ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1272-1283

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Nutraceutical Enrichment of Cracker with Incorporation of Bamboo Shoot and Green Gram Flour

RITISMITA DEVI[1], MANASH PRATIM SARMA[2]*, GIRIDHAR GOUDAR[3] and DAISY SHARMA[4]

[1]Programme of Biochemistry, Faculty of Science, Assam down town University, Guwahati, Assam, India.
[2]Programme of Biotechnology, Faculty of Science, Assam down town University, Guwahati, Assam, India.
[3]Food Quality Analysis & Biochemistry Division, Biochem Research & Testing Laboratory, Dharwad, Karnataka, India.
[4]Programme of Food Nutirtion and Dietetics, Faculty of Science, Assam down town University, Guwahati, Assam, India.

Abstract

Functional food products in the form of ready to eat (RTE) foods are gaining attention in recent years. Consumers are looking for the RTE with enhanced nutritional components and nutraceutical properties. In the present study, a functional food of cracker (RTE) is formulated by combination of Joha rice, bamboo shoot and green gram flours as main ingredients. The results revealed that protein and dietary fiber contents of the cracker were found to be 17.05 and 15.77 g/100g, respectively. The B-group vitamins of B3, B5, B6 and B9 in cracker were found to be 2.04, 2.50, 0.57 and 12.59 mg/100 g, respectively. Among the fat-soluble vitamins, the predominant vitamin in the cracker was vitamin E (11.655 µg/1000g) followed with vitamin A (24.09 µg/1000g) and vitamin K (0.59 µg/1000g). The predominant fatty acids in crackers were linoleic acid (46.28%) and palmitic acid (22.34%). Polyphenolic profile analysis revealed the higher content of gallic acid followed with protocatechuic acid, ferulic acid and luteolin in the cracker. The phenolic composition was analyzed for total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), total proanthocyanidin content (TPAC) and total carotenoid content (TCC). Antioxidant activity was analyzed by the DPPH, ABTS, FRAP and ORAC assays. Enzyme inhibition activity for α-amylase and α-glucosidase enzymes was found to be 62.43

Article History
Received: 23 July 2024
Accepted: 28 November 2024

Keywords
Antioxidant Activity;
Cracker;
Functional Food;
Minerals;
Polyphenol;
Vitamins.

CONTACT Manash Pratim Sarma manash3268@gmail.com Programme of Biotechnology, Faculty of Science, Assam down town University, Guwahati, Assam, India.

Doi: "https://dx.doi.org/10.12944/CRNFSJ.12.3.5

and 47.97 of IC50 µg GAE/mL in the cracker, respectively. The developed functional food (cracker) was found with higher retention percentage of prime nutrients including protein, dietary fiber and vitamins along with vital polyphenols.

Introduction

A functional food has been defined as any food product developed which include the conventional wholesome foods, which are enriched or enhanced to provide health beneficial properties in addition to basic nutrients , Another food, which is commonly consumed across Assam in India, is bamboo shoot (*Dendrocalamus hamiltonii*) with its health beneficial properties (*Behera & Balaji*,[3] The composition of bamboo shoot has the benefits of improving digestion, relieving sweating and hypertension, preventing cardiovascular diseases and cancer because bamboo shoots are rich in functional nutrients such as high quantity of protein, vitamin, dietary fibre and bioactive compounds, but low in fat and sugar contents.

In recent years, fresh nutritional value, however, its tissue lignification and flesh browning lead to rapid deterioration of its edible quality during postharvest.4 Bamboo shoot has immense medicinal value owing to their rich bioactive compounds. The major organic acid in bamboo shoot was oxalic acid , ranging from 157- 462 mg /100 g fresh weight. Citric acid was rich in the upper half while malic was rich in the lower quarter Fructose, glucose and sucrose were contained with approximately equal amount in the top quarter section, the former two sugars were also abundant in the lower half to balance one of the important nutrients (protein) which is deficient in *Joha* rice, a legume grain (green gram) was incorporated along with bamboo shoot. Selection of proper ingredients for preparing the flours will have an impact on the manufacturing of a functional food.[5]

In recent years, ready to eat (RTE) foods have been gaining popularity due to their easy preparation, availability, and convenience.[8 ,6] Several RTE foods have been developed by food technologists depending on their local availability and composition. Most of the foods developed are based on the taste perception as a major factor. The crackers, which are traditionally prepared using rice as a main component, are the deep-fried snack foods. Usually, the steamed rice is given circular shape and sundried, then they are deep-fried in oil to prepare the final product. In recent years the consideration of the consumers' health consciousness, the deep-fried foods need to be avoided and replaced by healthy snack food which can serve as a functional food.

The present study was undertaken to develop a functional food containing *Joha* rice, green gram and bamboo shoot flour as major ingredients. The functional foods which can serve as a snack food with nutraceutical properties are limited in context of Indian population. Different combinations were optimized using the *Joha* rice, green gram and bamboo shoot flours and the crackers were developed using the optimized composition of flour mix, which were baked using oven toaster grill (OTG) - microwave oven instead of deep-frying in oil. The developed product of cracker was assessed for the nutritional, polyphenolic and antioxidant composition.

Materials and Methods

Chemicals and Reagents

The chemicals used for the analysis were analytical grade (Merck KGaA, [Darmstadt], Germany). The reference standards were HPLC grade and purchased from Sigma-Aldrich Co. St. Louis, USA. The ultra-pure water was used for the reagent preparation during the analysis (MilliQ®, Merck KGaA, [Darmstadt], Germany).

Sample Preparation

The samples of bamboo shoot (*Dendrocalamus hamiltonii*), *Joha* rice (*Oryza sativa* L) and green gram (*Vigna radiata*) were dried at 50 °C using hot air oven ,manufacturer mercury and homogenized using the laboratory grinder to obtain the flours of the samples (Supplementary Table 3). All the three main ingredient of flour samples were mixed in different combinations and the composition was optimized by sensory evaluation score of the formulated crackers (Supplementary Table 1 & 2). The flour samples prior to mixing were equilibrated at room temperature for 30 min.

