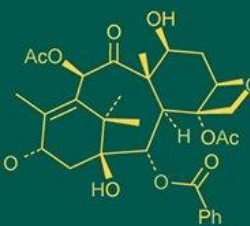
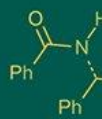


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Physicochemical, functional and bioactive profiling of underutilised distiller's rice spent grain and buckwheat in relation to conventional rice and wheat flours

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Abstract

Agro-industrial by-products and underutilised grains have considerable potential as alternative sources for developing nutritionally enriched and functionally enhanced food ingredients. Dried distiller's rice spent grain (DDRSG), a protein-rich by-product of ethanol production, and dehulled buckwheat are promising alternative resources with considerable nutritional and functional potential. This study comparatively evaluated the nutritional composition, functional properties, colour attributes and bioactive potential of DDRSG, dehulled buckwheat, rice and wheat flours. Among the evaluated samples, DDRSG showed the highest protein content ($54.24 \pm 0.67\%$), substantially exceeding that of dehulled buckwheat ($14.15 \pm 0.20\%$), wheat ($10.69 \pm 0.41\%$) and rice flour ($7.18 \pm 0.21\%$), and also had the highest fat ($4.50 \pm 0.08\%$) and ash ($3.38 \pm 0.22\%$) content. Its carbohydrate content was considerably lower ($36.33 \pm 0.63\%$) than that of the cereal flours. Distinctly favourable functional and powder-flow characteristics were evident in DDRSG, as reflected by its highest water-holding capacity (3.38 ± 0.06 g/g) and oil-holding capacity (1.51 ± 0.16 g/g), as well as the lowest compressibility ($3.37 \pm 0.19\%$) and Hausner ratio (1.03 ± 0.00). Dehulled buckwheat exhibited the highest antioxidant activity ($80.40 \pm 1.37\%$ DPPH inhibition), total phenolic content (4.96 ± 0.11 mg GAE/g) and total flavonoid content (7.26 ± 0.07 mg QE/g). Colour analysis indicated that DDRSG had substantially lower L^* and higher a^* , b^* and chroma values than the conventional flours, reflecting the darker and more intensely coloured appearance. Overall, DDRSG had higher crude protein content, whereas dehulled buckwheat showed superior antioxidant and bioactive properties, highlighting the complementary potential of both as value-added food ingredients. Their incorporation into suitable food formulations may contribute to nutritional enhancement, functional improvement and utilisation of underused agricultural resources.

Keywords: Dried distiller's rice spent grain, dehulled buckwheat flour, functional properties, bioactive compounds, powder-flow characteristics

1. Introduction

Cereal-derived foods represent a significant element of the worldwide food network, with rice and wheat acting as crucial providers of nutritional energy and foundational ingredients for flour-based food products. Nonetheless, the dominance of a few staple grains has augmented interest in diversifying food supplies by leveraging nutritionally rich underutilized crops and agro-industrial by-products. This diversification is especially pertinent to the advancement of sustainable food systems as it can concurrently boost resource efficiency, elevate nutritional quality, and generate value from materials that are presently underutilized.

Among different novel food ingredients, distiller's rice spent grain, produced during the manufacture of rice-based ethanol, constitutes a significant although relatively under-explored agro-industrial by-product. In the process of bioethanol production, a significant amount of easily fermentable rice starch is consumed, whereas other constituents such as protein become concentrated in the residue, i.e. distiller's grain. By-products from rice distillation have garnered interest as prospective sources of proteins and various nutrients. Rice distiller's dried grains with solubles (DDGS) are rich in protein, with significant

essential amino acids found in its protein fractions, notably glutelin and prolamin, underscoring the opportunity for utilizing rice distillery by-products for the extraction of protein (Singh *et al.*, 2019) [27]. Other studies have also recognized rice-derived distiller's grain as nutrient-dense substances with significant crude protein levels (Taranu *et al.*, 2019) [30] and potential for its incorporation into various food products, but their composition may differ based on the cereal source and ethanol production methods.

The utilization of distiller's rice spent grain is especially appealing from the viewpoint of a circular bioeconomy. Rather than disposing it as landfill this low - value by-product, has the potential for integration into human food products. Distiller's rice spent grain are generally important ingredient for their high crude protein content and varied amino acids, indicating their potential as alternative plant-based food sources. Nonetheless, although there is a growing interest in the valorization of distillery by-products, rice-derived spent grain is still much under-researched for direct food applications compared to traditional cereal flours, highlighting the necessity for a comprehensive evaluation of its nutritional, physical and functional attributes.

Simultaneously, buckwheat (*Fagopyrum spp.*) is an underutilized pseudocereal with significant nutritional and physiological characteristics. Buckwheat, however classified under the Polygonaceae family rather than true cereals, is processed and utilized in ways akin to cereal grains. It is acknowledged for its superior protein quality, dietary fiber, minerals, unsaturated fats, and bioactive substances, including as phenolic compounds and flavonoids. Pseudocereals like buckwheat typically exhibit unique nutritional characteristics in contrast to traditional cereals and offer opportunities for diversifying cereal-centric diets. Buckwheat contains 2-5 times higher levels of phenolic compounds compared to oats and barley, and its bran and hulls demonstrate antioxidant properties that are 2-7 times more effective than those of barley, triticale, and oats (Huda *et al.*, 2021; Zou *et al.*, 2021) [13, 33]. The principal bioactive category in buckwheat is flavonoids, with rutin being the predominant glycoside, alongside quercetin and C glycosyl flavones; these flavonoids are significantly associated with antioxidant, anti-inflammatory, and cardio metabolic effects (Matsui and Walker, 2020) [20]. Therefore, the nutritional attributes of buckwheat also provide a scientifically relevant benchmark for evaluating alternative cereal-derived ingredients. Comparative studies have demonstrated differences among buckwheat, rice and wheat flours in protein, fat, starch, ash, particle size and other physicochemical characteristics.

Rice and buckwheat flours were analyzed revealing notable disparities in protein/starch content, particle size, damaged starch, and color attributes (Torbica *et al.*, 2012) [31]. In a similar type of investigation, it was reported that wheat, buckwheat, and rice flours possess unique physicochemical and pasting attributes, with each flour significantly affecting technological properties (Yilmaz *et al.*, 2015) [32]. These results suggest that the source of the plants significantly affects the functional properties and processing characteristics of cereal and pseudocereal flour.

