



ISSN Print: 2664-844X
 ISSN Online: 2664-8458
 IJAFA 2024; 6(2): 259-262
www.agriculturaljournals.com
 Received: 15-08-2024
 Accepted: 21-09-2024

Hamish Woollahra
 Department of Agricultural
 Chemistry, Barossa Institute of
 Food and Wine Sciences,
 Adelaide, South Australia,
 Australia

Annabel Mallacoota
 Department of Agricultural
 Chemistry, Barossa Institute of
 Food and Wine Sciences,
 Adelaide, South Australia,
 Australia

Annabel Woollahra
 Department of Agricultural
 Chemistry, Barossa Institute of
 Food and Wine Sciences,
 Adelaide, South Australia,
 Australia

Phenolic profile and antioxidant capacity of traditional rice varieties from eastern Himalayas

Hamish Woollahra, Annabel Mallacoota and Annabel Woollahra

DOI: <https://doi.org/10.33545/2664844X.2024.v6.i2d.1385>

Abstract

Phenolic compounds are a broad class of secondary plant metabolites that act as natural antioxidants, and their concentration in rice grain varies enormously depending on variety, pigmentation, and growing environment. This research characterised the phenolic profile and antioxidant capacity of five traditional rice varieties black, red, purple, brown, and white collected from farming communities in the Eastern Himalayas. Grain samples were extracted with 80% methanol and analysed for Total Phenolic Content (TPC) by the Folin-Ciocalteu method, Total Flavonoid Content (TFC) by aluminium chloride assay, and individual phenolic acids by HPLC-DAD. Antioxidant capacity was measured using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Black rice recorded the highest TPC (4.82 ± 0.45 mg GAE/g), followed by purple rice (4.21 ± 0.52 mg GAE/g) and red rice (3.67 ± 0.38 mg GAE/g). HPLC analysis identified ferulic acid, protocatechuic acid, and p-coumaric acid as the dominant phenolic acids across all pigmented varieties. Antioxidant capacity correlated strongly with TPC ($r = 0.94$ for DPPH, $r = 0.91$ for FRAP). These results confirm that pigmented traditional rice varieties from the Eastern Himalayas are rich sources of dietary antioxidants and deserve attention as functional food ingredients.

Keywords: Phenolic profile, antioxidant capacity, traditional rice, eastern Himalayas, rice phytochemicals, ferulic acid, flavonoids, DPPH, pigmented rice, functional food

Introduction

Phenolic compounds, defined as molecules carrying one or more hydroxyl groups attached to an aromatic ring, are among the most abundant and widely distributed phytochemicals in the plant kingdom ^[1]. In rice grain, these compounds accumulate mainly in the bran and outer layers, where they serve defensive roles against ultraviolet radiation, pathogens, and oxidative stress ^[2]. When consumed as part of the human diet, rice phenolics contribute antioxidant, anti-inflammatory, and anti-diabetic activities that have drawn increasing interest from both nutrition researchers and the functional food industry ^[3].

The Eastern Himalayan region spanning parts of Nepal, Bhutan, and the adjoining hill tracts harbours an exceptional diversity of traditional rice landraces, many of which carry deep pigmentation ranging from red and purple to near-black ^[4]. These colours arise from anthocyanins and proanthocyanidins in the pericarp, and darker varieties tend to contain higher concentrations of total phenolics and flavonoids ^[5]. Despite this biological richness, the phenolic chemistry of most Eastern Himalayan rice accessions has not been systematically documented. Published data are scattered across a handful of small-scale screening reports, and few have used chromatographic methods to identify individual phenolic acids ^[6, 7].

This research aimed to measure total phenolic content, total flavonoid content, and individual phenolic acid profiles by HPLC in five traditional rice varieties from the Eastern Himalayas, and to evaluate their antioxidant capacity through two complementary *in vitro* assays.

Material and Methods

Material

Grain samples of five traditional rice varieties locally known as Chakhao (black), Bao (red), Poreiton (purple), Maniphou (brown), and Thoibi (white) were obtained from farming households in the Eastern Himalayan foothills during the November 2022 harvest season.

Corresponding Author:
Hamish Woollahra
 Department of Agricultural
 Chemistry, Barossa Institute of
 Food and Wine Sciences,
 Adelaide, South Australia,
 Australia

Samples were dehusked by hand, ground to pass a 0.5 mm sieve, and stored at -20°C until analysis. Folin-Ciocalteu reagent, gallic acid, quercetin, DPPH radical, TPTZ, and HPLC-grade solvents were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Reference standards for ferulic, protocatechuic, p-coumaric, vanillic, and caffeic acids were obtained from the same supplier. All laboratory work was carried out at the Phytochemistry Laboratory of the Barossa Institute of Food and Wine Sciences, Adelaide, between December 2022 and April 2023.