The optimization of formulated cracker was obtained from composition flour (F4) consisted of *Joha* rice (55%), green gram (30%) and bamboo shoot powder (15%). The composite flour was mixed with required quantity of water to prepare evenly kneeled dough by mixing a pinch of table salt. The dough was rested for approximately 10 min and flattened manually on a tray. The flattened dough was given circular shape with having size of 1.4 cm (diameter) and 0.2 cm (thickness). These circular doughs were baked in a LG model microwave oven (OTG) at 120 °C for 20 min, monitored for non-charring and the crackers were developed. The sample obtained was cooled to room temperature, homogenized to give flour and subjected for further analysis.

Proximate and Dietary Fiber Components

The proximate components were analyzed using the AOAC methods. Protein content was analyzed using Kjeldahl method by multiplying the obtained nitrogen content with 6.25 (AOAC 978.04). Soxhlet method was used to analyze the fat content (AOAC 963.15). Ash content is analyzed by incinerating the samples at 550 °C using muffle furnace, Bionics Scientific (AOAC 930.05). Total carbohydrate content was analyzed by the phenol-sulphuric acid metCalorific value of the food combusted was used to calculate the energy (total calories) of the sample using the bomb calorimeter(R.A. Scientific instruments). Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were analyzed by the enzymatic gravimetric method (AOAC 991.43). The total dietary fiber (TDF) was obtained by adding the SDF and IDF.The results obtained were reported as g/100 g and total energy as Kcal/100 g on dry weight basis (DWB) of sample.

Carbohydrate Components

Reducing and non-reducing sugars in the samples were analyzed by UV-Vis spectrophotometric method. The amylose and resistant starch content was analyzed using the Megazyme enzymatic kit method. 7,10.

Vitamin Analysis

Analysis of B-group vitamins (B3, B5, B6, B9, C) and fat-soluble vitamins (A, D, E, K) was carried out by HPLC method. [8,20] The HPLC system consisted of autosampler, binary pump and diode array detector (DAD) (1260 Infinity II, Agilent Technologies Inc., Santa Clara, USA). The C18 column of 250 × 4.6 mm with 5 µm (Hypersil BDS, Thermo Scientific, Waltham, MA USA) was used for the analysis. B-group vitamins were separated by two mobile phases of solvent A – phosphate buffer (pH 4) and acetonitrile (98:2, v/v) and solvent B – methanol and deionized water (50:50, v/v) with the gradient elution and a flow rate of 0.8 mL/min. Fat soluble vitamins were separated using isocratic elution with mobile phase consisting of acetonitrile: methanol (98:2, v/v) and flow rate of 1 mL/min.

Mineral Estimation

Calcium (Ca), iron (Fe), zinc (Zn) and manganese (Mn) elements were analyzed as per the AAS method.[9,21] Sample (0.5 g) was digested in microwave digestor using 65% HNO_3:H_2O_2 (3:1 v/v). The digested sample extract was diluting using 3% HNO_3 and analyzed for mineral composition using flame atomic absorption spectroscopy (FAAS) (240FS AA, Agilent Technologies Inc., Santa Clara, USA). The results obtained were expressed as mg/100g of flour on dry weight basis.

Fatty acid profile

The fatty acid profile in the samples was evaluated by GC-FID according to the previously reported method . 10 The results obtained were reported as % of fatty acid methyl ester (FAME). Sample (1.0 g) was digested with KOH and methanol containing 0.05% of butylated hydroxyl toluene (BHT) by incubating for 1 h 30 min at 55 °C. After addition of 24 N H_2SO_4 samples were incubated at 55 °C for further 1 h 30 min. Sample extracts were vortexed after addition of n-hexane and centrifuged to separate n-hexane layer which was collected in separate tube containing sodium sulphate. The solvent extract was evaporated using nitrogen gas, then dichloromethane was added to dissolve the residue obtained. The sample extract was filtered using 0.2 µm PVDF membrane and analyzed with GC-FID manufactured by biobase. The separation was done using GC capillary column (75 × 0.18 mm, 0.14 µm) (SP 2560 column, Supelco, Sigma-Aldrich, St. Louis MO USA). The injector temperature was 250 °C with split ratio of 1:100 and carrier gas (H2) was 0.6 mL/min.

Polyphenol Profile

Extraction of Polyphenols

The extraction of polyphenols was done as per the standard protocol of already conducted experiments.[11] 1.0 g of Sample was extracted with

80% hydro methanol (10 mL) for 3 h under dark condition with intermittent vortexing every 30 min. The sample extracts were centrifuged at 2236 ×g (10 ºC, 12 min) and the supernatant were collected. The sample residue was re-extracted with 80% methanol and the extraction process repeated for two times. The supernatants obtained were pooled and evaporated using a rotary evaporator manufactured by Trident under vacuum. The dried residue was reconstituted using 5 mL of methanol, filtered with 0.2 µm filter membrane and used for HPLC analysis of polyphenols.

The polyphenol profile of extracted samples was evaluated by HPLC system (1260 Infinity II, Agilent Technologies Inc., Santa Clara, USA) attached with binary solvent system and diode-array detection (DAD) . The quantification of individual polyphenols was performed using a C18 column (150 × 2 mm, 3 µm) (Luna, Phenomenex). The HPLC conditions were; column temperature - 30 ºC, mobile phase – solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), injection volume - 5 µL, gradient elution, flow rate 0.9 mL/min and λ max 280 nm. The results obtained were expressed as mg/100 g of flour on DWB.