Rice and wheat flours, on the other hand, are recognized as traditional cereal components with clearly defined nutritional and technological properties. Wheat flour contains gluten proteins that create a unique viscoelastic

network essential for certain baking processes, while rice flour is inherently gluten-free and exhibits specific starch-related functional attributes. Variations in protein composition, starch, particle dimensions, and inherent lipids lead to differences in nutritional and other functional attributes of cereal flours. The differing characteristics of rice and wheat flour serve as valuable traditional benchmarks for assessing the nutritional and functional qualities of alternative components.

A direct comparative analysis of dried distiller's rice spent grain in relation to underutilized pseudocereal i.e., buckwheat and traditional rice and wheat flours is still inadequately investigated. Specifically, an integrated comparison encompassing nutritional composition, functional properties, and bioactive characteristics could provide a comprehensive understanding of the extent to which distiller's rice spent grain shares similarity with the buckwheat, while also elucidating their differences from conventional cereal flours. Such a comparative approach may further identify key attributes that support the incorporation of these materials into value-added and nutritionally enhanced food formulations.

The comparative approach was designed to elucidate the nutritional, functional, and bioactive similarities and differences among the four flours, while assessing the potential of dried distiller's rice spent grain and buckwheat as alternative food resources. Furthermore, this analysis can provide an integrated assessment of the relationships among the evaluated attributes and facilitate the differentiation and positioning of the four flours based on their overall nutritional, physicochemical, and technological characteristics.

2. Materials and Methods

2.1 Sample Collection

Dried distiller's rice spent grain (DDRSG) was obtained from a rice-based ethanol distillery, India Glycols Limited, Kashipur, Uttarakhand, India, and transported to the Department of Food Science and Technology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, for further analysis. Buckwheat groats, wheat flour, and rice flour were procured from a local market in Pantnagar, Uttarakhand, India. The dehulled buckwheat groats were milled using a laboratory-scale mill, and the resulting dehulled buckwheat flour was passed through a 420 µm sieve to ensure uniform particle size. All samples were appropriately packaged and stored under suitable conditions until further analysis.

2.2 Chemicals and Reagents

All chemicals, reagents, and solvents employed in the present investigation were of analytical grade and obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The reagents included sulphuric acid (H₂SO₄), hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium sulphate (K₂SO₄), copper sulphate (CuSO₄), boric acid, ethanol, acetone, hydrogen peroxide (H₂O₂), and other analytical-grade reagents required for the nutritional, functional and other analyses.

2.3 Physicochemical Analysis

2.3.1 Moisture Content

For moisture determination, approximately 5 g of each sample was weighed into a clean, dry, and pre-weighed

moisture dish. The dishes containing the samples were placed in a hot-air oven and maintained at 130 ± 2 °C for 1 h. Upon completion of the drying period, the dishes were removed, cooled to ambient temperature in a desiccator, and weighed again. The moisture content was calculated based on the reduction in sample mass resulting from oven drying, in accordance with AACC International Approved Method 44-15.02 (AACC International, 1999) [1].

2.3.2 Crude Protein content

Crude protein content was determined using the Kjeldahl method. Approximately 0.2 g of sample was accurately weighed into Kjeldahl digestion tubes in triplicate. The samples were digested with 3 g of a catalyst mixture consisting of K_2SO_4 and $CuSO_4$ (5:1, w/w) in the presence of 10 mL concentrated H_2SO_4 . Digestion was carried out at 420 °C in a digestion block until the contents became clear and green, signifying complete mineralization of the organic matter. After cooling, the resulting digest was diluted appropriately with distilled water and transferred for steam distillation in a KELPLUS distillation unit. Prior to distillation, 40 % NaOH was added to liberate ammonia from the ammonium salts formed during digestion. The evolved ammonia was quantitatively absorbed in 25 mL of 4 % boric acid containing a mixed indicator and subsequently quantified by titration with standardized 0.1 N HCl. A reagent blank was subjected to the same digestion, distillation, and titration procedures to account for background nitrogen. The nitrogen concentration was calculated from the corrected titration values, and crude protein was expressed by applying a nitrogen-to-protein conversion factor of 5.95 for dried distiller's rice spent grain, rice flour, and wheat flour, and 6.25 for buckwheat flour.

$$\text{Nitrogen (\%)} = \frac{(V_s - V_b) \times N \times 14.007 \times 100}{W \times 100}$$

Where:

- V_s : Volume of acid used for the sample (mL)
 - V_b : Volume of acid used for the blank (mL)
 - N: Normality of the standard acid
 - 14.007: Atomic weight of nitrogen
 - W: Weight of the sample (g)
- $$\text{Crude Protein (\%)} = \text{Nitrogen (\%)} \times F$$

Where:

- Nitrogen (%): Percentage of nitrogen in the sample

2.4.4 Crude Fat

The lipid fraction of the samples was quantified using Soxhlet solvent extraction according to the method of Ranganna (1986) [23]. Briefly, 2 g of each sample, was weighed into a cellulose extraction thimble and extracted continuously with petroleum ether using a Soxhlet extraction system. The extraction was maintained at 70 °C for 90 min to facilitate efficient recovery of the lipid fraction. Following extraction, the solvent was recovered by heating at 150 °C. The extraction flasks containing the extracted lipids were subsequently subjected to oven drying at 70 °C for 1 h to eliminate any remaining solvent. The flasks were then cooled in a desiccator to room temperature and weighed. The crude fat content was expressed

gravimetrically based on the increase in mass of the extraction vessel attributable to the recovered lipid fraction.

2.4.5 Ash

Ash content was quantified using the dry ashing method described by AOAC International (2005) [2]. Briefly, approximately 2 g of each sample was weighed into cleaned, dried, and weighed porcelain crucibles. The samples were first gently charred on a hot plate to facilitate gradual removal of volatile constituents and minimise excessive fuming. The crucibles were subsequently transferred to a muffle furnace maintained at 550 °C and incinerated until a consistent light grey or white residue was obtained, confirming the removal of organic matter. The crucibles containing the ash were then cooled to room temperature in a desiccator and weighed. Ash content was determined gravimetrically based on the mass of inorganic residue obtained following complete incineration.

2.4.6 Total carbohydrate

The carbohydrate content of the samples was determined by difference. It was calculated by deducting the respective percentages of moisture, protein, fat, ash, and crude fibre from the total sample mass (100 %). The resulting value represented the estimated carbohydrate content.

