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Nutritive, Polyphenolic, and Antioxidative Properties of Locally Produced Cereals and Pseudograins

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Abstract

Cereals and pseudograins are known for their bioactive components and hence used as functional food worldwide. Recently, locally grown cheena, kaon, quinoa, and chia are available in Bangladeshi markets. The study aimed to determine the macronutrients, polyphenols, and flavonoids contents of selected cereals (bajra, zob, cheena, and kaon) and pseudograins (quinoa and chia) along with their antioxidant properties. Cheena (13.48%) and kaon (13.55%) showed higher protein content among cereals, whereas chia of commercial and local sources showed high quantities of protein (22.40% and 22.35%, respectively) among pseudograins. A high amount of carbohydrate was found in kaon and quinoa (64.57% and 59.79%, respectively), whereas chia contained a high amount of fat (20.88 to 29.77%) and dietary fiber (31.46% to 36.85%). Locally grown bajra exhibited a markedly higher concentration of total phenolics (135.75±0.33 mg/g GAE dw) and total flavonoids (17.44±0.05 mg/gm QE dw) compared to other foods studied. Regarding antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and half-maximal inhibitory (IC_{50}) values, chia from Dinajpur field showed the best results. Therefore, cereals and pseudograins studied and reported here could provide utilizable nutritional and functional bioactive polyphenolic components that may have the potential in the nutritional management of noncommunicable diseases.

Keywords: Cereals, Pseudograins, Macronutrients, Polyphenols, Antioxidant Property.

Introduction

The World Health Organization estimated that overweight and obesity kill more people than underweight around the world. Obesity raises various health risks, including cardiovascular disease, cancer, diabetes, osteoarthritis, and chronic renal disease (Hales *et al.*, 2020). Bangladesh currently faces a triple burden of malnutrition: undernutrition, overnutrition, and micronutrient deficiencies. Banik *et al.* (2018) conducted a comprehensive review of the literature and discovered a growing trend in overweight and obesity among Bangladeshi children, adolescents, and adults across time.

The use of bioactive dietary components has recently been proposed as a novel method to the prevention and control of lifestyle illnesses, specifically obesity. The most typical tactic for these approaches is the development of inhibitors of nutritional digestion and absorption. For example, a growing body of

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research suggests that polyphenols found in vegetables, fruits, cereals, and pseudograins can inhibit digestive lipase *in vitro* (De La Garza *et al.*, 2011). Plant secondary metabolites were evaluated for biological features in order to find biologically active Lipase inhibitors from natural resources (Scalbert *et al.*, 2005; Pandey *et al.*, 2009; Rahim *et al.*, 2015).

Although the beneficial effects of polyphenols on human health are well recognized, it is critical to analyze them quantitatively and qualitatively in local agricultural produce. There are several studies on cereals and pseudocereals (Kaur *et al.*, 2023); however, investigations on locally cultivated pseudograins are few. It is worth noting that pseudocereals, often known as pseudograins, are non-grass seeds with grain-like applications around the world. A recent ethnographic investigation (Rupa and Rahim, 2023) found that, despite Bangladesh's government support for good agricultural practices and significant agroeconomic benefits, pseudograin production, distribution, use, and consumption remain subpar when compared to other cereals. However, a significant amount of land has been used locally to grow buckwheat (*Fagopyrum esculentum*), quinoa (*Chenopodium quinoa*), and chia (*Salvia hispanica*). Furthermore, they are now widely available in retail markets as imported goods.

This analytical note describes the compositional data and bioactive properties of polyphenols in locally grown and selected cereals and pseudograins. The data presented here may be useful for nutritional management of noncommunicable diseases through diet therapy.