Phenolic Composition and Antioxidant Properties

Total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), total proanthocyanidin (TPAC) and total carotenoid content (TCC) were analyzed for phenolic composition Goudar.[13]

2,2-diphenyl-1-picrylhydrazyl - radical scavenging activity (DPPH-RSA), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) - radical scavenging activity (ABTS-RSA), ferric reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) were analyzed to assess the antioxidant properties Sharma.[14]

Enzyme Inhibition Assay

A-Amylase Inhibition Activity

The α-amylase inhibitory activity was assessed by reacting varying concentrations of phenolic extracts with α-amylase in a phosphate buffer, followed by incubation with soluble starch. The reaction was terminated using 3,5-dinitrosalicylic acid reagent and heated, then diluted, and the absorbance measured at 540 nm. Acarbose served as a positive control, and the percentage inhibition was calculated based on the difference in absorbance between the control and sample.[15]

$$\% \text{ Inhibition} = (A_{control} - A_{sample} / A_{control} \times 100$$

Where, $A_{control}$ is the absorbance of blank solution, A_{sample} is the absorbance of the sample

A-Glucosidase Inhibition Activity

The α-glucosidase inhibitory activity of the samples was determined using a spectrophotometric assay. Phenolic extracts were incubated with α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (PNPG) at 37 °C, and the reaction was terminated with Na2_2CO3_3. The absorbance was measured at 410 nm to quantify the enzyme activity. Acarbose served as a positive control, and the percentage inhibition was calculated based on the difference in absorbance between the control and the samples.[16]

$$\% \text{ Inhibition} = 1\text{-}A_{sample} / A_{control} \times 100$$

where, $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample. The IC_{50} value is referred to the concentration of sample extract required for 50% inhibition.

Statistical Analysis

All the analyses were carried out in triplicate and the results were reported as mean ± standard deviation (SD). The one-way statistical analysis of variance (ANOVA) was analyzed to obtain significant difference between means ($p<0.05$) (IBM SPSS Statistics, Version 24.0., IBM Corp., Armonk, USA). The heatmap was generated using the GraphPad – Prism 8 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Proximate and Dietary Fiber Components

The results obtained for the proximate and dietary fiber components for the analyzed samples are presented in Table 1. The protein content in the developed product (cracker) was found to be 17.05 g/100 g. A reduction in protein content was observed in the cracker in comparison to Bamboo shoot and green gram samples ($p<0.05$). Since, green gram is known to be nutrient dense, especially the protein content (26.40%),[17] it has been incorporated to increase the protein content in

functional food (cracker) in the present study. *Joha* rice was previously reported for a similar content of protein ,[11] which was in accordance with the study.[17] reported for protein content ranging between 7.13 to 9.86 g/100 g in the crackers developed from corn and sesame. Fat content of the crackers in the present study was found to be 3.07 g/100 g. However, fat content among the ingredients used in preparation of crackers was found between 0.19 to 2.11 g/100g (Table 1). The crackers formulated using water chestnut and barley flour (70:30%) were found to have fat content of 3.80 g/100g.[17] Green gram is known to have a wide range of fat content ranging between 0.12 to 2.31%.[18, 21] Total minerals (ash) content in the analyzed samples was ranging between 1.35 to 5.88 g/100g, with the highest and lowest content found in bamboo shoot and *Joha* rice flour, respectively (Table 1). The variation observed for the protein, fat and ash content reported among various studies might be due to the varietal differences of the rice and green gram samples.

Table 1 Proximate, dietary fiber & carbohydrate (per 100g) components of analyzed samples

Parameter	Raw flour samples of functional food			Functional food
	Joha rice	Green gram	Bamboo shoot	Cracker
Protein (g)	10.68±0.10^{a}	21.87±0.30^{d}	21.01±0.50^{c}	17.05±0.16^{b}
Fat (g)	2.11±0.06^{c}	1.13±0.06^{b}	0.19±0.03^{a}	3.07±0.12^{d}
Ash (g)	1.35±0.09^{a}	3.50±0.22^{c}	5.88±0.25^{d}	2.72±0.31^{b}
Carbohydrates (g)	66.55±1.56^{d}	48.82±0.36^{b}	44.43±1.06^{a}	58.38±0.15^{c}
Energy (Kcal)	330±7.10^{c}	291±1.53^{b}	268±3.06^{a}	289±4.10^{b}
SDF (g)	0.47±0.13^{a}	2.89±0.11^{b}	2.96±0.16^{b}	2.81±0.13^{b}
IDF (g)	1.93±0.05^{a}	12.51±0.26^{b}	18.68±0.07^{d}	12.96±0.13^{c}
TDF (g)	2.4±0.08^{a}	17.43±0.43^{c}	21.64±0.37^{d}	15.77±0.25^{b}
Reducing sugars (g)	1.82±0.06^{c}	0.88±0.08^{b}	0.23±0.02^{a}	2.46±0.03^{d}
Non reducing sugars (g)	12.8±0.21^{c}	7.26±0.30^{b}	4.03±0.21^{a}	14.47±0.10^{d}
Amylose (g)	18.92±0.62^{c}	15.29±0.37^{a}	21.01±0.50^{d}	16.21±0.27^{b}
Resistant starch (g)	2.61±0.26^{a}	5.90±0.22^{d}	3.08±0.06^{b}	4.21±0.29^{c}

Carbohydrate components of the analyzed samples were reported in Table 1. Total carbohydrate content in the crackers was found to be 58.38 g/100 g, which was comparatively less to the carbohydrate of *Joha* rice (66.55 g/100 g) ($p<0.05$). TDF in bamboo shoot and green gram flour was found to be 21.64 and 17.43 g/100 g, respectively, interestingly, the retention of TDF in the developed product (cracker) was 72.88 and 90.48% in comparison to bamboo shoot and green gram samples, respectively. Comparatively, a study reported for lesser TDF in the crackers developed from the wheat of Brazil and USDA with 2.5 and 4.5 g/100 g, respectively. Among the dietary fiber fractions analyzed in the study, higher SDF was recorded in green gram and bamboo shoot flour with 2.89 and 2.96 g/100 g, respectively. The retention of SDF in developed product (cracker) was ~94-97% in comparison with the green gram and bamboo shoot flour. Since, dietary fiber (DF) is nutritionally significant and plays an important role, in particular the SDF is involved to increase the viscosity of stomach, whereas the bowel movement of the food mass bulk is increased by IDF. The effective proportion of SDF and IDF fraction in foods is considered to be in the ratio of 1:2.[18 .18] Reducing and non-reducing sugars in the analyzed samples varied from 0.23 to 2.46 and 4.03 to 14.47 g/100 g, respectively. Comparatively less content of reducing and non-reducing sugars was reported in *Joha* rice by another study with 0.14 and 0.50%, respectively.[18, 11] The amylose content in the analyzed samples was ranging between 15.29 and 21.01 (%) with crackers containing 16.21 % amylose. Resistant starch was found to be 4.21 % in crackers, whereas in the raw samples used to formulate product it was ranging from 2.61 to 5.90 %. Different

