Comparative study of biochemical properties, anti-nutritional profile, and antioxidant activity of newly developed rye variants

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ABSTRACT
The present research was planned to explore the biochemical properties and antioxidant activity of newly developed Pakistani rye variants. For this purpose, four different rye variants (Gp-1, Gp-2, Gp-3, and Gp-4) were procured from the forage section of Ayub Agriculture Research Institute, Faisalabad. In first phase, each sample was milled to convert it into powder and was analyzed for their phytocchemical profile. In second phase, the mineral contents were explicated through flame photometer and atomic absorption spectrophotometer. In the last phase, the qualitative (tannin, phenols, saponin, and terpenoids) and quantitative screening of the phytoc hemicals (TPC, total flavonoids, DPPH, FRAP, and ABTS) were carried out through their respective methods. Results showed that the maximum total dietary fiber (TDF) content (24.47%), insoluble dietary fiber (IDF) (17.8%), and soluble dietary fiber (SDF) content (6.67%) were elucidated in the Gp-2 followed by Gp-4, Gp-3, and Gp-1. The maximum TPC (2.73 mg/g), DPPH (4.25 mg/g), FRAP (1.25 mg/g), ABTS (1.92 mg/g), and total flavonoids (12.31 mg/g) were observed in Gp-2; meanwhile, minimum TPC (1.05 mg/g), DPPH (1.05 mg/g) FRAP (1.017 mg/g), ABTS (1.02 mg/g), and total flavonoids (6.51 mg/g) were observed in Gp-4. The anti-nutritional compounds were not found in the Gp-1 and Gp-2, while Gp-3 and Gp-4 showed anti-nutritional compounds. The flavonoids and phenols were present in all the rye variants except Gp-3 and Gp-4, respectively. Conclusively, among the four rye variants, the Gp-2 rye variant was more nutritious and had good bioactive potential as it contained more antioxidants and biochemical profile than the rest of the variants.

Introduction
Cereal rye (Secale cereal L.) is a widely farmed grass as a grain, cover, and fodder crop. It belongs to the wheat family (Triticeae) and is associated with barley (Hordeum) and wheat (Triticum). Rye is a relatively new cereal that was first grown in Northern Europe. It is considered to have sprung from wild rye varieties that grow as weeds in wheat fields in Asia. Rye grains are dark in color, smaller in size, and affect well due to their morphological features. For marketing reasons, hectoliter
(hL) weight is typically around 70–75 kg, with a minimum of 70 kg/hL and a maximum moisture of 12%. Rye grain protein is lower compared to wheat protein. The dough does not have the elastic qualities of wheaten dough. It has been reported that rye bread is firm in texture and faint taste.\textsuperscript{[2]}

The weight of grain or test weight per unit volume has been used to determine the quality of rye grain. Test weight is an important criterion for rye.\textsuperscript{[3]} Per capita consumption (32.4 kg/capita) of the rye is maximum in Poland followed by the Baltic, and Nordic countries are also high consumption. The consumption of rye in the European Union is about 5.6 kg/capita, whereas the whole world only eats 0.9 kg/capita.\textsuperscript{[4]}

Among cereals, rye includes the most dietary fiber and a diverse range of bioactive components that have direct health impacts.\textsuperscript{[5]} The consumption of rye has a long history in numerous Nordic nations. Dietary fibers have been studied for their role in regulating physiological mechanisms such as reducing blood cholesterol, improving GI mobility, regulating lipid and glucose metabolism, stimulating bacterial metabolic activity, detoxifying colon luminal content, and maintaining the integrity of the intestinal mucosa.\textsuperscript{[6]} Whole-grain flour is recommended for baking with all cereals, especially rye, because the whole grains are high in fiber, minerals, vitamins, as well as bioactive phytochemicals.\textsuperscript{[7,8]} Rye is rich in fiber content, which has a beneficial role in promoting human health. However, dietary fiber alone cannot explain the favorable physiological activities of rye, and so other variables, such as bioactive chemicals, have been proposed to play an important role.\textsuperscript{[9]} Phytochemicals are plant-derived substances that serve plant activities such as cell signaling and defense. Bran layer, i.e. aleurone cells, is a major source of phytochemicals.\textsuperscript{[7]} The rye cereal is a good source of bioactive chemicals with biologically active properties. Increased intake of rye as a whole grain or whole-grain flour may result in an increased intake of phytochemicals that are good for the customer’s health.\textsuperscript{[10]} Bioactive chemicals identified in rye comprise phenolic acids, benzoazinoids, alkylresorcinols, and lignans. However, the number of phytochemicals discovered in rye is constantly growing, and it now includes nearly 2000 chemical species. Bioactive phytochemical density is higher in plant portions essential for reproduction. As a result, recent epidemiological studies have indicated that, when consumed as part of a regular diet, such foods are highly protective against various diseases.\textsuperscript{[8,11]} Rye grains provide proteins, carbohydrates, fiber, amino acids, especially lysine content, as well as vitamin E, the vitamins B complex including folic acid, riboflavin, thiamine, and pantothenic acid and the mineral contents, i.e. phosphorus, potassium calcium, and iron.\textsuperscript{[12]} Rye has a low gluten content as compared to wheat.\textsuperscript{[7]}