Materials and Methods

Sample Collection

Food samples were collected from the grower's field from different parts of the country. The collected samples were fresh, well-shaped, and free from insect bites and other deformities. They were selected at random from the cultivation fields or from a stockpile of traders. Emphasis was given to collecting food samples during their pick seasons. zob, cheena, and kaon were collected from Bangladesh Agricultural Research Institute (BARI), but bajra was collected from a commercial source. Chia seeds were collected from the research field of BARI located in Bogra, Mymensingh, and Dinajpur, while quinoa was collected from the Bangladesh Agricultural University (BAU) research field. Lists of collected samples were presented in Table 1.

Laboratory Sample Preparation

Figure 1 describes the flow diagram of laboratory sample preparation for chemical analysis. Briefly, collected food samples were cleanly washed with tap water, and edible portions were separated, followed by drying in open air for half an hour. For moisture, ash, and TDF estimations on a fresh weight basis, clean and air-dried sample pieces were weighed, grinded to powder, and processed for laboratory analysis. Powdered samples were sieved and distributed in small ziplock sachets, and kept in desiccators at room temperature until analysis.

Table 1. Description of collected foodstuffs

Food Type	Food Name		Botanical Name	Sources
	Local Name	English Name		
Cereals	Bajra	Pearl millet	*Pennisetum glaucum* (L.) R. Br.	Market
	Zob	Barley	*Hordeum vulgare* L.	Field
	Cheena (BARI)	Proso/White millet	*Panicum miliaceum* L.	Field
	Kaon	Foxtail millet	*Setaria italica* (L.) P. Beau vois	Field
Pseudograins	Chia seeds	Chia seed	*Salvia hispanica* L.	Field and Market
	Quinoa	Quinoa	*Chenopodium quinoa* willd.	Field and Market

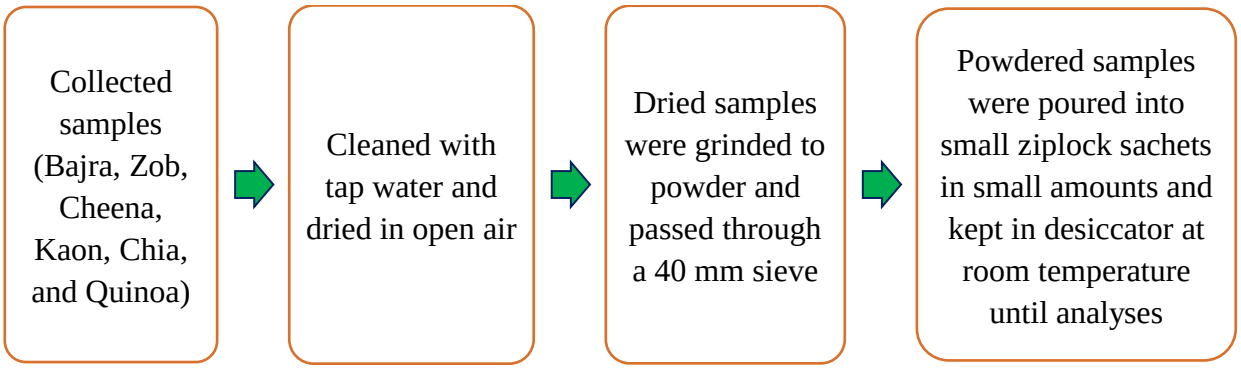

Figure 1. Flow chart showing the preparation of sample flours for lab analyses.

Analyses of Proximate Composition

All methods used for proximate analyses were recommended methods (AOAC, 2000). The moisture content (method no. 925.09) was determined by weight loss on drying of the sample in an oven at 105 °C for 4 hours (h). Following repeated weighing after each 30 minutes (min) until the weight becomes constant. Ash content (method no. 923.03) was estimated by heating the moisture-free sample at 200 °C for charring about 2 h, followed by heating at 600 °C for 5 h in a muffle furnace. The nitrogen content was determined by the micro-Kjeldahl method of AOAC (2000). The protein content was then calculated by multiplying the nitrogen values with Jones factors (XN) for cereals, *i.e.*, 6.25. Crude fat content (method no. 991.36) was derived by the continuous extraction method using Soxhlet apparatus. Total dietary fiber (TDF) was determined by using a total dietary fiber assay kit based on enzymatic-gravimetric determination of both soluble and insoluble non-starch polysaccharides of Prosky methods (methods no. 985.29). Available carbohydrate content of each sample was calculated by difference using Atwater's conversion factors (Atwater and Bryant, 1902). Finally, the energy content was determined applying the following equation:

