of 4 μl was taken with a flow rate of 0.9 ml/min for a run time of 52 min. HPLC with diode-Array Detection (HPLC-DAD) analysis was performed at four wavelengths such as 250, 270, 320 and 370 nm. The ultraviolet spectra and retention times were compared with the corresponding standards to identify the specific phenolic compounds and the data was quantified using the corresponding calibration curves.

Measurement of total antioxidant activity:

The antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Charmforoshan *et al.*[29] with slight modifications whereas 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and Ferric Reducing Antioxidant Power (FRAP) assays were performed following the method described by Tachakittirungrod *et al.*[27] with slight modifications.

DPPH assay: 1 g sample was extracted with 10 ml of water or methanol, vortexed and centrifuged at 4000 rpm for 10 min. The supernatant obtained (150 μl) was mixed with 2 ml of methanolic DPPH solution. Methanol was added to make it up to 3 ml. The sample mixture was incubated for 40 min at room temperature and the Optical Density (OD) was measured at 517 nm using a spectrophotometer (SPECORD S 600, Germany)[29]. The free radical scavenging activity was expressed as a percentage of inhibition. The percentage of inhibition and half-maximal Inhibitory Concentration (IC_{50}) was measured using the following formulas

% inhibition of DPPH=(control OD-extract OD)/ control OD×100 (1)

IC_{50}=concentration of extract/% inhibition of DPPH×50 (2)

FRAP assay: About 1 g of sample was extracted either with water or methanol independently and centrifuged at 4000 rpm for 10 min after vortexing for 2 min. The supernatant was collected for analysis. FRAP reagent was prepared using sodium acetate buffer (300 mmol), 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mmol/l), HCl (40 mmol/l) and iron (III) chloride (20 mmol/l) respectively, at a ratio of 10:1:1 v/v/v. Milli-Q water (240 µl) was taken in a screw cap tube and 2.4 ml of TPTZ buffer and 80 µl of the extracted sample was added into each tube and mixed using vortex. The OD readings were taken at 593 nm in a spectrophotometer (SPECORD S 600, Germany) after 4 min of incubation at 37°. TPTZ buffer (2.4 ml) was added into five screw cap tubes, followed by 20, 40, 60, 80 and 100 µl of Ferrous sulfate ($FeSO_4$) solution as standards. The standard solutions were made up to 320 µl using Milli-Q water and mixed using a vortex. The OD readings were taken at 593 nm. The standard graph with different standard concentrations was plotted and the results were calculated and expressed as mM equivalents of $FeSO_4$/100 g sample[27].

ABTS assay: To prepare the radical cation solution, 2.45 mM potassium persulphate was mixed with 7 mM ABTS stock solution. The mixture was left for 4-16 h until the completion of the reaction and a stable absorbance was obtained. ABTS solution was diluted using Milli-Q water till an absorbance of 0.700 was measured at 734 nm. The photometric assay was carried out on a mixture of 200 µl of sample extract and 1.8 ml of ABTS in a spectrophotometer (SPECORD S 600, Germany)[24]. The percentage of inhibition and IC_{50} was measured using the following formulas.

% inhibition of ABTS=(control OD-extract OD)/ control OD×100 (3)

IC_{50}=concentration of the extract/% inhibition of ABTS×50 (4)

Statistical analysis:

Descriptive statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) software version 16.0 (Chicago, USA). To determine the significance of differences within the analysed samples, a one-way ANOVA was performed, followed by the Scheffe test as a post hoc analysis with a significance level set at $p<0.05$. The results for brown and polished rice were presented as the mean±Standard Deviation (SD) of triplicate analyses (n=3), while for bamboo rice, the mean±SD was based on six collected samples (n=6).

RESULTS AND DISCUSSION

Dietary fats and fatty acids play an important role in human lipid metabolism. Each fatty acid follows its own metabolic pathway and therefore knowing the fatty acid composition of foods is essential[30,31]. In this study, we have quantified comprehensive fatty acid profiles of bamboo rice along with commonly consumed brown and polished rice (10 % DoM) (fig. 1), which are presented in Table 1. These data are particularly significant, because to the best of our knowledge, no prior studies have detailed the comprehensive fatty acid composition of bamboo rice (*Bambusa arundinacea* Willd.). The levels of Polyunsaturated Fatty Acids (PUFA) were similar across all the rice samples. Similarly, the difference in PUFA levels in polished rice and brown rice was not statistically significant as observed previously[32].