processing treatments were shown to have minimal effect on the nutritional components in green gram (mung bean),[19] hence the green gram was selected for inclusion into cracker. The retention percentage of important components which include protein, SDF, amylose and resistant starch was found to be optimum in the developed product (cracker), which are vital in maintaining proper health.

Micronutrient Composition

The micronutrients analyzed in the samples include the water-soluble vitamins (B3, B5, B6, B9, C), fat-soluble vitamins (A, D, E, K) and minerals (Ca, Fe, Zn, Mn) (Figure 1). Among the B-group vitamins, vitamin B9 was predominantly found in the analyzed samples ranging from 0.08 mg/100g (bamboo shoot) to 1.5283 mg/100g (green gram) with the crackers containing vitamin B9 of 1.259 mg/100g ($p<0.05$). The crackers developed with corn and sesame seeds were reported with vitamin B9 content ranging between 10.04 to 12.12 µg/100g.[19] Vitamin B3 content in the analyzed samples was varying between 0.07 to 3.10 mg/100g with the highest and lowest content found in *Joha* rice and bamboo shoot, respectively. Whereas, the developed cracker was reported with vitamin B3 content of 2.04 mg/100g. Another study on cracker developed with different combinations of wheat and quinoa flour reported for a similar content of vitamin B3 ranging from 0.95 to 5.43 mg/100 g.[19 .15] The content of vitamin B5 was ranging from 0.15 to 2.50 mg/100g in the analyzed samples, with the crackers containing the highest amount of vitamin B5 ($p<0.05$). In comparison to the analyzed B-group vitamins, vitamin B6 was found with the least content in the analyzed samples which ranged between 0.25 to 0.57 mg/100g. Similarly, another study on the crackers formulated with wheat reported for vitamin B6 content which ranged from 0.13 to 0.17 mg/100 g Vitamin C was detected only in bamboo shoot flour and cracker with 0.69 and 0.63 mg/100 g, respectively. Similarly, vitamin A was also found only in bamboo shoot flour and cracker with 0.293 and 0.241 µg/100g expressed as retinol equivalent (RE). Various bamboo species have been reported to contain vitamin C content ranging between 3.00 to 12.90 %, with *D. hamiltonii* having highest content. 19Among the fat-soluble vitamins analyzed in the samples, the least detected vitamin was vitamin D ranging between 2.95 to 4.19 µg/100g. Whereas, vitamin E was predominantly found in the samples with *Joha* rice having the highest content (2.799 µg/100 g) followed with the cracker (1.16 µg/100 g). Vitamin K in the analyzed samples was ranging between 0.81 to 10.93 µg/100 g, with the highest and the lowest content found in the green gram and bamboo shoot flour. However, the vitamin K content in the cracker was found to be 0.59 µg/100 g. Similar content of vitamin K was reported in the saltine and cheese crackers which was ranging between 8.0 to 20.5 µg/100g.[19] have reported that the shoots of *D. hamiltonii* were found to have higher contents of nutrients especially the water and fat-soluble vitamins in comparison to other species of bamboo shoots.

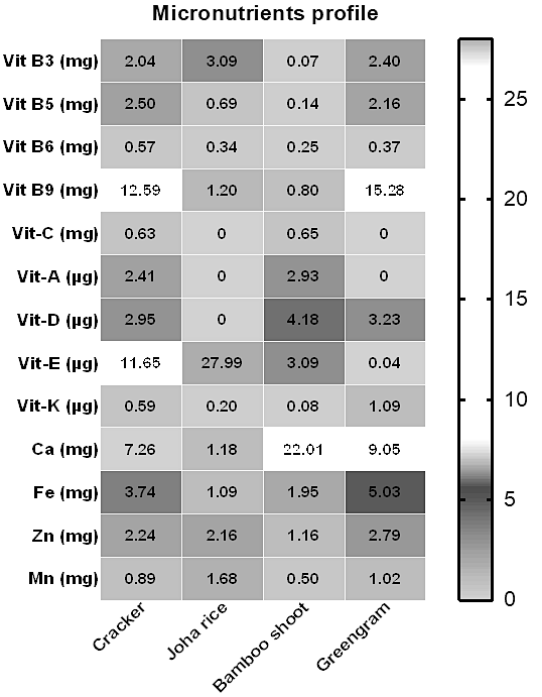

Fig. 1 Micronutrients profile of the analyzed samples. All values are reported per 100 g of sample except for Vit-B9, Vit-A, Vit-E, Vit-K and Ca parameters which are reported per 1000g of sample

Among the minerals analyzed in the samples were Ca, Fe, Zn and Mn (Figure 1). Ca was found to be highest in the bamboo shoot powder (2.20 mg/100 g) and the crackers were having Ca of 7.26 mg/100 g. The Fe, Zn and Mn content in the raw samples utilized for formulating the functional food (cracker) was ranging between 1.09 to 5.03, 1.16 to 2.79 and 0.50 to 1.68 mg/100 g, respectively ($p<0.05$). The contents of Fe, Zn and Mn in the cracker was found

to be 3.74, 2.24 and 0.89 mg/100 g, respectively. A reduction in the Fe content due to the oxidation of porphyrin ring and release of iron in soluble form from 5.07 – 5.42 to 3.84 – 4.09 mg/100 g after processing (heat treatment) in green gram (mung bean) cultivars was reported,[20] Raghuvanshi imilar to the present study. Another study reported for a similar content of Ca in *D. hamiltonii* shoots with 150 mg/100 g.[21] The content of Ca and Fe in the crackers might have enhanced due to the incorporation of bamboo shoot and green gram flours, respectively, since they are found to be rich source of these minerals. As the minerals play a vital part for several physiological processes in the body and are required for proper metabolism process. Hence the minerals retained in the cracker will be beneficial in maintaining the healthy lifestyle, which is an essential part of a functional food.