Energy (Kcal/g) = [4 × protein (g) + 4 × carbohydrates (g) + 9 × fat (g) + 2 × TDF (g)]

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Solvent extraction of solid samples is one of the most commonly used and easiest methods of extraction for phenolics (Pekić *et al.*, 1998; Lapornik *et al.*, 2005). Briefly, 50 g of sample flour was added to 125 mL of HPLC-graded 99.9% methanol. The mixture was then shaken repeatedly and rested for maximization of phenolic extraction. Followed by filtration through Whatman No. 1 filter paper, the extraction process was repeated two more times, and the final extract was stored in a dark glass bottle. After 72 h, the total extract was collected and immediately evaporated by a rotary evaporator at 40 ºC to get crude methanol extract. Finally, collected concentrated extracts were stored in a refrigerator at 4 ºC for further determination of total contents of phenolics, flavonoids, and antioxidant activity.

Total soluble phenolics in the extracts were determined with Folin-Ciocalteau reagent using gallic acid as a standard according to the method of Singleton (1965) with suitable modification. About 1 mg of extract was mixed with 1 mL of ethanol. After that, 15.8 mL of deionized water and 0.1 mL of 50% of Folin-Ciocalteau reagent were added into 0.2 mL of ethanol-extract solution. The mixture was then shaken and left to stand for 8 min, followed by adding 3 mL of 2% sodium carbonate and shaken for 3 min. The mixture was then kept in the dark for 2 h at room temperature. Finally, the absorbance was taken using a UV-Vis spectrophotometer (Uviline 9400, Secomam, France) at 765 nm.

TPC was determined in gallic acid equivalents (GAE) from the following formula and expressed as mg of gallic acid equivalent (GAE) per 100 g of sample: A = (C × V)/M. Where A = total phenolic content of extract equivalent to gallic acid; C = concentration of the gallic acid obtained from the calibration curve (mg/mL); V = volume of extract solution (mL); M = weight of extract (g).

Total flavonoid content (TFC) was determined after Sinay *et al.* (2022) with slight modification. About 0.5 mg of extract was mixed with 2 mL of 96% methanol, followed by adding 2 mL of 2% aluminum chloride. Subsequently, the absorbance of the mixture was measured using a UV-Vis spectrophotometer (Uviline 9400, Secomam, France) at 430 nm. Quercetin was used as a standard. TFC was measured by the following equation and expressed as mg of quercetin equivalent (QE) per 100 g of sample: $A_1 = (C_1 \times V1)/M1$. Where A_1 = total flavonoid content of extract equivalent to quercetin; C_1 = concentration of the quercetin obtained from the calibration curve (mg/mL); V_1 = volume of extract solution (mL); M_1 = weight of extract (g).

Determination of Antioxidant Activity of the Study Samples

activity test (Sinay *et al.*, 2022) with a slight adjustment. About 2.5 mL of methanol extract of the sample at different concentrations (2.5 µg mg^{-1}, 5.0 µg mg^{-1}, 10 µg mg^{-1}, 20 µg mg^{-1}, 40 µg mg^{-1}, 60 µg mg^{-1}, 80 µg mg^{-1}, 100 µg mg^{-1}, 250 µg mg^{-1}, and 500 µg mg^{-1}) was added to 1.0 mL of 0.3 mM DPPH methanol solution. For the blank, 2.5 mL of methanol replaced the methanol extract of the sample. The sample and the blank were then allowed to stand in a dark chamber at room temperature for 30 min. After changing the color from deep violet to light yellow, the absorbance was measured at 514 nm using a UV-Vis spectrophotometer (Uviline 9400, Secomam, France). Ascorbic acid was used as a control for its antioxidant activity. The free radical sequestering ability was expressed as a percent inhibition of oxidation of the radical and calculated according to the following equation:

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

The half-maximal inhibitory (IC_{50}) value expresses the sample concentration required to scavenge 50% DPPH radical. It was calculated from the plot of inhibition (%) against the concentration of the sample extract. The inhibition (%) was plotted against the methanol extract of the sample. The IC_{50} was calculated after developing a linear regression graph, which means the amount of sample necessary to decrease the absorbance of DPPH by 50%.