So, keeping in view, the present study was planned to characterize the nutritional, biochemical, and antioxidant profile of different rye variants available in Pakistan.

Materials and methods

The current study was carried out in the Advanced Food Analysis Lab at the Department of Food Sciences, Government College University, Faisalabad. The Rye seeds were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Four different rye variants, namely, RJS-10001, RJS-10002, RJS-10003, and RJS-10004, were coded as GP-1, GP-2, GP-3, and GP-4. All the required chemicals for the analysis were analytical grade. All chemicals used for this study were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Germany).

Milling

The grains of the rye variant were cleaned manually to remove seeds from other crops, dust particles, dirt, and other outside matter. Hammer-type laboratory mill LM-120, perton, Sweden was used for milling of rye grains available at Ayub Agriculture Research Institute, Faisalabad, Pakistan, as described in our previous work.\textsuperscript{[13]} For this purpose, Sieves size (0.5–2.0 mm) is used.
Morphological traits

Agronomic traits (test weight and thousand kernel weight) of the rye grain were carried out by the AACC[14] method. Schopper Chondrometer (OHAVS; Chicago) was used to calculate the test weight of different variants of rye seed and stated in kilogram per hectarliter (kg/hl), whereas a hundred gram sample of each rye seed variant was taken and a thousand kernel weight was calculated by counting the sound, clean, and unbroken seeds. The kernel weight was indicated as g/1000 kernels.

Total dietary fiber (TDF)

According to AACC,[14] total dietary fiber was measured in rye flour samples. The flour was disseminated in a buffer solution and incubated for 40 minutes at 95–100°C with heat-stable α-amylase.

Soluble dietary fiber (SDF)

The flour samples were tested for soluble dietary fiber using the Megazyme assay kit (Megazyme, Ireland) described in AACC.[14]

Insoluble dietary fiber (IDF)

The IDF contents in all the flour samples were calculated using the method described in AACC[14] method No. 32–20. The samples were disseminated in a buffer solution and incubated for 35 minutes at 95–100°C with heat-stable α-amylase. After cooling, the contents were incubated at 60°C for 30 minutes with the addition of 100 μl protease enzyme, followed by incubated for 30 minutes at 60°C with amylloglucosidase enzyme.

Mineral contents

The method described in AOAC[15] was used to measure the mineral contents of rye flour, such as K, Na, Ca, Fe, Zn, Mg, Cu, and Mn. For Ca, K, P, and Mg sample preparations, the digestion method of Lierop[16] was employed, while Gorsuch[17] was applied for Zn, Na, Fe, Cu, and Mn sample preparations. Zn, Mn, Cu, Mg, and Fe contents were measured by flame absorption on the Varian Spectra AA-30 Spectrophotometer, while Na and K contents were detected by flame emission. The quinalizarin method was used to assess boron content, while the molybdovanado phosphoric acid method was used to estimate P using a Technicon AutoAnalyzer II and the MgNO3 method was used to detect sulfur content using the method given in AOAC.[15]

Qualitative phytochemical screening

Test for flavonoids (Alkaline reagent test): The flavonoid content in the rye flour sample was determined as previously described by Prabhavathi et al.[18] Briefly, 2 ml extract was mixed with 20% sodium hydroxide. Intense yellow color formation occurs. The yellow color disappeared when a few drops of 70% hydrochloric acid were added. Color changes designate the existence of flavonoids in the sample.