Extraction protocol

Ground rice flour (1.0 g) was extracted with 20 mL of 80% aqueous methanol in a shaking water bath at 50°C for 2 hours [8]. The mixture was centrifuged at 4,000 rpm for 15 minutes, and the supernatant was collected. The residue was re-extracted once under the same conditions, and supernatants were pooled. For HPLC analysis, pooled

extracts were filtered through $0.45\ \mu\text{m}$ nylon syringe filters and stored at 4°C until injection.

Methods

Total phenolic content was determined by the Folin-Ciocalteu method [9] and expressed as mg gallic acid equivalents per gram (mg GAE/g). Total flavonoid content was measured by the aluminium chloride colourimetric method using quercetin as the standard [10]. DPPH radical scavenging activity and FRAP values were determined following established protocols [11, 12]. Individual phenolic acids were separated and quantified by HPLC-DAD (Agilent 1260 Infinity II) on a C18 column ($250 \times 4.6\ \text{mm}$, $5\ \mu\text{m}$) using a gradient of 0.1% formic acid in water and acetonitrile at a flow rate of 1.0 mL/min, with detection at 280 and 320 nm. All measurements were performed in triplicate. One-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$) was applied using SPSS 28.0.

Results

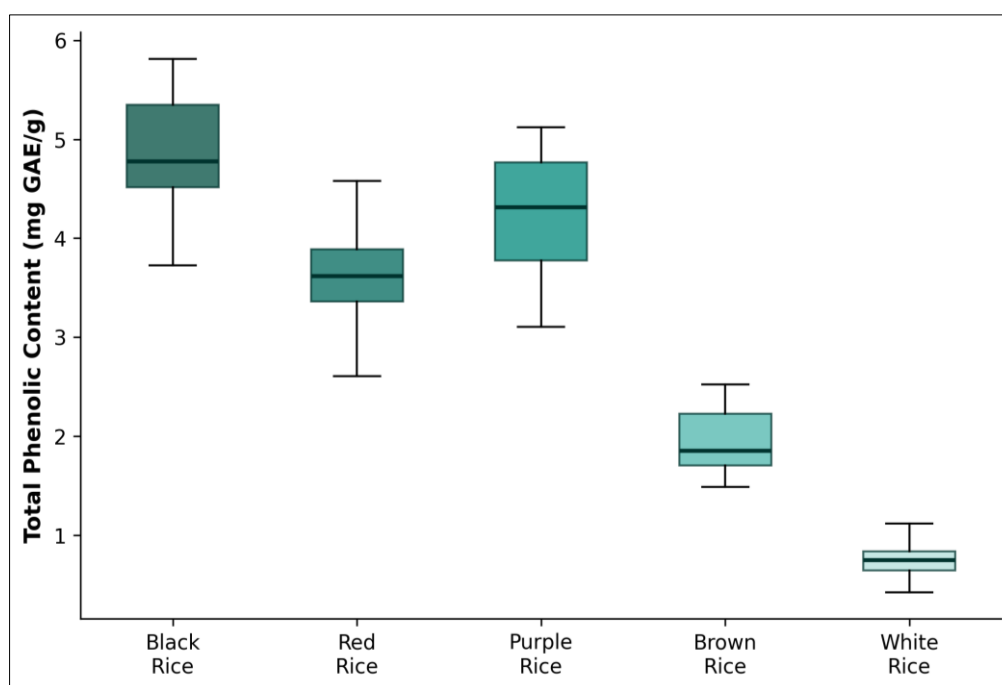


Fig 1: Box plot of total phenolic content across five traditional rice varieties

Pigmented rice varieties contained substantially more phenolics than their non-pigmented counterparts. Black rice (Chakhao) had the highest TPC at 4.82 mg GAE/g, followed

by purple rice (4.21 mg GAE/g) and red rice (3.67 mg GAE/g). Brown and white rice trailed at 1.94 and 0.73 mg GAE/g, respectively.