2.5 Colour characteristics

The colour profile of the untreated and treated DDRSG samples was assessed through digital image processing under controlled illumination conditions. Samples were placed in petri plates and positioned inside a specially constructed imaging chamber with a black inner surface to minimise background reflection and the influence of white light. Consistent illumination was achieved using white LED light sources, while a digital camera positioned vertically above the samples was used to capture the images at a fixed distance. The acquired images were analysed to obtain RGB colour values using Microsoft Paint. These RGB values were subsequently transformed into the CIE L^* , a^* , and b^* colour coordinates using ColorDesigner.io. To improve the representativeness of the measurements, colour values were recorded from several randomly selected positions within each sample, and the corresponding mean values were calculated for further statistical evaluation.

2.5.1 Chroma Index and Hue Angle

For a detailed assessment of the colour characteristics of untreated and hydrogen peroxide-decolourised DDRSG, the derived colour parameters, chroma (C^*) and hue angle (h°), were calculated from the experimentally determined CIE L^* , a^* , and b^* coordinates. These parameters were used to further characterize the intensity and tonal attributes of the samples, with calculations performed according to the following equations:

$$\text{Chroma index} = \sqrt{a^2 + b^2}$$

$$\text{Hue angle} = \tan^{-1} \left(\frac{b}{a} \right)$$

2.6 Functional Properties

2.6.1 Water holding capacity

Water-holding capacity (WHC) was assessed using the method reported by Beuchat (1977) [3], with minor modifications where applicable. Briefly, 1 g of the sample

was accurately weighed into a pre-weighed 50 mL centrifuge tube, and 10 mL of distilled water was added. The contents were thoroughly dispersed using a vortex mixer for 30 min and subsequently equilibrated at room temperature for another 30 min to allow maximum hydration of the sample matrix. The resulting suspension was centrifuged at $3000 \times g$ for 25 min to separate the unbound water from the hydrated sample fraction. The supernatant was carefully decanted, and the remaining hydrated material was weighed. WHC was calculated from the quantity of water retained by the sample and expressed as grams of water retained per gram of dry sample (g/g).

2.6.2 Oil Holding Capacity

The oil-holding capacity (OHC) of DDRSG was determined with slight modifications (Rodríguez-Ambriz *et al.*, 2005) [24]. Approximately 1 g of DDRSG was accurately weighed into a centrifuge tube, followed by the addition of 25 mL of groundnut oil. The sample was thoroughly dispersed using a vortex mixer for 2 min and subsequently allowed to equilibrate at room temperature for 1 h to facilitate oil absorption. The sample-oil mixture was then centrifuged at $3000 \times g$ for 20 min to separate the excess oil. The supernatant oil was carefully decanted, and the centrifuge tubes were inclined at approximately 45° for 10 min to allow excess oil to drain completely. The amount of oil retained by the sample was determined gravimetrically, and OHC was expressed as grams of oil retained per gram of dry sample (g/g).

2.6.3 Bulk and Tapped Density

A known mass of approximately 1 g of the sample was transferred carefully into a 10 mL graduated cylinder, ensuring that the powder was not subjected to intentional compaction during filling. The volume occupied by the loosely filled sample was recorded as the initial volume, and the corresponding bulk density was calculated from the sample mass-to-volume ratio. For determination of tapped density, the graduated cylinder was subjected to repeated gentle tapping until a constant volume was attained, indicating complete settling of the powder bed. The final settled volume was recorded, and tapped density was calculated from the ratio of sample mass to the settled volume. The resulting bulk and tapped density values were expressed in kg/m^3 (Jinaponget *et al.*, 2008) [15].

2.6.4 Compressibility

The flowability-related compressibility characteristics of DDRSG were assessed by determining Carr's compressibility index from the measured bulk and tapped densities. The compressibility index was calculated according to the approach proposed by Carr (1965) [6], using the following equation:

$$\text{Compressibility (\%)} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100$$

2.6.3 Hausner Ratio

The Hausner ratio of DDRSG was evaluated as an indicator of its powder flow characteristics using the measured bulk and tapped bulk densities. The ratio was calculated in accordance with the method proposed by Hausner (1967)

[12], based on the relationship between the tapped and bulk density values, as represented by the following equation:

$$\text{Hausner ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

2.7 Bioactive Properties

2.7.1 Preparation of the Extracts

Phenolic and antioxidant compounds were extracted following the procedure reported by Dossa *et al.* (2023) [10], with minor modifications. Briefly, 1 g of each sample was accurately weighed into a conical flask, and 10 mL of 70 % (v/v) methanol was added. The conical flasks were capped and subjected to continuous agitation using a magnetic stirrer (IDL, Freising, Germany) for 30 min to facilitate the extraction of bioactive constituents. Following extraction, the mixtures were filtered through Whatman No. 1 filter paper. The resulting filtrates were collected and used for the determination of total and individual polyphenols, total flavonoid content, and antioxidant activity.

2.7.2 Antioxidant Activity

The free-radical-scavenging potential of the samples was assessed using the DPPH assay (Ciulcaet *et al.*, 2021) [7]. For the assay, 2.0 mL of each sample extract was mixed with 3 mL of 0.004% (w/v) DPPH solution. The reaction mixtures were homogenized using a vortex mixer and subsequently incubated at room temperature for 30 min under dark conditions to prevent light-induced degradation of the DPPH reagent. After incubation, the absorbance of each reaction mixture was recorded at 517 nm using a spectrophotometer (Thermo Fisher Scientific). Gallic acid was used as a reference, and each determination was carried out in triplicate. The percentage inhibition of DPPH radicals was calculated using the following expression:

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

where ControlAbsorbance refers to the absorbance values of the control and SampleAbsorbance to the absorbance values of the sample.

2.7.3 Total Phenolic Content

The total phenolic content (TPC) of the samples were quantified using the prepared extracts following the Folin-Ciocalteu colorimetric method, with appropriate modifications (Cocan *et al.*, 2022) [8]. Each extract (0.1 mL) was transferred into labelled test tubes, followed by the addition of 0.4 mL of the respective extraction solvent and 1.25 mL of Folin-Ciocalteu reagent (HiMedia), previously diluted 1:10 (v/v) with glass-distilled water. The reaction mixtures were allowed to incubate at room temperature for 5 min, after which an aqueous sodium carbonate (Na_2CO_3) solution (6 g/100 mL) was added. The resulting mixtures were incubated at 50°C for 30 min in a thermostatically controlled environment. Subsequently, absorbance was measured at 750 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific). Quantification was performed using a calibration curve prepared with gallic acid standards in the concentration range of 50-250 mg/L. The TPC was

expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g).