In the present study, bamboo rice contained 23 % higher Monounsaturated Fatty Acids (MUFA) than polished rice. MUFA was significantly different in all the rice samples analysed with the highest content found in brown rice (36.58 % FAME). Similarly, in brown rice varieties, highest proportion of MUFA (45.5 % of total fatty acids) was observed previously among all fatty acids[33]. Kim *et al*.[32] also reported that MUFA in brown rice was higher by 27 % when compared to polished rice. MUFA was previously reported to significantly decrease upon polishing the rice to 10 % DoM[34]. Among the monounsaturated fatty acids, methyl 11-eicosenoic acid was found to be 0.90±0.12 % FAME in bamboo rice, which was 54 % and 73 % higher than brown rice and polished rice, respectively. These findings align with previously reported proportions of MUFA in brown rice where the relative proportion of oleic acid, eicosenoic acid and palmitoleic were in the order of 44.75, 0.6 and 0.15 % weight of total fatty acids, respectively[33]. Oleic acid in polished rice was lesser than bamboo rice and brown rice by 22 % and 40 %, respectively. Similarly, previous studies found that oleic acid decreased after milling of rice by 10 %[34].

The total Saturated Fatty Acids (SFA) in bamboo rice is comparable to both brown and polished rice while there was a significant difference between Total Saturated Fatty Acids (TSFA) in brown (30.39 % FAME) and

polished rice (41.80 % FAME). Similarly, Chen *et al.*[35] reported that the TSFA concentrations in bamboo rice (*P. edulis* and *D. asper*) were comparable to those in the Chinese rice varieties Jiayou5, Fengliangyou4 and Liangyoupeijiu. Among SFA, caprylic acid in bamboo rice was lower than polished rice by 12 %. Myristic acid was lowest in bamboo rice (0.59±0.19 % FAME) when compared to both brown rice and polished rice. Compared to polished rice, myristic acid in bamboo rice was less by 73 %.
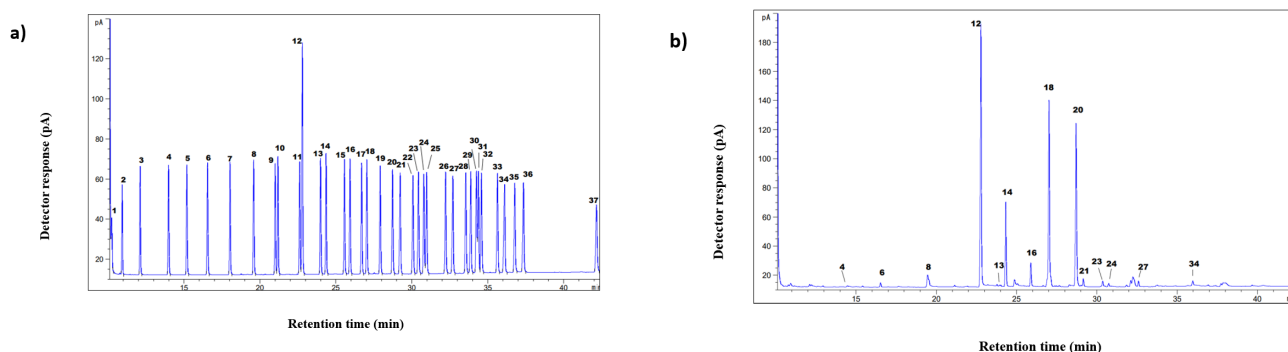

Fig. 1: Chromatogram of, (a): Fatty acid standard mix and (b): Fatty acids in bamboo rice
Note: 1: Butyric acid (C4:0); 2: Caproic acid (C6:0); 3: Caprylic acid (C8:0); 4: Capric acid (C10:0); 5: Undecanoic acid (C11:0); 6: Lauric acid (C12:0); 7: Tridecanoic acid (C13:0); 8: Myristic acid (C14:0); 9: Myristoleic acid (C14:1); 10: Pentadecanoic acid (C15:0); 11: Cis-pentadecenoic acid (C15:1); 12: Palmitic acid (C16:0); 13: Palmitoleic acid (C16:1); 14: Heptadecanoic acid (C17:0); 15: Cis-heptadecenoic acid (C17:1); 16: Stearic acid (C18:0); 17: Elaidic acid (C18:1n9t); 18: Oleic acid (C18:1n9c); 19: Linoelaidic acid (C18:2n6t); 20: Linoleic acid (C18:2n6c); 21: Gamma linolenic acid (C18:3n6); 22: Arachidic acid (C20:0); 23: Linolenic acid (C18:3n3); 24: Cis-11-eicosenoic acid (C20:1n9); 25: Heneicosanoic acid (C21:0); 26: Cis-11,14-eicosadienoic acid (C20:2); 27: Cis-8,11,14-eicosatrienoic acid (C20:3n6); 28: Behenic acid (C22:0); 29: Erucic acid (C22:1n9); 30: Cis-11,14,17-eicosatrienoic acid (C20:3n3); 31: Arachidonic acid (C20:4n6); 32: Tricosanoic acid (C23:0); 33: Cis-13,16-docosadienoic acid (C22:2); 34: Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3); 35: Lignoceric acid (C24:0); 36: Nervonic acid (C24:1n9); 37: Cis-4,7,13,16,19-docosahexaenoic acid (C22:6n3)