Table 2. Fatty acid composition (% fatty acid methyl ester-FAME) of analyzed samples

Fatty acid (%FAME)	Raw flour samples of functional food			Functional food
	Joha rice	Green gram	Bamboo shoot	Cracker
Myristic (C14:0)	3.15±0.42^{c}	ND	2.36±0.23^{b}	0.83±0.10a
Palmitic (C16:0)	27.75±0.95^{c}	23.62±1.83^{b}	33.05±1.78^{d}	22.34±1.25^{a}
Stearic (C18:0)	3.61±0.49^{c}	4.41±0.48^{d}	3.35±0.54^{b}	1.97±0.56^{a}
Arachidic (C20:0)	0.35±0.43^{b}	ND	0.93±0.14^{c}	0.25±0.02^{a}
Behenic (C22:0)	0.19±0.10^{a}	ND	0.72±0.05^{c}	0.29±0.05^{b}
Lignoceric (C24:0)	0.25±0.06^{a}	ND	0.37±0.24^{b}	0.25±0.03^{a}
Palmitoleic (C16:1)	0.28±0.13^{a}	ND	1.23±0.03^{b}	0.27±0.01^{a}
Oleic (C18:1n9c)	22.55±0.54^{c}	28.91±1.08^{d}	19.05±1.54^{b}	15.44±1.05^{a}
Eicosenoic (C20:1n9)	0.93±0.03^{b}	ND	ND	0.25±0.02^{a}
Erucic (C22:1n9)	ND	ND	ND	ND
Linoleic (C18:2n6c)	48.28±0.51^{d}	43.65±0.18^{b}	29.29±0.52^{a}	46.28±0.12^{c}
γ-Linoleic (C18:3n6)	ND	ND	ND	ND
α-Linolenic (C18:3n3)	15.56±0.17^{b}	18.21±0.25^{c}	13.42±0.25^{ab}	11.83±0.89a
Eicosatrienoic (C20:3n3)	ND	ND	ND	ND
Arachidonic (C20:4n6)	ND	ND	ND	ND
∑SFA 103.15	67.75	82.69	25.93	
∑MUFA	27.28	28.90	20.28	15.96
∑PUFA	63.81	61.85	42.67	58.11
∑PUFA/∑SFA	0.61	0.91	0.51	2.24
ω-6/ω-3	3.10	2.39	2.18	3.91

Mean values followed by different superscript letter are significantly different ($p< 0.05$) compared within rows for the analyzed samples, ND-not detected, SFA-saturated fatty acid, MUFA-mono unsaturated fatty acid, PUFA-polyunsaturated fatty acid

Fatty Acid Composition

The fatty acids (FAs) detected in the analyzed samples include myristic, palmitic, stearic, arachidic, behenic, lignoceric, palmitoleic, oleic, eicosenoic, linoleic, and α-linolenic acids. Whereas, erucic, γ-linoleic, eicosatrienoic and arachidonic acids were not detected in any of the analyzed samples (Table 2). The predominant FAs found in the crackers were linoleic acid (46.28%), palmitic acid (22.34%), oleic acid (15.44%) and α-linolenic acid (11.83%) ($p<0.05$). The total saturated fatty acid (∑SFA) and monounsaturated fatty acid (∑MUFA) content in the cracker was found to be 25.93 and 15.96%, respectively. Interestingly, the polyunsaturated fatty acid (∑PUFA) was found with higher content in cracker (58.11%). A study on the crackers developed from brown rice, hemp flour, chia seeds and canola oil reported for ∑MUFA and ∑PUFA ranging between

10.73 to 11.42 and 7.62 to 9.84%, respectively.[21] The ratio of omega-6/omega-3 (ω-6/ω-3) in the crackers was found to be 3.91 and the recommended value for ω-6/ω-3 is 5-10 as per the World Health Organization (WHO) (WHO, 2008). It may be concluded that the crackers are good sources of ω-3 and ω-6 FAs which are having higher retention after processing and through diet they provide adequate amounts of ω-3 and ω-6 FAs.

Table 2. Fatty acid composition (% fatty acid methyl ester-FAME) of analyzed samples

Polyphenol	Raw flour samples of functional food			Functional food
	Joha rice	Green gram	Bamboo shoot	Cracker
Phenolic acids				
Gallic acid	22.28±1.91^{a}	219.66±5.57^{d}	192.43±2.74^{c}	29.63±0.6^{b}
Caffeic acid	4.32±0.38^{a}	28.57±0.47^{c}	19.63±0.47^{b}	ND
Ferulic acid	6.51±0.40^{b}	4.20±0.19^{a}	42.69±0.47^{d}	8.59±0.41^{c}
p-Coumaric acid	ND	2.37±0.15^{a}	ND	2.72±0.11^{b}
o-Coumaric acid	1.68±0.24^{a}	15.31±0.44^{d}	5.57±0.41^{c}	2.44±0.41^{b}
Protocatechuic acid	2.55±0.16^{a}	18.44±0.48^{c}	ND	17.56±0.46^{b}
4-hydroxy benzoic acid	8.33±0.44^{b}	ND	ND	2.27±0.12^{a}
Sinapic acid	1.87±0.15^{b}	9.75±0.36^{c}	27.46±0.68^{d}	1.09±0.14^{a}
Flavonoids				
Catechin	ND	8.53±0.36^{b}	20.35±0.52^{c}	4.07±0.17^{a}
Diadzein	2.39±0.12^{a}	3.55±0.38^{a}	134.42±3.47^{b}	2.81±0.10^{a}
Myricetin	9.24±0.32^{b}	10.07±0.22^{c}	60.54±0.63^{d}	5.02±0.14^{a}
Naringenin	ND	6.54±0.11^{b}	11.53±0.33^{c}	2.38±0.19^{a}
Luteolin	11.29±0.19^{c}	ND	2.45±0.30^{a}	6.44±0.60^{b}
Total polyphenols	**70.44**	**326.97**	**517.05**	**84.99**