2.7.4 Total Flavonoid Content

The total flavonoid content (TFC) of the samples was determined using the colorimetric method (Cocan *et al.*, 2022) [8], with slight modifications. Each sample extract (1 mL) was transferred into a test tube, followed by the addition of 0.3 mL of 5% sodium nitrite (NaNO₂) and 0.3 mL of 10% aluminium chloride (AlCl₃) solution. The reaction mixture was allowed to stand for 6 min at room temperature, after which 2 mL of 1 M sodium hydroxide (NaOH) was added. The mixture was subsequently incubated for an additional 15 min at room temperature, and the absorbance was measured at 510 nm using a UV-VIS spectrophotometer (Thermo Fisher Scientific). A 70 % (v/v) methanol solution was used as the control. Quantification was carried out using a quercetin calibration curve prepared over the concentration range of 50-1000 mg/L. The TFC was expressed as milligrams of quercetin equivalents per gram of dry sample (mg QE/g).

2.8 Statistical Analysis

All experimental determinations were carried out in three independent replicates, and the obtained data were reported as mean $\pm$ standard deviation (SD). Statistical evaluation was performed using OriginPro 2026b (OriginLab Corporation, Northampton, MA, USA). The data corresponding to the physicochemical and functional attributes were subjected to one-way analysis of variance (ANOVA) to determine the significance of variation among the samples. Significant differences between treatment means were further assessed using Tukey's honestly significant difference (HSD) test for multiple pairwise comparisons. A probability level of $p < 0.05$ was considered indicative of statistically significant differences.

3. Results and Discussion

3.1. Nutritional Composition

The nutritional composition showed significant differences among dried distiller's rice spent grain, buckwheat, rice and wheat flour. The moisture content ranged from 10.14 % in rice flour to 12.57 % in DDRSG. The values are within the general range reported for cereal flours, although moisture can vary considerably according to drying conditions, storage and milling. The moisture content of all the samples falls within the moisture range generally considered suitable for storage (Shad *et al.*, 2021).

DDRSG exhibited a distinct nutritional profile characterised by exceptionally high crude protein ($54.24 \pm 0.67\%$ dwb), relatively high fat ($4.50 \pm 0.08\%$ dwb) and ash ($3.38 \pm 0.22\%$ dwb) (Table 1), together with comparatively low carbohydrate ($36.33 \pm 0.63\%$ dwb). This composition clearly differentiates DDRSG from the conventional cereal flours and indicates a substantial concentration of non-starch components during the ethanol-production process.

The protein contents of DDRSG, buckwheat, wheat and rice flour were 54.24 %, 14.15 %, 10.69 % and 7.18 %, respectively, on a dry-weight basis. The presence of high protein content in DDRSG was due to the starch hydrolysis and fermentation during ethanol production, whereas proteins and other non-fermentable constituents remain concentrated in the residual spent grain (Liu, 2011) [18]. The nutritional composition of dried distiller's rice spent grain

depends on the rice variety, fermentation efficiency, drying, solubles recovery and processing. Thus, DDRSG is a high-protein source possessing potential for incorporation into various food products.

The protein content (14.15 %) in buckwheat flour (Table 1) was significantly higher than that in wheat and rice. The results are in line with the previous reports showing approximately 9.06 - 14.88% protein among different *Tartary* buckwheat varieties (Bhinder *et al.*, 2020) [4]. The crude protein content in buckwheat, particularly depends on the cultivar and milling fraction. Buckwheat protein is predominantly composed of globulins, particularly 13S globulin, followed by albumins, whereas glutelins and prolamins constitute comparatively smaller fractions (Tang and Wang *et al.*, 2010) [29]. Hence, buckwheat and DDRSG possess higher protein content in comparison to conventional wheat and rice flour.

The fat content followed the order DDRSG > buckwheat > wheat > rice. DDRSG contained 4.50% fat, indicating retention/concentration of lipid components during fermentation. Buckwheat also contained appreciably more fat (3.41%) than wheat (1.71%) and rice (0.96%). The fat content of buckwheat flour was $3.41 \pm 0.13\%$ (dry basis), which falls within the range of 2.02-3.60% reported for flours from different *Tartary* buckwheat varieties (Bhinder *et al.*, 2020) [4].

The ash content varied significantly among the samples, with DDRSG exhibiting the highest ash content ($3.38 \pm 0.22\%$ dwb), followed by buckwheat ($2.03 \pm 0.09\%$ dwb), wheat ($1.23 \pm 0.17\%$ dwb), and rice flour ($0.75 \pm 0.06\%$ dwb). The elevated ash content of DDRSG indicates a greater concentration of mineral constituents in the spent grain. During ethanol production, the major fraction of grain starch is hydrolysed and subsequently fermented to ethanol and carbon dioxide, whereas minerals and other relatively non-fermentable constituents are retained and consequently concentrated in the residual distiller's grain. The starch is converted to glucose and subsequently ethanol and CO₂ during dry-grind ethanol processing, while most other grain components remain and become concentrated in Dried distiller's with solubles (Liu, 2011) [18].

The ash content of buckwheat flour (2.03 %) was within the range reported for *Tartary* buckwheat. In 23 *Tartary* buckwheat varieties, reported ash contents ranged from 1.76 to 2.80 %, with substantial variation among varieties (Bhinder *et al.*, 2020) [4]. Buckwheat is characterized by the presence of K, Mg and Ca which makes it a source of minerals. The relatively higher ash content of buckwheat than wheat and rice flour in the present study may therefore be associated with its greater mineral content. In contrast, the lower ash content of rice flour (0.75%), has reflected the use of milled flour fractions in which mineral-rich outer grain tissues are preferentially removed. Similar variation has been reported among rice flours, with commercial rice flour showing substantially lower ash than less-refined brown rice flours (Oppong *et al.*, 2021) [22].

The carbohydrate content also differed significantly among the samples, ranging from $36.33 \pm 0.63\%$ dwb in DDRSG to $88.23 \pm 0.21\%$ dwb in rice flour, with wheat and buckwheat containing 86.28 ± 0.33 and $80.35 \pm 0.21\%$, respectively. The high carbohydrate contents observed in rice and wheat are characteristic of cereal flours because starch constitutes the major storage carbohydrate in cereal endosperm. In a study of different rice flours, carbohydrate contents of 85.79 - 93.32% dwb were reported, which encompasses the value

obtained for rice flour in the present investigation (Oppong *et al.*, 2021) ^[22]. Similarly, carbohydrate contents of 72.23 - 79.35% have been reported among different wheat cultivars, although differences in cultivar and milling fraction can result in considerable variation (Siddiqi *et al.*, 2020) ^[26].