TABLE 1: FATTY ACID COMPOSITION OF BAMBOO RICE AND COMMONLY CONSUMED BROWN AND POLISHED RICE

Fatty acids (% dry weight)	Brown rice	Polished rice	Bamboo rice
Caprylic acid (C10:0)	0.02±0.005[a]	0.08±0.007[b]	0.07±0.01[b]
Lauric acid (C12:0)	0.06±0.01[a]	0.22±0.11[a]	0.21±0.13[a]
Myristic acid (C14:0)	0.76±0.05[a]	2.18±0.45[b]	0.59±0.19[a]
Palmitic acid (C16:0)	26.86±0.12[a]	36.03±0.55[b]	30.62±5.08[ab]
Stearic acid (C18:0)	1.81±0.05[a]	2.66±0.48[b]	3.06±0.46[b]
Arachidic acid (C20:0)	0.42±0.01[a]	0.30±0.04[a]	0.97±0.14[b]
Methyl behenate (C22:0)	0.17±0.004[a]	0.10±0.01[a]	0.72±0.12[b]
Methyl lignocerate (C24:0)	0.28±0.01[a]	0.22±0.03[a]	0.74±0.10[b]
Palmitoleic acid (C16:1)	0.22±0.001[b]	0.25±0.008[b]	0.14±0.03[a]
Oleic acid (C18:1n9c)	35.95±0.18[c]	21.34±0.56[a]	27.55±1.97[b]
Methyl 11-eicosenoate (C20:1)	0.41±0.00[b]	0.24±0.03[a]	0.90±0.12[c]
Linoleic acid (C18:2n6c)	31.97±0.29[a]	35.11±1.01[a]	33.76±6.07[a]
alpha linolenic acid (C18:3 n3)	1.05±0.005[b]	1.28±0.053[b]	0.68±0.17[a]
TSFA	30.39±0.17[a]	41.80±1.53[b]	36.97±6.01[ab]
Total Monounsaturated Fatty Acids (TMUFA)	36.58±0.18[c]	21.82±0.58[a]	28.59±2.01[b]
Total Polyunsaturated Fatty Acid (TPUFA)	33.02±0.29[a]	36.38±0.97[a]	34.44±6.22[a]

Note: The values are mean±SD (n=3 for brown and polished rice; n=6 for bamboo rice) and different superscript alphabets in the same row indicates the statistically significant (p<0.05) difference among rice samples

The proportion of palmitic acid was found to be higher by 25 % in polished rice which is in agreement with the findings of Longvah *et al.*[34] where it was observed that milling increases the proportion of palmitic acid in rice. Among all the analysed unsaturated fatty acids, bamboo rice was rich in oleic acid and linoleic acid. In the present study, polished rice contained higher levels of SFA than brown rice and bamboo rice. A similar increase in SFA content was observed in polished rice in previous studies[32,34]. Palmitic, oleic, myristic and stearic acids were higher in polished rice than in brown rice which is similar to the trend observed by Kim *et al.*[32]. The observed differences in fatty acids between polished rice, brown rice and bamboo rice may be attributed to milling, environmental factors, genetic factors and cultivation area which can significantly influence the lipid and fatty acid content in rice[36].

Amino acids, the fundamental components of proteins, are crucial for numerous biological functions, including growth, repair and maintenance of body tissues. Understanding the amino acid composition of rice is crucial for assessing its nutritional value[37]. Recent studies using inflammatory bowel disease models have identified peptides such as glutamine and arginine, which demonstrate chemoprotective and anti-apoptotic properties within the intestinal mucosa. These properties are crucial for reducing inflammation and restoring mucosal homeostasis[38]. In the present study, 18 different amino acids were quantified (Table 2). The total and essential amino acids in all the samples were similar but the Limiting Amino Acid (LAA) score (lysine) was significantly higher in bamboo rice (78) when compared to brown (68) and polished rice (61). The lysine content in brown, polished and bamboo rice were 3.96 g/100 g, 3.55 g/100 g and 4.52 g/100 g protein, respectively. Chen *et al.*[35] have observed that the lysine content in two different species of bamboo seeds namely *Passiflora edulis* (*P. edulis*) and *Dendrocalamus asper* (*D. asper*) were 4.55 g/100 g and 5.16 g/100 g protein, respectively. Lysine content in the International Rice Research Institute's world collection of rice showed a variability of not more than 3 g/100 g protein at any protein level[39]. In bamboo rice, the lysine content was positively correlated with the protein content[20].