Mean values followed by different superscript letter are significantly different ($p< 0.05$) compared within rows for the analyzed samples, ND-not detected

Polyphenol Profile

Among the various bioactive compounds obtained from the food sources, polyphenols are considered as important due to their antioxidant properties which play a vital role in counteracting with the various types of reactive oxygen species (ROS). The polyphenols evaluated in the present study included eight phenolic acids and five flavonoids (Table 3). Various phenolic acids and flavonoids among the samples were ranging from 1.09 to 219.66 and 2.38 to 134.42 µg/g, respectively ($p<0.05$). The predominantly detected phenolic acid among the raw ingredients of crackers was gallic acid (22.28 – 219.66 µg/g), followed with ferulic acid (4.20 – 42.69 µg/g) and sinapic acid (1.87 – 27.46 µg/g). Whereas, the highest detected flavonoids in the raw ingredients of developed cracker include diadzein and myricetin which ranged between 2.39 to 134.42 and 9.24 to 60.54 µg/g, respectively. The major phenolic acids detected in the cracker were gallic acid (29.63 µg/g) and protocatechuic acid (17.56 µg/g). Whereas, the flavonoids in the cracker were ranging between 2.81 and 6.44 µg/g with major flavonoids of luteolin, myricetin and catechin. Cereals and legumes are known to be a good source of various polyphenols including hydroxybenzoic acids, hydroxycinnamic acids, isoflavonoids, and anthocyanins.[22 .14;34] The health beneficial properties of bioactive compounds in bamboo shoots for developing functional foods has been reviewed earlier.[22] Due to the combination of *Joha* rice, green gram and bamboo shoot, the composition of polyphenols also

varied in the developed functional food of cracker. However, the studies on the polyphenol components of crackers are limited, hence the present data on polyphenolic profile of healthy cracker will be useful for the product.

Phenolic Composition, Antioxidant Potential and Enzyme Inhibition Activity

The samples analyzed for TPC, TFC, TAC, TPAC and TCC were reported in Figure 2. TPC in the analyzed samples was ranging between 129.18 to 321.72 mg GAE/100g, with the highest and the lowest content found in green gram and *Joha* rice ($p<0.05$). Similarly, different genotypes of green gram from China were reported for TPC ranging between 287 to 560 mg GAE/100 g .[34] The cracker and the bamboo shoot were reported with the TPC of 263.81 and 243.08 mg GAE/100g. Another study on cracker developed from lentil reported for TPC of 78 to 333 mg GAE/100 g.[22 .24] TFC in the analyzed samples was ranged between 95.79 to 321.50 mg CE/100g and the crackers were found with the TFC of 176.20 mg CE/100g ($p<0.05$). Different genotypes of Chinese green gram were reported with TFC of 125 to 352 mg RE/100 g. 22 Compared to TPC and TFC, the TAC was found with less content in the analyzed samples ranging between 12.17 to 21.61 µg Cy-3-glu/g. The highest and the lowest content of TPAC was found in bamboo shoot (8.37 mg CE/100 g) and *Joha* rice (0.75 mg CE/100 g). The highest TCC was found in bamboo shoot flour (350.48 µg/100 g) followed with the green gram, crackers and *Joha* rice samples ($p<0.05$).

The antioxidant activity by DPPH and ABTS radical scavenging assay of developed crackers was found to be 47.21 and 37.02 % inhibition (Figure 2) ($p<0.05$). A study reported for bamboo shoot (*D. hamiltonii*) and its processed product for DPPH and ABTS antioxidant activity ranging between 62.8 to 283.2 and 117.6 to 334.5 IC50 of radical scavenging activity (µg/mL) [22] Dadwal.[10] FRAP and ORAC antioxidant activity among the raw ingredients of crackers was ranging between 1.87 to 2.19 µmol Fe (II)/g and 41.15 to 51.24 µmol TE/g, with the highest activity found in bamboo shoot among them. The crackers developed in the present study was reported with FRAP and ORAC antioxidant activity of 2.88 µmol Fe (II)/10g and 56.37 µmol TE/g, respectively ($p<0.05$).