The comparatively lower carbohydrate content of buckwheat flour (80.35 %) relative to rice and wheat may be attributed to its greater contribution of protein, lipid, and minerals to the overall dry matter. The nutritional composition of buckwheat substantially depends on the variety of buckwheat used.

The particularly low carbohydrate content of DDRSG (36.33%) can be explained by the biochemical

transformations occurring during ethanol production. In the dry-grind process, starch, the principal carbohydrate of rice, is hydrolysed into fermentable sugars and subsequently converted by microorganisms into ethanol and carbon dioxide. Consequently, a substantial proportion of the original starch is removed from the grain matrix through fermentation, while proteins, minerals, lipids and other relatively non-fermentable components remain in the residual solids. Thus, the substantially reduced carbohydrate concentration of DDRSG compared with rice flour can reasonably be attributed to starch hydrolysis and subsequent fermentation during ethanol manufacture, rather than simply to differences in the original rice composition.

Table 1: Nutritional composition of DDRSG, Dehulled buckwheat, rice and wheat flour

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
DDRSG	12.57 ± 0.34 ^b	54.24 ± 0.67 ^d	4.50 ± 0.08 ^d	3.38 ± 0.22 ^d	36.33 ± 0.63 ^a
Buckwheat	12.48 ± 0.10 ^b	14.15 ± 0.20 ^c	3.41 ± 0.13 ^c	2.03 ± 0.09 ^c	80.35 ± 0.21 ^b
Rice	10.14 ± 0.09 ^a	7.18 ± 0.21 ^a	0.96 ± 0.07 ^a	0.75 ± 0.06 ^a	88.23 ± 0.21 ^d
Wheat	12.53 ± 0.09 ^b	10.69 ± 0.41 ^b	1.71 ± 0.15 ^b	1.23 ± 0.17 ^c	86.28 ± 0.33 ^c

3.2. Colour characteristics

The colour characteristics of DDRSG, buckwheat, rice and wheat flours are presented in Table 2. Significant differences ($p < 0.05$) were observed among the samples for all colour parameters, including L*, a*, b*, chroma (C*) and hue angle (h°).

3.2.1 L* (Lightness value)

The L* value differed significantly among the samples, ranging from 45.73 ± 4.38 in DDRSG to 91.59 ± 0.17 in rice flour. Rice flour exhibited the highest L* value, followed by wheat flour (87.93 ± 0.27) and buckwheat flour (79.63 ± 2.54), whereas DDRSG showed the lowest value. The high L* value of rice flour indicates its relatively white appearance, characteristic of milled rice flour that predominantly contains the starchy endosperm. In contrast, the lower L* values of dehulled buckwheat and whole wheat flour may be associated with the presence of naturally occurring pigments and bran, respectively.

The significantly lower L* value of DDRSG indicates its substantially darker appearance compared with the conventional flours. This darker colour can be attributed to the production of melanoidins during saccharification and liquefaction, which promote the formation and retention of Maillard reaction products. These compounds are strongly associated with brown colouration and can substantially reduce flour lightness. The darker colour of distillers' grains compared with the original grain has also been reported in previous studies (Song and Shurson, 2013) ^[28].

3.2.2 a* value (Redness-greenness)

The a* values ranged from 0.21 ± 0.03 for rice flour to 12.20 ± 1.51 for DDRSG. Significantly higher positive a* values were observed in DDRSG than in all other samples, indicating the presence of coloured compounds. Buckwheat (2.06 ± 0.08), wheat (2.12 ± 0.11), and rice (0.21 ± 0.03) exhibited significantly lower a* values. The elevated positive a* value of DDRSG, together with its low L* value, suggests non-enzymatic browning reactions, particularly Maillard reactions between reducing sugars and amino groups during the high-temperature stages of ethanol manufacture. The relatively low a* value of dehulled buckwheat flour reflects its lighter and less reddish appearance.

3.2.3 b* value (Yellowness - blueness)

The b* value was highest in DDRSG (23.23 ± 3.09), followed by wheat (13.23 ± 0.96), buckwheat (7.80 ± 0.40) and rice flour (6.38 ± 0.14). All samples exhibited positive b* values, indicating that the colour of the flours was shifted toward the yellow region rather than blue. The considerably higher b* value of DDRSG indicates a stronger yellow component, which, in combination with its high a* value, contributes to its characteristic yellowish-brown to brown appearance. The relatively low b* values of rice and buckwheat indicate weaker yellow colour intensity.

3.2.4 Chroma (C*)

Chroma represents the intensity or saturation of colour. Significant differences were observed among the samples, with values ranging from 6.38 ± 0.14 in rice flour to 26.29 ± 2.77 in DDRSG. DDRSG exhibited the highest chroma, followed by wheat (13.40 ± 0.97), buckwheat (8.07 ± 0.40) and rice (6.38 ± 0.14). The substantially higher chroma of DDRSG reflects its more intense and saturated colour and is primarily attributable to its considerably higher a* and b* values. In contrast, the low chroma of rice flour indicates a relatively weak colour saturation and a lighter appearance. The intermediate chroma of wheat suggests greater colour intensity than buckwheat and rice but considerably less than DDRSG.

3.2.5 Hue angle (h°)

Hue angle (h°) denotes the predominant colour tone of a sample and is expressed as the angular position of its colour coordinates within the CIELAB a*-b* chromaticity plane. The values ranged from 62.11 ± 4.58° for DDRSG to 88.12 ± 0.23° for rice flour. Rice flour showed the highest hue angle, followed by wheat (80.89 ± 0.41°), buckwheat (75.19 ± 0.80°) and DDRSG. The hue angle of rice flour, close to 90°, indicates a predominantly yellow hue with very little red contribution, consistent with its low a* value. Wheat and buckwheat exhibited intermediate hue angles, reflecting greater contributions from both yellow and red components. DDRSG exhibited the lowest hue angle, indicating a shift away from the predominantly yellow region toward brown colour tones. This observation agrees with its simultaneously high a* and b* values and low L* value.