TABLE 2: AMINO ACID COMPOSITION OF BAMBOO RICE AND COMMONLY CONSUMED BROWN AND POLISHED RICE

Amino acids (g/100 g protein)	Brown rice	Polished rice	Bamboo rice
Aspartic acid	8.82	8.63	9.38
Threonine	3.63	3.45	3.73
Serine	5.09	5.08	4.54
Glutamic acid	17.70	18.44	16.74
Proline	6.23	6.66	6.48
Glycine	4.64	4.38	4.98
Alanine	5.62	5.39	5.52
Cystine	1.44	1.35	1.59
Valine	5.88	5.86	5.90
Methionine	1.70	1.69	1.73
Isoleucine	4.16	4.19	3.94
Leucine	7.55	5.98	6.87
Tyrosine	5.27	5.89	4.41
Phenylalanine	6.06	6.90	5.98
Histidine	2.85	3.01	2.56
Lysine	3.96	3.55	4.52
Arginine	7.88	5.48	7.88
TAA	98.48	95.92	96.76
TEA	39.66	38.86	38.67
LAA score (lysine)	68	61	78

Note: TAA: Total Amino Acid and TEA: Total Essential Amino acid

Similarly, Cagampang *et al.*[40] have observed that the relative increase in the lysine content was concomitant with the increase in the protein content of polished rice, but at a substantially lesser rate than other amino acids. Rice protein has gained popularity due to its lysine content being up to 30 % higher than that of other cereals and grains such as corn, wheat, millet and sorghum[41]. In this context, it is noteworthy that bamboo rice contains a relatively higher lysine content compared to commonly consumed brown and polished rice. The concentrations of essential amino acids, namely lysine and threonine were higher in bamboo rice by 12 % and 2.6 % when compared to brown rice and by 21 % and 7.5 % compared to polished rice respectively. In the present study, histidine and phenylalanine content in bamboo rice were 2.56 g/100 g and 5.98 g/100 g protein, respectively. Gopalan *et al.*[42], have also reported similar concentrations for histidine and phenylalanine which were 2.93 g/100 g and 6.09 g/100 g protein, respectively. However, the concentrations of arginine, lysine, methionine, threonine, leucine, isoleucine and valine were found to be comparatively lower than the values reported in the Nutritive Value of Indian Foods (NVIF) by Gopalan *et al.*[42]. This variation could be due to the difference in the analytical methodology used.

The Essential Amino Acids (EAAs) in bamboo rice were found to be in the decreasing order of leucine, phenylalanine, valine, lysine, isoleucine, threonine, histidine and methionine with respective concentrations of 6.87, 5.98, 5.9, 4.52, 3.94, 3.73, 2.56 and 1.73 g/100 g protein. Chen *et al.*[35] reported that the EAAs in *P. edulis* species of bamboo rice were present in the decreasing order of leucine, phenylalanine, valine, lysine, isoleucine, threonine, methionine and histidine with respective concentrations of 8.99 g/100 g, 7.25 g/100 g, 6.63 g/100 g, 4.55 g/100 g, 4.44 g/100 g, 3.93 g/100 g, 2.81 g/100 g and 2.75 g/100 g protein. However, in *D. asper* species of bamboo rice, the decreasing order of EAA was leucine, phenylalanine, valine, lysine, isoleucine, threonine, histidine and methionine with respective concentrations of 8.70 g/100 g, 6.85 g/100 g, 6.70 g/100 g, 5.16 g/100 g, 4.31 g/100 g, 3.85 g/100 g, 2.85 g/100 g and 2.39 g/100 g protein[35]. In the present study, EAAs in brown rice were distributed in the order of leucine, phenylalanine, valine, isoleucine, lysine, threonine, histidine, methionine with respective concentrations of 7.55 g/100 g, 6.06 g/100 g, 5.88 g/100 g, 4.16 g/100 g, 3.96 g/100 g, 3.63 g/100 g, 2.85 g/100 g and 1.7 g/100 g protein. Moongngarm *et al.*[43] reported the brown rice EAAs contents in the order leucine, valine, lysine, phenyalanine, threonine,

histidine, isoleucine and methionine with respective concentrations of 9.96 g/100 g, 7.72 g/100 g, 4.74 g/100 g, 4.73 g/100 g, 3.65 g/100 g, 3.17 g/100 g, 1.95 g/100 g and 1.48 g/100 g protein. In this study, the concentrations of aspartic acid, valine, methionine and lysine were found to be higher in bamboo rice compared to brown and polished rice. Similarly, Chen *et al.*[35] observed that the concentrations of these essential amino acids in bamboo rice were significantly higher than those in the Chinese rice varieties Jiayou5, Fengliangyou4 and Liangyoupeijiu.