Test for phenols (FeCl3 test): Aqueous ferric chloride (5%) was added to 2 ml extracted sample. The appearance of blue color indicates the existence of phenols in the extracted sample.[18]
**Qualitative anti-nutrient screening**

Test for Saponins (froth test): A 0.5 ml of rye flour extract was added to a clean test tube. 10 mL distilled water was transfereed to the test tube, tightly sealed, and thoroughly shaken for 30 seconds. The tubes were then let to stand and the existence of foam, which lasted for at least an hour, was accepted as proof of Saponins’ presence.[19]

Test for tannin (FeCl3 test): Aqueous ferric chloride (5%) was added to 2 ml extracted sample. The development of blue-black color designates the presence of tannin in the sample extract.[20]

Test for terpenoids (salkowski test): Two milliliters of chloroform was added to each 0.5 mL of extract. To produce a layer, 3 ml of concentrated sulfuric acid was carefully applied. A reddish-brown coloring of the interface indicates the presence of terpenoids.

**In Vitro quantitative antioxidant characterization, total phenolic compounds, and total flavonoids**

Various antioxidant assays were performed for DPPH, ABTS, TPC, FRAP, and total flavonoid assay estimation.

Total Phenolic Compounds (TPC): TPC content was determined as reported by Sengul et al.,[21] using gallic acid equivalent (mg gallic acid/g). The absorbance was measured at 760 nm using a spectrophotometer (IRMEOC, U2020).

Total Flavonoids (TF): A mixture of sample, water, NaNO2, and 10% AlCl3 was added in a ratio of 0.1, 4 0.3 ml, and 5%, respectively, and rested for 6 minutes and added 1.0 M NaOH. The final absorbance was carried out at 430 nm by adapting the guidelines of Ghasemzadeh & Jaffar.[22]

2,2-diphenyl-1-picrylhydrazyl (DPPH) Activity: Sample extract (4 ml) and DPPH (1 ml) were added to the test tube. The test tube containing the reactant was placed in the darkroom for 30 minutes. The absorbance was recorded using the UV/Visible Spectrophotometer at 520 nm.[23]

2,2'−azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Activity: ABTS assay was estimated conferring to the method outlined by Hossain et al.[24]

Ferric Reducing Antioxidant Power (FRAP): The FRAP assay of the samples was carried out following the method Hameed et al.,[25] Spectrophotometer was used to determine the FRAP content, and reading was recorded at 700 nm.

**Statistical design**

Analyses were accomplished in triplicate to investigate the different parameters of the rye varieties. The data were analyzed statistically by CRD using SPSS software by Steel.[26] The results were presented as mean ± S.D.

**Results and discussion**

**Physico-chemical properties of rye varieties**

The mean values regarding the physico-chemical properties of rye variants are presented in Table 1. Results showed that the test weight in the rye grains ranged from 70.64 to 74 kg hl⁻¹. The maximum test weight of the rye variant (74 kg hl⁻¹) was observed in Gp-2 followed by the Gp-1 (71.3 kg hl⁻¹) and Gp-4 (70.69 kg

| Parameter | Test weight (kg hl⁻¹) | Thousand kernel weight (g) |
|-----------|-----------------------|-----------------------------|
| Gp-1      | 71.3 ± 1.5b           | 41.03 ± 1.3                 |
| Gp-2      | 74 ± 1.2a             | 41.63 ± 1.5                 |
| Gp-3      | 70.64 ± 2.1c          | 40.66 ± 0.9                 |
| Gp-4      | 70.69 ± 3.2c          | 41.43 ± 2.3                 |
hl$^{-1}$), while the lowest test weight (70.64 kg hl$^{-1}$) was exhibited in Gp-3. The results are in agreement with Hansen et al.,$^{[27]}$ during the study of the different nutritional and functional properties of the rye, which is influenced by the harvest year and genotype. These results are also in line with the study of McLeod et al.,$^{[28]}$ who reported that the test weight of the rye ranges from 68.9 to 71.9 kg hl$^{-1}$. Another study conducted by the Banu$^{[29]}$ suggested that the test weight of the rye ranged from 44.20 to 76.10 kg hl$^{-1}$. The thousand kernel weights of different rye grains were in the range of 40.66–41.63 g. The maximum thousand kernel weight (41.633 g) was observed in the Gp-2 followed by the Gp-4 (41.43 g) and Gp-1 (41.03 g), whereas the minimum thousand kernel weight (40.66) of the rye grains was observed in Gp-3. The average kernel weight of rye grain ranged between 15.7 and 33.7 g/1000 kernels, as reported by Grabiński et al.$^{[1]}$