Table 2: Colour characteristics of DDRSG, dehulled buckwheat, rice and wheat flour

Sample	L*	a*	b*	Chroma (C*)	Hue (h°)
DDRSG	45.73 ± 4.38 ^a	12.20 ± 1.51 ^a	23.23 ± 3.09 ^c	26.29 ± 2.77 ^c	62.11 ± 4.58 ^a
Buckwheat	79.63 ± 2.54 ^b	2.06 ± 0.08 ^b	7.80 ± 0.40 ^a	8.07 ± 0.40 ^a	75.19 ± 0.80 ^b
Rice	91.59 ± 0.17 ^c	0.21 ± 0.03 ^b	6.38 ± 0.14 ^a	6.38 ± 0.14 ^a	88.12 ± 0.23 ^c
Wheat	87.93 ± 0.27 ^c	2.12 ± 0.11 ^b	13.23 ± 0.96 ^b	13.40 ± 0.97 ^b	80.89 ± 0.41 ^b

3.3. Functional Properties

3.3.1. Water holding capacity

The water holding capacity (WHC) varied significantly among the samples, with the highest value observed for DDRSG (3.38 ± 0.06 g/g), followed by, buckwheat (1.17 ± 0.04 g/g), wheat (1.06 ± 0.05 g/g) and rice flour (0.95 ± 0.07 g/g) (Table 3). The markedly higher WHC of DDRSG may be associated with its substantially greater protein content. Proteins contain polar functional groups capable of interacting with water through hydrogen bonding. Water Holding Capacity is influenced by protein and carbohydrate, with proteins capable of binding water through polar groups of the polypeptide chains.

The high protein content of DDRSG observed in the present study (54.24 % dwb) may therefore have contributed substantially to its superior water-holding capacity. In addition, processing during ethanol production may alter the physical structure of the residual grain matrix, potentially increasing the accessibility of hydrophilic groups to water. The considerably greater WHC of DDRSG indicates its potential for applications where water retention, viscosity development and moisture management are desirable, such as bakery products, dough systems and meat or plant-based food formulations. Buckwheat flour has also been reported to exhibit higher water absorption in comparison to wheat and rice flour, although values vary depending on cultivar and processing conditions (Culetu *et al.*, 2021) [9].

3.3.2. Oil holding capacity

The oil holding capacity (OHC) ranged from 0.78 ± 0.05 g/g in rice flour to 1.51 ± 0.16 g/g in DDRSG. The highest OHC was observed in DDRSG, whereas buckwheat (0.84 ± 0.08 g/g), rice (0.78 ± 0.05 g/g) and wheat (0.88 ± 0.04 g/g) were statistically comparable. The higher oil-holding ability of DDRSG may be related to its high protein concentration and the availability of hydrophobic regions within proteins that can interact with lipid molecules. The physical structure and surface characteristics of flour particles can also influence oil entrapment.

Differences in OHC among flours can arise from variations in the presence and exposure of non-polar amino-acid side chains, which facilitate interactions with hydrocarbon chains of oil (Jan *et al.*, 2015) [14]. Buckwheat flour has also been reported to possess higher oil absorption capacity than wheat flour, with values varying according to cultivar and processing conditions (Breslauer *et al.*, 2023) [5]. The relatively high OHC of DDRSG may be advantageous in food formulations because oil-binding capacity can contribute to flavour retention, mouthfeel and structural characteristics of products.

3.3.3. Bulk density

Bulk density differed significantly among the samples and ranged from 546.79 ± 6.53 kg/m³ for DDRSG to 678.61 ± 5.52 kg/m³ for rice flour. DDRSG exhibited the lowest bulk

density, followed by wheat (563.07 ± 15.00 kg/m³), buckwheat (668.41 ± 5.18 kg/m³) and rice flour. Bulk density is influenced by particle size, particle shape, moisture content, porosity and the chemical composition of flour. A lower bulk density indicates that a greater volume of powder is occupied by a given mass, generally reflecting greater interparticle void spaces or a more porous particle structure.

The relatively low bulk density of DDRSG may be associated with its heterogeneous particle structure and high protein content, which can produce a less compact powder matrix. Processing, including drying and milling, can further modify particle morphology and packing behaviour. In contrast, the comparatively high bulk density of rice flour suggests more efficient particle packing. Considerable differences in bulk density among cereal and pseudocereal flours, with buckwheat flour reported to have bulk densities around 0.79-0.81 g/mL in different investigations (Culetu *et al.*, 2021) [9].

3.3.4. Tapped density

The tapped density followed the order buckwheat (770.97 ± 2.48 kg/m³) > rice (765.53 ± 12.25 kg/m³) > wheat (718.91 ± 4.98 kg/m³) > DDRSG (565.83 ± 5.65 kg/m³). Tapped density represents the density attained after mechanical tapping and therefore reflects the ability of powder particles to rearrange and occupy available interparticle spaces.

The relatively small increase between bulk and tapped density in DDRSG indicates that its particles were already relatively resistant to further packing, whereas the greater increase observed for buckwheat and wheat indicates greater rearrangement during tapping. Differences in particle morphology, size distribution and surface characteristics can substantially affect powder packing (Klunklin and Savage, 2018) [16].

The high tapped density of buckwheat may be associated with its relatively compact particle structure and efficient packing, whereas the lower tapped density of DDRSG may reflect greater particle irregularity and porosity.

3.3.5. Compressibility index

The compressibility index, calculated from bulk and tapped densities, varied considerably among the samples. DDRSG showed the lowest value (3.37 ± 0.19 %), followed by rice (11.35 ± 0.93 %), buckwheat (13.30 ± 0.42 %) and wheat (21.69 ± 1.55 %).

Compressibility provides an indication of the extent to which a powder can consolidate upon tapping. A lower compressibility index indicates a smaller difference between bulk and tapped states and generally reflects better flowability, whereas a higher value indicates greater interparticle cohesion and/or a greater capacity for rearrangement during tapping.

Thus, the very low compressibility index of DDRSG suggests excellent flow characteristics and relatively low

susceptibility to consolidation. Rice and buckwheat exhibited comparatively greater compressibility but remained substantially lower than wheat. The relatively high value for wheat indicates greater powder consolidation upon tapping, which may be associated with stronger interparticle interactions and differences in particle-size distribution.

3.3.6. Hausner ratio

A similar trend was observed for the Hausner ratio, which ranged from 1.03 ± 0.00 for DDRSG to 1.28 ± 0.01 for wheat. The lowest Hausner ratio had been observed in DDRSG, followed by rice (1.13 ± 0.01), buckwheat (1.15 ± 0.01) and wheat flour (1.28 ± 0.01).