Arginine is known to be one of the most significant non-essential amino acids involved in synthesising other nitrogenous compounds vital for physiological viability[44]. In this study, bamboo rice contained arginine at 7.8 g/100 g protein which was similar to that in brown rice but higher by 30 % compared to polished rice. However, the concentrations of histidine and phenyl alanine in bamboo rice were lower than in brown and polished rice (Table 2). The concentrations of all amino acids analysed in brown and polished rice in this study were comparable to the values reported by Longvah *et al.*[34,45].

A combination of plant sterols and stanols make up phytosterols and are generally present in small quantities. They are structurally closer to cholesterol while it is different by the side chain i.e., the presence or absence of double bonds[46]. Cereals and their by-products are reported to be significant sources of phytosterols. Common cereals such as wheat, corn, rice and oats contain notable amounts of phytosterols particularly β-sitosterol, campesterol, and stigmasterol[47]. These phytosterols were quantified in brown, polished and bamboo rice (fig. 2 and Table 3). Among all the samples analysed, β-sitosterol (22.26 mg/100 g) was the predominant phytosterol in bamboo rice. β-sitosterol content in brown and polished rice were 19.72±0.28 mg/100 g and 7.90±0.11 mg/100 g, respectively. Piironen *et al.*[48] reported 37.5 mg/100 g and 19.5 mg/100 g of β-sitosterol in brown and polished rice, respectively. This finding confirms that β-sitosterol is the dominant phytosterol in brown rice compared to polished rice. The concentrations of campesterol and stigmasterol in brown rice were 8.18±0.27 mg/100 g and 6.03±0.19 mg/100 g, respectively which was double the amount in bamboo rice (4.10±0.20 mg/100 g and 3.20±0.39 mg/100 g, respectively). However, the concentration of β-sitosterol and ergosterol were not significantly different among bamboo rice (22.26±1.86 mg/100 g and 4.77±0.68 mg/100 g respectively), and

brown rice (19.72±0.28 mg/100 g and 4.25±0.31 mg/100 g respectively), Although the concentration of ergosterol in polished rice (5.50±0.43 mg/100 g) was comparable with brown rice and bamboo rice, it had the lowest levels of all the other phytosterols analysed. The campesterol, sitosterol and stigmasterol content in rice were reported as 14.6 mg/100 g, 37.5 mg/100 g and 10. 4 mg/100 g previously[49]. Furthermore, the phytosterol content in brown rice and bamboo rice was 50 %-55 % higher than in polished rice. This is because phytosterols are predominantly located in the germ and bran layers of cereals, making wholegrain products excellent sources of these sterols[46,50]. Therefore, increasing the consumption of cereals, especially whole grains, can be considered a natural way to increase the intake of phytosterols.

Phenolic acids include a group of cinnamic and benzoic acid derivatives that occur in both free and bound forms in plant-based foods[51]. Regular consumption of phytochemicals, particularly phenolic compounds found in cereals, has been shown to potentially reduce the risk of degenerative diseases, such as heart disease and cancer[46,52]. In the current study, the TPC was extracted with methanol and water independently, analysed and presented in Table 4.
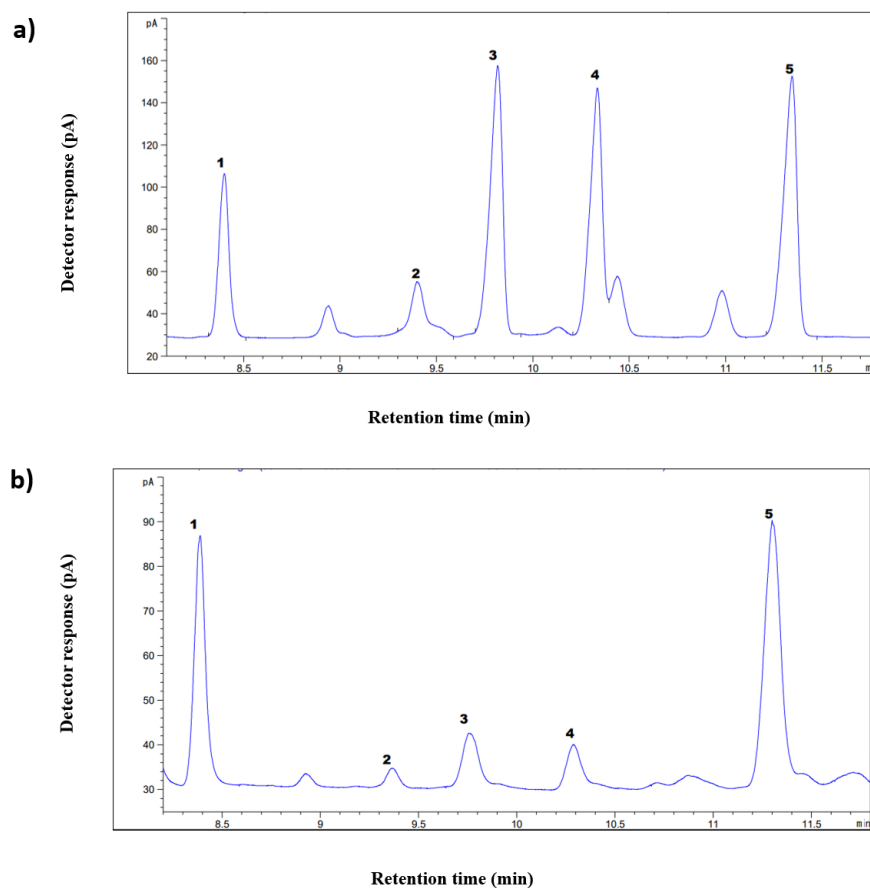