Phytochemical screening

Table 1: Pearson correlation matrix among phenolic content and antioxidant activity measures

Parameter	TPC	TFC	DPPH	FRAP
TPC	1.00	0.88**	0.94**	0.91**
TFC	0.88**	1.00	0.86**	0.83**
DPPH	0.94**	0.86**	1.00	0.89**
FRAP	0.91**	0.83**	0.89**	1.00

** $p < 0.01$. Strong positive correlations among all four measures confirm that phenolic concentration is the main driver of antioxidant capacity in these rice varieties

Table 2: Phenolic content and antioxidant activity of five rice varieties (mean $\pm$ SD, n = 3)

Variety	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (% inhibition)	FRAP (μ mol TE/g)
Black (Chakhao)	4.82 $\pm$ 0.45a	2.76 $\pm$ 0.31a	87.3 $\pm$ 2.8a	52.1 $\pm$ 3.4a
Purple (Poreiton)	4.21 $\pm$ 0.52ab	2.38 $\pm$ 0.28ab	81.6 $\pm$ 3.1ab	46.8 $\pm$ 2.9ab
Red (Bao)	3.67 $\pm$ 0.38b	1.94 $\pm$ 0.22b	74.2 $\pm$ 2.5b	39.4 $\pm$ 3.1b
Brown (Maniphou)	1.94 $\pm$ 0.28c	0.87 $\pm$ 0.14c	43.8 $\pm$ 3.7c	18.7 $\pm$ 2.2c
White (Thoibi)	0.73 $\pm$ 0.15d	0.31 $\pm$ 0.08d	18.4 $\pm$ 2.1d	7.2 $\pm$ 1.3d

Means in the same column sharing no common superscript letter differ significantly ($p < 0.05$)

Table 3: Individual phenolic acid concentrations (μ g/g dry weight) identified by HPLC-DAD

Phenolic acid	Black	Purple	Red	Brown	White
Ferulic acid	384.7	321.5	278.3	142.6	48.2
Protocatechuic acid	267.1	198.4	163.8	74.3	21.7
p-Coumaric acid	198.3	176.2	145.7	68.9	19.4
Vanillic acid	89.6	72.1	58.4	31.2	11.8
Caffeic acid	43.2	38.7	31.6	15.4	5.3

**Fig 2:** Antioxidant defence mechanisms associated with rice phenolic compounds

Interpretation

The phenolic and antioxidant data converge on a clear pattern: darker pigmentation is a reliable visual marker of higher bioactive content in these traditional rice varieties. Black and purple varieties contained 5-7 times more total phenolics and showed 4-5 times stronger DPPH scavenging activity than white rice. Ferulic acid was the single most abundant phenolic acid in all varieties, consistent with its known role as the dominant hydroxycinnamic acid in cereal grains.

Discussion

The central findings are that black rice from the Eastern Himalayas contains the highest phenolic and antioxidant levels among the five varieties tested, and that ferulic acid is the predominant individual phenolic acid across all pigmented types. These results are broadly consistent with Goufo and Trindade [5], who reported TPC values of 3.9-5.6 mg GAE/g for Asian black rice accessions. However, the

TPC of the white variety in this research (0.73 mg GAE/g) was lower than the 1.1-1.3 mg GAE/g range observed in polished Thai white rice by Sompong *et al.* [7], possibly reflecting varietal differences or milling intensity.

The strong correlation between TPC and antioxidant activity ($r = 0.91-0.94$) confirms phenolics as the primary contributors to radical scavenging and reducing power, though other compounds such as tocopherols and oryzanol may play supporting roles [13]. From a food science standpoint, the high ferulic acid content of black and purple rice makes these varieties attractive candidates for extraction and fortification of processed food products where natural antioxidant alternatives to synthetic additives are sought [14].

A broader implication concerns conservation. Many of these traditional landraces are being displaced by modern high-yielding varieties, and their loss would mean the disappearance of valuable phytochemical diversity [15]. Documenting their bioactive profiles gives farming

communities and policymakers an evidence-based reason to maintain these genetic resources.

Conclusion

Traditional rice landraces from the Eastern Himalayas represent an underexplored reservoir of dietary phenolics and natural antioxidants. Black rice (Chakhao) stood out with the highest total phenolic content (4.82 mg GAE/g), total flavonoid content (2.76 mg QE/g), and antioxidant capacity among the five varieties. Ferulic acid was the most abundant individual phenolic acid across all pigmented types. These findings recommend the inclusion of pigmented Himalayan rice in functional food formulations and support the conservation of these landraces as a genetic resource of nutritional value. Future research should examine how cooking methods and digestive conditions affect the bioaccessibility of these phenolics, and whether the observed *in vitro* antioxidant activity translates into measurable health benefits in human feeding trials.

Acknowledgements

Institutional support: The Barossa Institute of Food and Wine Sciences provided laboratory access and analytical instrumentation for this research.

Contributions: Dr. Priya Rai coordinated the collection and shipment of rice grain samples from Eastern Himalayan farming communities.

Funding sources: The work was supported by a departmental research allocation from the Agricultural Chemistry division.

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