The Hausner ratio is derived from the relationship between tapped and bulk density and provides an indication of interparticle friction and powder flowability. Lower values indicate better flow, whereas higher values generally indicate greater cohesion and poorer flow. Accordingly, the Hausner ratio of 1.03 for DDRSG indicates very good flowability, while rice and buckwheat exhibited relatively good flow characteristics. The highest ratio for wheat indicates comparatively poorer flow and greater susceptibility to particle rearrangement and consolidation. The trends in Hausner ratio correspond closely with those obtained for compressibility index, confirming the distinct packing and flow behaviour of the four flour samples.

Table 3: Functional properties of DDRSG, dehulled buckwheat, rice and wheat flour

Sample	Water Holding Capacity (g/g)	Oil Holding Capacity (g/g)	Bulk density (kg/m ³)	Tapped Density (kg/m ³)	Compressibility (%)	Hausner Ratio
DDRSG	3.38 ± 0.06^c	1.51 ± 0.16^b	546.79 ± 6.53^a	565.83 ± 5.65^a	3.37 ± 0.19^a	1.03 ± 0.00^a
Buckwheat	1.17 ± 0.04^b	0.84 ± 0.08^a	668.41 ± 5.18^b	770.97 ± 2.48^c	13.30 ± 0.42^b	1.15 ± 0.01^b
Rice	0.95 ± 0.07^a	0.78 ± 0.05^a	678.61 ± 5.52^a	765.53 ± 12.25^c	11.35 ± 0.93^b	1.13 ± 0.01^b
Wheat	1.06 ± 0.05^{ab}	0.88 ± 0.04^a	563.07 ± 15.00^b	718.91 ± 4.98^b	21.69 ± 1.55^c	1.28 ± 0.01^c

3.4. Bioactive properties of DDRSG, Buckwheat, Rice and Wheat flour

3.4.1. Antioxidant activity

The DPPH radical-scavenging activity differed significantly among the samples, ranging from 40.33 ± 0.69 % in rice flour to 80.40 ± 1.37 % in buckwheat flour (Table 4). Buckwheat exhibited the highest antioxidant activity, followed by DDRSG (67.91 ± 0.81 %) and wheat flour (67.81 ± 0.35 %), which were statistically insignificant, whereas rice exhibited the lowest activity. The markedly higher radical-scavenging activity of buckwheat can be attributed to its abundance of phenolic acids and flavonoids, particularly rutin, quercetin and other flavonoid compounds possessing radical-scavenging properties (Sakač *et al.*, 2011) [25].

The DPPH radical-scavenging activity of DDRSG was 67.91%, indicating its high antioxidant potential. Distiller's grains can retain and concentrate phenolic compounds because starch is preferentially hydrolysed and fermented during ethanol production, thereby increasing the relative concentration of non-starch constituents in the residual material. Research on distillers' grains has demonstrated that phenolic compounds and other constituents contribute substantially to their antioxidant potential.

The comparatively low DPPH activity of rice flour (40.33 %) may be related to its lower concentration of phenolic and flavonoid compounds. Similar observations have been reported in comparative studies in which buckwheat exhibited considerably greater antioxidant and DPPH-radical-scavenging activity than rice flour (Sakač *et al.*, 2011) [25].

3.4.2. The total phenolic content (TPC)

The total phenolic content (TPC) showed a marked variation among the samples. Buckwheat possessed the highest TPC (4.96 ± 0.11 mg GAE/g), followed by DDRSG (2.53 ± 0.06 mg GAE/g), whereas wheat and rice exhibited considerably

lower values of 1.22 ± 0.12 and 1.03 ± 0.06 mg GAE/g, respectively. Buckwheat was significantly different from all other samples, while DDRSG also exhibited significantly higher TPC than rice and wheat (Giménez-Bastida *et al.*, 2017) [11].

The high TPC of buckwheat is consistent with its recognized richness in phenolic compounds. Buckwheat contains a diverse range of phenolic acids and flavonoids, including rutin, quercetin, catechin and related compounds. The concentration of these compounds can vary considerably according to species, cultivar, milling fraction and processing conditions. Studies on buckwheat flour have reported substantial concentrations of total phenolics and demonstrated that milling fractions containing more outer grain tissues can possess greater phenolic concentrations.

The TPC of DDRSG (2.53 mg GAE/g) was intermediate between buckwheat and the conventional cereal flours. This may be attributed to the concentration and/or liberation of phenolic compounds during ethanol fermentation and starch depletion. The relatively low TPC of rice (1.03 mg GAE/g) and wheat (1.22 mg GAE/g) indicates a lower abundance of extractable phenolic constituents under the conditions used in the present study. Wheat flour has generally been reported to exhibit lower total phenolic content than buckwheat, while reported TPC values for rice flour range from approximately 1.05 to 1.95 mg GAE/g, with variations attributable to cultivar and analytical conditions (Levent and Yuksel., 2022) [17].

3.4.3. Total flavonoid content

The total flavonoid content (TFC) ranged from 0.60 ± 0.03 mg QE/g in rice flour to 7.26 ± 0.07 mg QE/g in buckwheat flour. Buckwheat exhibited the highest content, although it was statistically comparable with DDRSG (6.69 ± 0.42 mg QE/g). Wheat flour showed an intermediate TFC (2.46 ± 0.16 mg QE/g), whereas rice flour had the lowest value (0.60 ± 0.03 mg QE/g).

The high flavonoid content of buckwheat is consistent with its characteristic phytochemical profile. Rutin is one of the

major flavonoids associated with buckwheat, together with quercetin, catechin and other flavonoid compounds. Rutin and quercetin possess substantial radical-scavenging capacity and can therefore contribute to the antioxidant activity of buckwheat extracts. Studies have also shown that flavonoid concentration can vary substantially among buckwheat fractions because phenolic and flavonoid compounds are unevenly distributed throughout the grain (Martín-García *et al.*, 2019) [19].

Wheat flour (2.46 mg QE/g) exhibited substantially lower TFC than DDRSG and buckwheat, while rice showed the lowest value (0.60 mg QE/g). The observed values are broadly consistent with the reported results describing buckwheat as a particularly flavonoid-rich pseudocereal compared with conventional cereal flours (Jan *et al.*, 2015) [14].