Fig. 2: Chromatogram of of, (a): Phytosterol standards and (b): Phytosterols in bamboo rice
Note: 1: Alpha cholesterol; 2: Ergosterol; 3: Campesterol; 4: Stigmasterol and 5: β-sitosterol

TABLE 3: PHYTOSTEROL CONCENTRATION IN BAMBOO RICE AND COMMONLY CONSUMED BROWN AND POLISHED RICE

Sample	Phytosterols (mg/100 g)			
	Ergosterol	Campesterol	Stigmasterol	β-sitosterol
Brown rice	4.25±0.31[a]	8.18±0.27[c]	6.03±0.19[c]	19.72±0.28[b]
Polished rice	5.50±0.43[a]	2.57±0.06[a]	1.38±0.01[a]	7.90±0.11[a]
Bamboo rice	4.77±0.68[a]	4.10±0.20[b]	3.20±0.39[b]	22.26±1.86[b]

Note: The values are mean±SD (n=3 for brown and polished rice and n=6 for bamboo rice) and different superscript alphabets in the same column indicates the statistically significant (p<0.05) difference among rice samples

TABLE 4: TPC AND INDIVIDUAL POLYPHENOL CONCENTRATION IN BAMBOO RICE AND COMMONLY CONSUMED BROWN AND POLISHED RICE

TPC (g GAE in 100 g)	Brown rice	Polished rice	Bamboo rice
TPC in water extract	0.091±0.001[b]	0.025±0.000[a]	0.09±0.010[b]
TPC in methanolic extract	0.071±0.000[a]	0.024± 0.001[b]	0.04±0.010[c]
Individual polyphenols (mg/100 g)			
Gallic acid	0.008±0.006[a]	BDL	0.043±0.015[b]
Protocatechuic acid	0.068±0.01[a]	BDL	0.426±0.259[b]
4-hydroxy benzoic acid	0.085±0.006[a]	0.086±0.011[a]	0.132±0.067[a]
Catechin	BDL	BDL	0.963±0.563[a]
Caffeic acid	0.032±0.003[a]	BDL	0.037±0.02[a]
Sinapic acid	0.146±0.013[a]	0.043±0.003[a]	0.349±0.245[a]
Ferulic acid	0.516±0.012[b]	0.066±0.003[a]	0.266±0.242[ab]
4-coumaric acid	0.117±0.002[a]	BDL	0.292±0.231[a]
Ellagic acid	BDL	BDL	0.024±0.018[a]
2-coumaric acid	0.026±0.003[a]	BDL	0.177±0.06[b]
Luteolin 7-O-glucoside	BDL	BDL	0.157±0.09[a]
Myricetin	BDL	BDL	0.272±0.105[a]

Note: The values are mean±SD (n=3 for brown and polished rice and n=6 for bamboo rice); different superscript alphabets in the same row indicate the statistically significant (p<0.05) difference among rice samples and BDL: Below Detectable Limit

It was found that water was more efficient than methanol in extracting polyphenols in brown, polished and bamboo rice. In aqueous medium extraction, the TPC content of bamboo rice was 0.090±0.010 g GAE/100 g, which is comparable to the TPC of brown rice (0.091±0.001 g GAE/100 g). In methanolic medium extraction, the TPC of brown rice (0.071 g GAE/100 g) was higher than bamboo rice (0.04 g GAE/100 g) and polished rice (0.024 g GAE/100 g). However, in a study by Haldipur *et al.*[16] bamboo rice showed significantly higher content of TPC (0.0647 g GAE/100g) when compared to brown rice (0.0485 g GAE/100 g) when extracted in a mixture of methanol, water and formic acid. This difference in the TPC of the bamboo rice in the present study could be due to the difference in the extraction medium. It has been proved that the amounts of phenolics and their antioxidant activity typically differ based on the extraction process and vary significantly among the aleurone, bran and whole grain with the aleurone layer containing the highest levels[53,54]. However, in this study, the TPC in brown rice is consistent with the findings of Gong *et al.*[55] who reported that the TPC of brown rice cultivars ranged from 0.072-0.120 g GAE/100 g. Polished rice had the

least content of TPC in both water and methanolic extracts with 0.025±0.0004 g GAE/100 g and 0.024±0.001 g GAE/100 g, respectively. This reduction in phenolic concentration is attributed to the removal of the outer layers of the grain during polishing[56]. The observed variations in the TPC content in brown rice, polished rice and bamboo rice could be ascribed to the difference in cultivation conditions[57].