Statistical Analyses

Data analysis was performed using a statistical package for the social sciences version 25.0 for Windows (SPSS Inc., Chicago, USA). The values were expressed as mean±standard deviation. One-way analysis of variance (ANOVA) with Tukey's test was used to determine the significant differences among the samples. The values of $P<0.05$ were considered as statistically significant.

Results and Discussion

Food Description

Table 1 presented in the 'Materials and Method' section described general food descriptions that were collected for chemical analysis. On the other hand, an example of a herbarium sheet for quinoa seeds is presented in Figure 2, which summarizes the botanical profile of the plant. Similar herbarium sheets were prepared for all collected food samples that described the local name, English name, binomial name, collector's name, herbarium identification number, and related descriptions.

Figure 2. Herbarium sheet of Quinoa.

Nutritional Analysis

Tables 2 and 3 show the content of proximate nutrient values of the cereals and pseudograins studied. Results are expressed as the mean of the duplicate analysis of each sample and expressed as grams per 100 grams of edible portion (EP). Notable values are shown in bold letters. Both cheena and kaon were found to be rich in protein, while zob was rich in dietary fiber. These values are in well agreement with literature values (Shaheen *et al.*, 2014).

Table 2. Proximate compositions of cereals (g/100 g EP)

Food Description	Energy (Kcal)	Moisture (g)	Protein (g)	Fat (g)	Dietary Fiber (g)	Available CHO (g)	Ash (g)
Bajra (Commercial/Local)	334.63	12.93	9.47	2.73	10.83	62.63	1.41
Zob (BARI-6 Field)	322.38	11.69	11.65	1.64	**15.11**	57.70	2.21
Cheena (BARI Field)	339.49	10.77	**13.47**	1.65	9.88	62.75	1.48
Kaon (BARI Field)	344.90	9.48	**13.65**	1.50	9.26	64.57	1.54

EP = Edible parts.

Table 3. Proximate compositions of local pseudograins (g/100 g EP)

Food Description	Energy (Kcal)	Moisture (g)	Protein (g)	Fat (g)	Dietary Fiber (g)	Available CHO (g)	Ash (g)
Chia (Bogra Field)	418.65	7.10	19.25	28.93	31.44	04.60	8.68
Chia (Dinajpur Field)	383.84	10.00	20.44	20.88	31.40	12.84	4.44
Chia (BAU Field)	436.03	7.00	**22.35**	**29.77**	**32.41**	03.47	5.00
Quinoa (SAU-1 Field)	328.13	12.36	16.04	4.39	13.37	49.06	4.41

EP = Edible parts. SAU = Sher-e-Bangla Agricultural University.

Buckwheat (*Fagopyrum esculentum*), chia (*Salvia hispanica*), and quinoa (*Chenopodium quinoa*) are well-known pseudograins in Bangladesh (Rupa and Rahim, 2023). Protein (20.44 to 22.57%), fat (20.88 to 29.77%), and dietary fiber contents (31.40 to 36.85 g%) of locally grown chia seeds were found to be almost similar to the ranges reported by Bartosz et al. (2019). Comparing to other pseudograins, quinoa contained lower proximate nutrients (Table 3). However, similar amounts of protein (12.43 to 16.04%) and fat (4.3 to 5.17%) in quinoa have been reported earlier (Filho *et al.*, 2017). Local chia seeds were thus revealed to be a good source of protein, fat, and dietary fiber compared among pseudograins cultivated and available in Bangladesh (Rupa and Rahim, 2023). Smith *et al.* (2011) reported that whole grains, especially cereal fiber, reduced mortality, cardiovascular disease, and type-2 diabetes. Genotypes and cultivation conditions had direct influence on the nutritional and polyphenolic composition of plants (Ghimire *et al.*, 2021). Apart from rich phytochemical content (Table 5), these two pseudograins showed excellent amounts of macronutrients (Table 3) also.