Table 4: Bioactive properties of DDRSG, dehulled buckwheat, rice and wheat flour

Sample	Antioxidant Activity (% Inhibition DPPH)	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
DDRSG	67.91 ± 0.81 ^b	2.53 ± 0.06 ^b	6.69 ± 0.42 ^c
Buckwheat	80.40 ± 1.37 ^c	4.96 ± 0.11 ^c	7.26 ± 0.07 ^c
Rice	40.33 ± 0.69 ^a	1.03 ± 0.06 ^a	0.60 ± 0.03 ^a
Wheat	67.81 ± 0.35 ^b	1.22 ± 0.12 ^a	2.46 ± 0.16 ^b

3.5. Multivariate Analysis of Physicochemical, Functional and bioactive Characteristics of DDRSG, dehulled buckwheat, rice and wheat flour

Principal component analysis (PCA) was performed to elucidate the relationships among the physicochemical, functional, colour and antioxidant attributes of DDRSG, buckwheat, rice and wheat flours. As illustrated in Fig. 1, the first two principal components accounted for 88.46% of the total variability, with PC1 explaining 72.01% and PC2 explaining 16.45%. The high cumulative variance indicates that the two principal components adequately represented the major variation present in the investigated characteristics.

PC1 was strongly influenced by the nutritional, functional and antioxidant attributes of the samples. Protein, fat, ash, moisture, antioxidant activity, total flavonoid content, water-holding capacity, oil-holding capacity, a* and chroma showed positive loadings on PC1, whereas carbohydrate content, bulk density, tap density, compressibility and Hausner ratio exhibited negative loadings. The strong positive loading of protein on PC1 reflects its importance in differentiating the flour samples based on their nutritional composition. Similarly, the positive association of antioxidant activity and total flavonoid content indicates that variation in bioactive constituents contributed considerably to sample discrimination.

PC2 explained 16.45 % of the total variation and was primarily associated with the antioxidant and phenolic characteristics. Total phenolic content exhibited a strong positive loading on PC2, together with antioxidant activity, total flavonoid content, moisture, fat and ash. In contrast, hue angle, protein, water-holding capacity, oil-holding capacity, a* and chroma showed negative associations with PC2. The close positioning of total phenolic content, total flavonoid content and antioxidant activity suggests a positive relationship among these attributes, indicating that the phenolic and flavonoid constituents may have contributed to the antioxidant potential of the samples.

The clustering of bulk density, tap density, compressibility and Hausner ratio in the negative PC1 region reflects their close interrelationship as powder-flow characteristics. This association is expected because compressibility and Hausner ratio are derived from bulk and tapped density and therefore describe related aspects of powder packing and flowability. Carbohydrate content was also positioned on the negative side of PC1, showing an opposing relationship with protein and several functional and antioxidant attributes. This pattern is consistent with the contrasting compositional characteristics of cereal flours and the protein-enriched DDRSG.

The colour parameters also contributed to the discrimination of the samples. The positive association of a* and chroma with PC1 reflects the contribution of redness and colour saturation to sample differentiation, whereas the lower hue angle was positioned in the opposite direction. This is particularly relevant to DDRSG, which exhibited a considerably lower L* value and higher a*, b* and chroma values than the conventional flours, indicating its darker and more intensely coloured appearance. The observed colour characteristics may be associated with the formation or retention of Maillard reaction products and melanoidins during ethanol processing, which contribute to the characteristic brown colouration of distillers' grain.

Overall, the PCA demonstrates that the investigated samples were differentiated by a combination of proximate composition, powder-flow characteristics, functional properties, colour attributes and antioxidant constituents. The high contribution of PC1, together with the substantial additional variation explained by PC2, indicates that these parameters collectively provide effective discrimination among the samples. In particular, the positioning of protein, antioxidant activity, phenolic and flavonoid contents, functional properties and colour parameters highlights the potential of DDRSG as a nutritionally and functionally distinct ingredient compared with conventional rice, wheat and buckwheat flours.

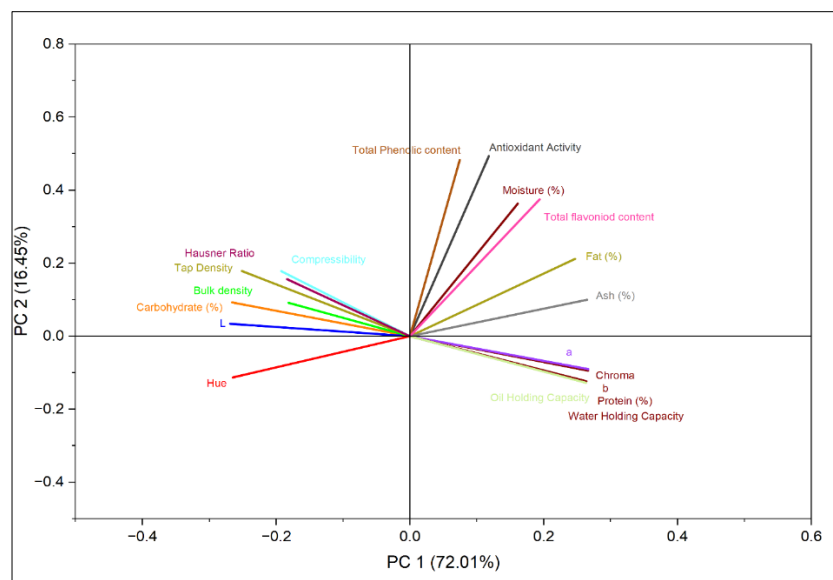


Fig 1: Principal component analysis (PCA) showing the relationships among physicochemical, functional, and bioactive properties of DDRSG, buckwheat, rice and wheat flours

4. Conclusion

The comparative analysis demonstrated that DDRSG and dehulled buckwheat possess distinct and complementary nutritional and functional advantages. The highest protein content of DDRSG, along with superior water- and oil-holding capacities and favourable powder-flow properties, highlights its potential as a protein-rich ingredient. In contrast, dehulled buckwheat exhibited the highest antioxidant activity, total phenolic content and total flavonoid content, indicating its strong bioactive potential. The darker colour of DDRSG, reflected by its lower L* and higher a*, b* and chroma values, may require consideration during food-product development. Collectively, DDRSG and dehulled buckwheat represent promising alternative ingredients for developing nutritionally enriched, antioxidant-rich and functionally improved food products.

Conflict of Interest

The authors declare no conflict of interest in connection with this article.

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