Polyphenols, naturally occurring in cereals like wheat, rice and oats, offer numerous health benefits including antioxidant and anti-inflammatory effects. Antioxidant compounds, predominantly phenolic compounds are located in both free and insoluble forms within the outer layers of cereals[58]. In cereals, the primary hydroxycinnamic acids present are sinapic acid, ferulic acid and p-coumaric acid[59]. In this study, 21 polyphenolic compounds were analysed in bamboo rice, brown rice and polished rice, out of which 12 compounds were detected and quantified (Table 4). The polyphenols that were detected and quantified in bamboo rice were catechin>protocatechuic acid>sinapic acid>4-coumaric acid>myricetin>ferulic acid>2-coumaric acid>luteolin7-o-glucoside>4-

hydroxy benzoic acid>gallic acid>caffeic acid>ellagic acid. Among these, catechin (0.963±0.563 mg/100 g), ellagic acid (0.024±0.018 mg/100 g), luteolin 7-O-glucoside (0.157±0.09 mg/100 g) and myricetin (0.272±0.105 mg/100 g) were exclusively detected in bamboo rice, with catechin emerging as the predominant polyphenol. Similarly, in a study by Haldipur *et al.*[16] where an untargeted phenolic profiling of bamboo rice was conducted by quadrupole time-of-flight liquid chromatography with tandem mass spectrometry, gallic acid, 4-hydroxybenzaldehyde, caffeic acid, 4-O-glucoside and p-coumaric acid were reported to be the most abundant among the 63 different phenolic metabolites identified[16]. Certain polyphenols in bamboo rice such as gallic acid, protocatechuic acid and 2-coumaric acid were found to be over 80 % higher than brown rice. Sinapic acid, 4-coumaric acid, 4-hydroxybenzoic acid and caffeic acid in bamboo rice were also abundant by 58 %, 59 %, 35 % and 13 % respectively when compared to brown rice. Moreover, polyphenols such as protocatechuic acid, sinapic acid and ferulic acid which were detected in polished rice were approximately 75 %-85 % lower than in bamboo rice. It was found that the concentrations of

polyphenols such as ferulic acid and caffeic acid were comparable with concentrations reported previously in brown rice and polished rice[60-62]. van Hung[63], also reported relatively higher concentrations of coumaric and ferulic acids and lower concentrations of gallic, caffeic and vanillic acids in brown and polished rice as observed in this study. These bioactive polyphenol compounds possess high antioxidant potential having significant pharmaceutical relevance due to their biological activities, such as anti-allergic, antimicrobial, anticancer and anti-inflammatory effects[6].

The antioxidant activities exhibited by the bamboo rice were investigated and compared with the commonly consumed polished and brown rice (fig. 3). The methanolic extract of bamboo rice exhibited 25 % higher antioxidant activity in the FRAP assay (185±36.79 mM $FeSO_4$ equivalents/100 g) and a 10 % higher activity in the DPPH assay (IC_{50} of 65.52±7.03 mg/ml) compared to its water extract, which showed 139±38.13 mM $FeSO_4$ equivalents/100 g and an IC_{50} of 73.07±9.89 mg/ml, respectively. However, the water extract of bamboo rice demonstrated approximately 32 % higher activity in the ABTS assay when compared to the methanolic extract.
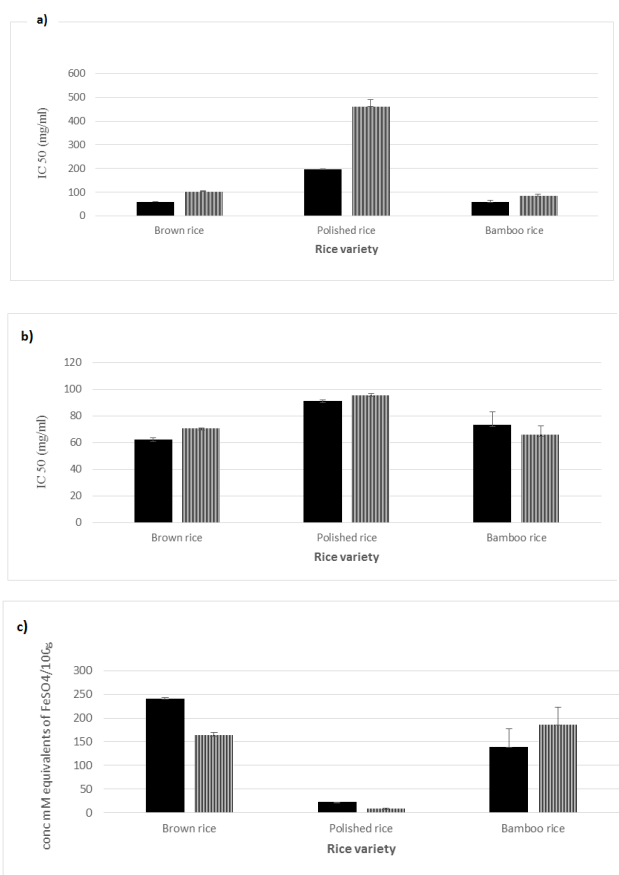