Total Polyphenol Content (TPC) and Total Flavonoid Contents (TFC)

Tables 4 and 5 show the TP and TF contents of selected minor cereals and pseudograins. Among the minor cereals studied, bajra was found to be rich in both TPC (135.75±0.33) and TFC (17.44±0.05) (Table 4). Samaila *et al*. (2022) found 20.14±0.14 mg/100 g TPC and 6.36±0.07 mg/100 g TFC in non-germinated pearl millet. Lower TPC was observed in cheena (22.97±0.04) while kaon showed lower TF (7.12±0.01) content (Table 4). A study analyzed twenty-five genotypes of Proso millet and found a maximum 0.10 mg/g dw TFC among them (Balli *et al.*, 2020). A recent study revealed that the methanolic extract of Foxtail millet flour exhibited significantly elevated amounts of TPC and TFC (51.35±1.35 mg GAE/100 g and 68.26±1.51 mg QE/100 g, respectively) in comparison to the other solvent extracts (Abedin *et al.*, 2022).

Table 4. Total polyphenols and flavonoids content in cereals (Mean±SD)

Sample	Total Polyphenols Content (TPC) (mg of, GAE*/gm of dry extract)	Total Flavonoids Content (TFC) (mg of QE**/gm dry extract)
Bajra	135.75±0.33	17.44±0.05
Zob	57.75±0.37	0.45 ±0.01
Cheena	22.97±0.04	7.71 ±0.01
Kaon	61.24±0.03	7.12±0.01

*Gallic acid equivalent; **Quercetin equivalent.

Table 5. Total polyphenols and flavonoids content in pseudograins (Mean±SD)

Sample	Total Polyphenols (mg of, GAE*/gm of dry extract)	Total Flavonoids (mg of QE**/gm dry extract)
Chia DP[#]	79.75±0.19	3.65±0.075
Quinoa SAU 1[$]	28.55±0.06	6.66±0.01

*Gallic acid equivalent; **Quercetin equivalent; [#]DP- Chia seeds from Dinajpur field; [$]SAU 1- Quinoa from Sher-e-Bangla Agricultural University.

The TPC (79.75±0.19) of Chia seeds was higher than quinoa, but interestingly, a higher content of TF (6.66±0.01) was found in quinoa than chia seeds (Table 5). These values are relatively higher than reported values in chia (2.6 mg GAE/gm of TPC and 1.17 mg QE/gm of TFC), but quinoa was shown to contain a very low amount of TPC (1.4 mg GAE/gm) and 0.9 mg QE/gm of TFC (Brend *et al.*, 2012). These reported values are much lower than the values obtained in this study.

Agronomic conditions (Naczk *et al.*, 2006) and extraction methods (Chandrasekara *et al.*, 2010) could be the reasons for these differences in the content of TPC and TFC in the seeds of chia and quinoa. Low temperatures may increase the production of phenolics by enhancing the synthesis of phenylalanine ammonia-lyase (PAL) in plants, while high altitude and long sunlight exposure with high UV radiation positively affect the synthesis of phenolic compounds (Kishore *et al.*, 2010). Thus, the differences in TPC and TFC content of the pseudograins in this study with the earlier studies may be due to the variations in the geographical location, different extraction/cultivation/sample processing used, and so forth (Ghias *et al.*, 2013). The relatively higher contents of TP and TF in locally grown minor cereals and pseudograins generates optimism to use them as healthful foods for the management of noncommunicable diseases.