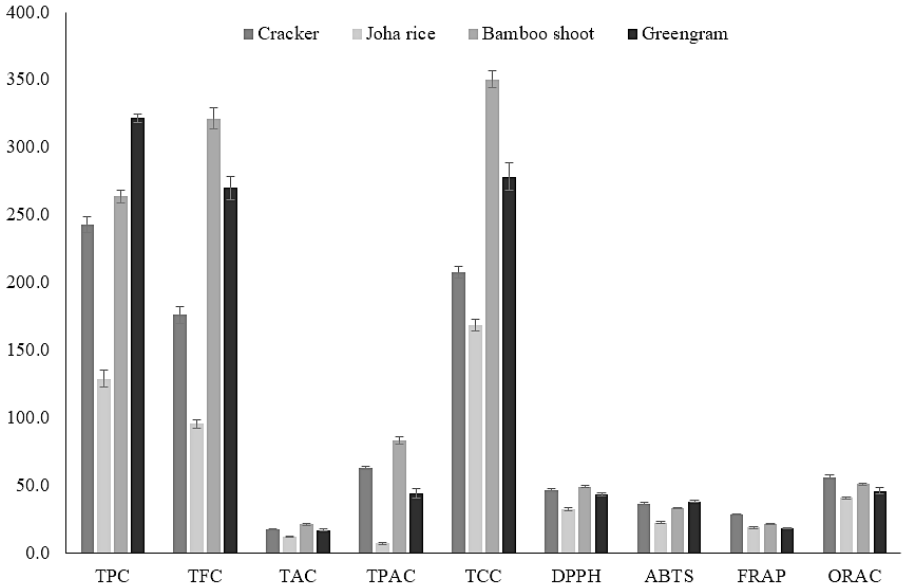

Fig. 2. Phenolic composition and antioxidant properties of the analyzed samples. TPC-total phenolic content (mg GAE/100g), TFC-total flavonoid content (mg CE/100g), TAC-total anthocyanin content (µgCy-3-glu/g), TPAC-total proanthocyanidin content (mg CE/kg), TCC-total carotenoid content (µg/100g), DPPH radical scavenging activity (% inhibition), ABTS radical scavenging activity (% inhibition), FRAP assay (µmol Fe(II)/10g), ORAC assay (µmol TE/g)

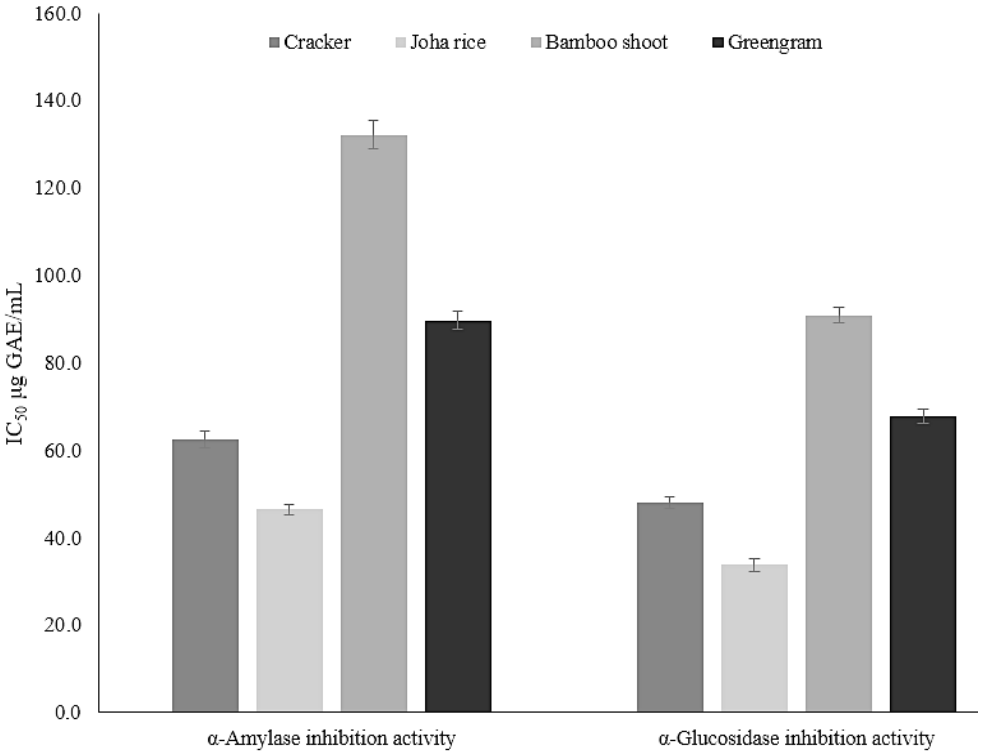

Fig. 3 Enzyme (α-amylase & α-glucosidase) inhibition activity of the analyzed samples

The results for the enzyme inhibition activity obtained for the analyzed samples is represented in Figure 3. The α-amylase inhibition activity for the analyzed samples was ranging between 46.41 to 132.12 IC_{50} µg GAE/mL, with the highest and the lowest activity found in bamboo shoot and *Joha* rice flour, respectively. This activity is a strategy to treat the disorders of carbohydrates uptake such as diabetes and obesity. The samples analyzed in the present study showed the α-glucosidase inhibition activity of 33.79 to 90.84 IC_{50} µg GAE/mL. This activity has been developed to treat type 2 diabetes. The functional food (cracker) was found to contain α-amylase and α-glucosidase inhibition activity of 62.43 and 47.97 IC_{50} µg GAE/mL, respectively. Another study on bamboo shoot reported for α-amylase and α-glucosidase inhibition activity in fresh and boiled bamboo shoot with 74.96 and 34.18 and 44.04 and17.32 % inhibition at 0.1 mg mL^{-1}. The bound polyphenolic extract of green gram was reported to have α-amylase and α-glucosidase inhibition activity of 372 and 167 IC_{50}.[22]The inhibition activity of carbohydrate-hydrolyzing enzymes (α-amylase & α-glucosidase) is due to the interaction of polyphenols with active site of enzyme involving hydrogen and hydrophobic bonding.[22]

Conclusion

Functional snack food (cracker) developed using *Joha* rice, bamboo shoot and green gram flours was known to contain high contents of protein and dietary fiber. Amylose and resistant starch retention in cracker were comparatively higher and most of the vital vitamins and minerals were enriched in the cracker with the inclusion of ingredients used in development of crackers. Linoleic and α-linolenic acids are detected in the crackers with optimum quantity, which are known for their health beneficial properties. Various polyphenolic components were found in the cracker with the dominance of gallic acid, protocatechuic acid, ferulic acid and luteolin , 16 conducted this study for which paddy (Variety: Jhelum) and wheat (variety: SKW – 355) were procured from mountain research centre for Field crops , Khudwani , SKUAST – Kashmir . Models for all the selected physical parameters were highly significant ($p<0.05$) with high coefficient of determination ($R2 = 0.99-0.67$) , which indicates that

developed models could be used to navigate the R2 were in reasonable agreement with each other In the result it was found that DCF had a significantly higher content of protein (22.10%), fat (8 .04 %), Ash (6.17%), crude fibre (17.69%) and low content of carbohydrate (41.06%) and moisture (10.66%). The crackers were also found with optimum antioxidant potential evaluated by different methods. A comprehensive report on nutrients and nutraceutical potential of RTE functional foods are very limited. Hence, the functional food of cracker which is developed in the study can be utilized as a healthy snack food which is rich in several nutrients and bioactive compounds.