Fig. 3: Antioxidant activity of bamboo rice and commonly consumed brown and polished rice by, (a): ABTS assay, (b): DPPH assay and (c): FRAP assay

Though bamboo rice exhibited a higher antioxidant activity with ABTS assay in both water and methanolic extracts (IC$_{50}$ of 57.62±7.30 mg/ml and 84.81±7.75 mg/ml, respectively), brown rice showed a better activity for both FRAP (241±1.82 mM equivalents of FeSO$_4$/100 g and 163±5.92 mM equivalents of FeSO$_4$/100 g, respectively) and DPPH assays (IC$_{50}$ of 61.96±1.61 mg/ml and 70.34±0.50 mg/ml, respectively). Notably, bamboo rice exhibited superior antioxidant activity compared to polished rice across all the assays. This is in agreement with studies that have demonstrated that darker grains possess higher levels of total phenolics and exhibit enhanced antioxidant activity[64,65]. According to Galanakis[66], phenolic compounds such as sinapic, gallic, syringic, chlorogenic, cinnamic, caffeic, ferulic, protocatechuic and vanillic acids can be utilized as additives in meat products to inhibit lipid oxidation and prolong shelf life. This is attributed to their capacity to neutralize radicals through hydrogen donation. Furthermore, Karimi et al.[6], underscored the potential of phenolic compounds and fatty acids, including palmitic acid, oleic acid, linoleic acid and γ-linolenic acid in contributing to antioxidant activity[6]. Therefore, the antioxidant activity observed in bamboo rice is likely due to the presence of various polyphenols and fatty acids. The biological activities of these compounds may be linked to the ease with which an aromatic hydroxyl group can donate a hydrogen atom to a free radical and the capacity of an aromatic compound to stabilize an unpaired electron. This mechanism is crucial, as compounds with antioxidant activity can safeguard cellular systems from the detrimental effects of metabolic processes that cause excessive oxidation. Additionally, these antioxidants can disrupt free radical chain reactions, scavenge free radicals and thereby help prevent cancer, heart disease, vascular disorders, and neurodegenerative diseases[6]. Therefore, the significant antioxidant activity in bamboo rice, driven by its rich polyphenol content, underscores its potential to protect against oxidative stress and related chronic diseases.

Conclusion:

Epidemiological data has demonstrated a positive correlation between the consumption of antioxidant-rich foods and improved human well-being. This study aimed to explore the BACs in bamboo rice, revealing a rich profile of essential BACs beneficial for human health. Bamboo rice contained a higher proportion of total MUFA and a lower proportion of SFA compared to polished rice. The concentrations of essential amino acids namely lysine and threonine were higher in bamboo rice compared to both brown and polished rice. The significant antioxidant activity exhibited by bamboo rice can be attributed to the higher concentrations of various individual polyphenolic acids analysed, including protocatechuic, gallic, 4-hydroxybenzoic, catechin, caffeic, sinapic, ferulic, 4-coumaric, ellagic, 2-coumaric, luteolin 7-O-glucoside and myricetin. Moreover, few polyphenols were exclusively found in bamboo rice when compared to brown and polished rice. The findings of this study can significantly contribute to further research aimed at enhancing the extraction and utilization of these BACs from bamboo rice. The data gathered can also help explore the mechanisms and pathways of these BACs and their interactions with human metabolism and physiology. These improvements can lead to the development of new functional foods, supplements, and nutraceutical products that offer health benefits such as lowering cholesterol, preventing oxidative stress, and improving protein quality. Importantly, this study can also support the conservation and use of bamboo rice as a traditional and nutritious food source for indigenous and rural communities in India and other regions where it naturally grows.

Conflict of interest:

The authors declare no conflict of interests.

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