Free Radical Scavenging Activity by DPPH

The capability of scavenging free radicals by antioxidants is to neutralize them from being able to reactively damage cellular lipids, proteins, enzymes, carbohydrates, and DNA (Fang *et al.*, 2002). Some phenolic compounds and flavonoids possess antioxidant activities because they are able to act as scavengers of singlet oxygen and free radicals in biological systems *(*Rice-Evans *et al.*, 1997). Phenolics are among the major phytochemicals that have been considered as bioactive compounds with health benefits based on clinical trials and epidemiological studies of oxidative stress-related diseases.

Therefore, assaying the free radical scavenging activity of chia and quinoa by DPPH was considered vital, and the data resulted in is presented in Table 6. The half-maximal inhibitory concentration (IC_{50}) value was chosen as an index of DPPH activity. It has been demonstrated that commercial bajra is more active, as evidenced by its lower IC_{50} value (141.11±5.33) compared to cultivated zob (415.27±1.09). The ethanolic extract of pearl millet (bajra) exhibited a comparable value (152.586 µg/mg) to the findings of the current study (Krishnan *et al.*, 2022). The findings of Fouad *et al.* (2021) showed IC_{50} values of four different zob extracts in acetone ranged from 2.031 to 3.428 mg/mL, which denoted much lower values compared to our results. The present study also observed higher antioxidant activity (low IC_{50} values) in chia than the study of Khursheed *et al.* (2023). However, lower IC_{50} values (3.6 mg/mL), *i.e.*, high antioxidant activity, for chia are in India (Namrata and Haripriya, 2022).

Table 6. Antioxidant activity of selected cereals and pseudograins by DPPH (% of inhibition by substrate concentration in µg mg^{-1})

DPPH Index/ Food	Bajra	Zob	Chia DP	Quinoa SAU 1	Standard (Ascorbic acid)
$^{*}IC_{50}$	141.11±5.33	415.27±1.09	70.57±2.33	355.56±3.77	5.58

*The half-maximal inhibitory concentration (IC_{50}) represents the concentration (mg/ML) at which a substance exerts half of its maximal inhibitory effect.

Quinoa has been shown about 80% DPPH radical scavenging activity (Namrata and Haripriya, 2022), which is comparatively higher than the findings of the present study (Table 6). Relatively higher free radical inhibition (IC_{50} = 70.57±2.33) was observed in chia from Dinajpur field, compared to other foodstuffs studied.

It is worth noting that comparing results obtained from the different geographical locations using different extraction methods is difficult as it may influence the chemical and functional properties of the pseudograins, which might be a possible reason behind the quite different findings of DPPH antioxidant activity in the present study compared to other reported studies (Klepacka *et al.*, 2021). Lower the IC_{50} value, more potent is the substance in food materials at scavenging DPPH, thereby, implies a more potential health benefit of these food materials in health and diseases. It has been reviewed that an additional strategy for lowering body weight and managing adipose tissue mass could be the use of phytochemicals with anti-obesity potential (Roy *et al.*, 2020).

Conclusions

TPC and TFC of the food samples studied were relatively higher than other reported values, indicating the effects of environmental factors such as sun exposure, soil type, and rainfall on the phenolic contents of plants (Manach *et al.*, 2004) across different agro-ecological regions. Within the stipulated opportunity, all collected food samples were not possible to analyze their TP and TF profiles. However, the free radical scavenging activity of DPPH found in Bajra, Chia, and Quinoa (Table 6) is encouraging to use them in diet therapy as well as in habitual diets of the general population.

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Declaration

The authors declare no conflict of interest.

Authors' Contributions

ATMAR conceived and designed the experiments, analyzed and interpreted the data, wrote the manuscript, contributed reagents, materials, and intellectual inputs; AZR conducted the experiments, analyzed and interpreted the data, and wrote the draft manuscript; KTA analyzed and interpreted the data. The completed manuscript has been read and approved by all authors.

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