Acknowledgement
The author acknowledges the laboratory support of Faculty of Science, Assam down town University along with the Seed Money Grant 2021-2022, Assam down town University.

Funding Sources
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest
The authors declare that there is no conflict of interest.

Data Availability Statement
This statement does not apply to this article.

Ethics Approval Statement
This research did not involve human participants , animal subjects or any material that requires ethical approval.

Informed Consent Statement
This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration
This research does not involve any clinical trials.

Author Contributions
- **Daisy Sharma:** participated in framing of the idea of the performed work.
- **Manash Pratim Sarma:** conceptualized and framed the manuscript with critical review of the same.
- **Ritismita Devi:** participated in the laboratory work, evaluation of the results and review of the results.
  Giridhar Goudhar: participated in quantitative re analysis and data evaluation of the samples.

References

1. Tecson-Mendoza EM. Development of functional foods in the Philippines. *Food Sci Technol Res.* 2007;13(3):179-186.
2. Lahkar L, Hazarika G, Tanti B. Proximate composition, physicochemical and antioxidant properties revealed the potentiality of traditional aromatic (*Joha*) rice as functional food. *Vegetos.* 2020;33:40-51.
3. Dadwal V, Sharma A, Joshi R, Gupta M. Assessment of nutritional properties and phenolic characterization of freshly harvested Dendrocalamus hamiltoni shoots and processed bamboo candy. *Food Sci Biotechnol.* 2023;32(6):769-778.
4. Li X, Xing Y. Quality of bamboo shoots during storage as affected by high hydrostatic pressure processing. *Int J Food Prop.* 2021;24.
5. Sansenya S, Payaka A, Mansalai P. Biological activity and inhibition potential against α-glucosidase and α-amylase of 2, 4-di-tert-butylphenol from bamboo shoot extract by *in vitro* and in silico studies. *Process Biochem*. 2023;126:15-22.
6. Chaffee O, McGillivray A, Duizer L, Ross CF. Identifying elements of a ready-to-eat meal desired by older adults. *Food Res Int.* 2022;157:111353.
7. Baggio A, Federici E, Gentilucci V, Brown rice and pulses for the development of shelf-stable and low glycemic index ready-to-eat meals. *J Funct Foods.* 2023;100:105364.
8. Mateeva A, Kondeva-Burdina M, Peikova L, Simultaneous analysis of water-soluble and

fat-soluble vitamins through RP-HPLC/DAD in food supplements and brewer's yeast. *Heliyon.* 2023;9 (1).

9. Mekkara nikarthil Sudhakaran S, Bukkan DS. A review on nutritional composition, antinutritional components and health benefits of green gram (*Vigna radiata* (L.) Wilczek). *J Food Biochem*. 2021;45(6):e13743.
10. Sagar S, Goudar G, Sreedhar M, Panghal A, Sharma P. Characterization of nutritional content and *in vitro* antioxidant properties of Plantago ovata seeds. *Int J Food Nutr Sci.* 2020;9:27-31.
11. Goudar G, Manne M, Pradeepkiran JA, Kumar S. Food bioactive compounds, sources, and their effectiveness during COVID-19. In: COVID-19 in Alzheimer's Disease and Dementia. 2023:91-108.
12. Raghuvanshi RS, Singh S, Bisht K, Singh DP. Processing of mungbean products and its nutritional and organoleptic evaluation. *Int J Food Sci Technol.* 2011;46(7):1378-1387.
13. Hussien H, El-Adly NA, Shams OS, Mohamed E. Production of High Nutritional Value Gluten Free Crackers with Sesame and Turmeric Powder. *Egypt J Food Sci.* 2020;48(2):291-302.
14. Hassan MH, Salem MA, Doweidar MM. Preparation and evaluation of healthy crackers by using flour mixes of different types of cereal. J Food Dairy Sci. 2021;12(2):23-32.
15. Hussain SZ, Beigh M, Qadri T, Ahmad I, Naseer B. Development of low glycemic index crackers from water chestnut and barley flour. *Br Food J.* 2020;122(4):1156-1169.
16. Basumatary A, Middha SK, Usha T, Basumatary AK, Brahma BK, Goyal AK. Bamboo shoots as a nutritive boon for Northeast India: an overview. 3 Biotech. 2017;7:1-6.
17. Chongtham N, Bisht MS, Santosh O, Bajwa HK, Indira A. Mineral elements in bamboo shoots and potential role in food fortification. *J Food Compos Anal.* 2021;95:103662.
18. Radočaj O, Dimić E, Tsao R. Effects of hemp (*Cannabis sativa* L.) seed oil press-cake and decaffeinated green tea leaves (Camellia sinensis) on functional characteristics of gluten-free crackers. *J Food Sci.* 2014;79(3):C318-C325.
19. Wang Y, Chen J, Wang D, A systematic review on the composition, storage, processing of bamboo shoots: Focusing the nutritional and functional benefits. *J Funct Foods.* 2020;71:104015.
20. Nirmala C, Bisht MS, Laishram M. Bioactive compounds in bamboo shoots: health benefits and prospects for developing functional foods. *Int J Food Sci Technol.* 2014;49(6):1425-1431.
21. Padovani RM, Lima DM, Colugnati FA, Rodriguez-Amaya DB. Comparison of proximate, mineral and vitamin composition of common Brazilian and US foods. *J Food Compos Anal.* 2007;20(8):733-738.
22. Albalasmeh AA, Berhe AA, Ghezzehei TA. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydr Polym.* 2013;97(2):253-261.