**Dietary Fiber in the Rye Flour:** Mean values regarding dietary fiber content have been shown in Table 2. The average total dietary fiber content was between 16.89% and 24.47%. The highest total dietary fiber content (24.47%) was observed in the Gp-2, while the lowest total dietary fiber content was observed in the Gp-1 (16.89%). The average soluble dietary fiber content was between 2.36% and 6.67%. The maximum soluble dietary fiber content (6.67%) was observed in the Gp-2 whilst, the minimum soluble dietary fiber content was observed in the Gp-1 (2.36%). The average insoluble dietary fiber content was between 14.53% and 17.8%. The highest insoluble dietary fiber content (17.8%) was observed in the Gp-2 whilst, the lowest insoluble dietary fiber content was observed in the Gp-1 (14.53%). The results are in accordance with the findings of Joye et al.,$^{[30]}$ who studied that the total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) in the rye flour ranged from 15.2 to 20.9, 11.1 to 15.9, and 3.7 to 4.5, respectively. Gartaula et al.$^{[31]}$ extracted the dietary fiber from three cereal flours (wheat, rye, and barley) and found health endorsing benefits.

**Mineral content in the Rye Flour:** Mean values regarding the mineral content of rye flour have been shown in Table 3. The average phosphorus content was between 2.8 and 2.86 mg/g. The maximum phosphorus content (2.86 mg/g) was observed in Gp-2, while the minimum phosphorus content was observed in the Gp-1 (2.8 mg/g). The average potassium content was between 4.51 and 4.57 mg/g. The highest potassium content (4.57 mg/g) was observed in the Gp-2, while the lowest potassium content was observed in the Gp-1 (4.51 mg/g). The average calcium content was between 0.32 and 0.37 mg/g. The highest calcium content (0.37 mg/g) was observed in Gp-2, while the minimum calcium content was observed in the Gp-1 (0.32 mg/g). The average magnesium content was between 1.22 and 1.31 mg/g. The maximum magnesium content (1.31 mg/g) was observed in the Gp-2, while the minimum magnesium content was observed in the Gp-4 (1.22 mg/g). The average sulfur content was between 1.43 and 1.48 mg/g. The maximum sulfur content (1.48 mg/g) was observed in the Gp-2, while the lowest was observed in the Gp-4 (1.43 mg/g).

The average iron content was between 0.05 and 0.067 mg/g. The maximum iron content (0.067 mg/g) was observed in the Gp-2, while the lowest was observed in the Gp-3 (0.05 mg/g). The average zinc content was between 0.024 and 0.044 mg/g. The maximum zinc content (0.044 mg/g) was observed in the Gp-2, while a minimum zinc content was observed in the Gp-4 (0.024 mg/g). The average manganese content was between 0.3 and 0.38 mg/g. The highest manganese content (0.38 mg/g) was observed in the Gp-2, while the lowest was observed in the Gp-4 (0.3 mg/g). The average boron content was between 0.01 and 0.023 mg/g. The maximum boron content (0.023 mg/g) was observed in Gp-2, while the minimum boron content was observed in the Gp-4 (0.01 mg/g). The average copper content was between 0.01 and 0.04 mg/g. The highest copper content (0.04 mg/g) was observed in the Gp-2, while the lowest was observed in the Gp-3 (0.01 mg/g).

### Table 2. Dietary fiber in the rye flour.

| Parameter | Total dietary fiber (%) | Soluble dietary fiber (%) | Insoluble dietary fiber (%) |
|-----------|-------------------------|---------------------------|-----------------------------|
| Gp-1      | 16.89 ± 3.08d           | 2.36 ± 1.7d               | 14.53 ± 2.7 cd              |
| Gp-2      | 24.47 ± 1.3a            | 6.67 ± 4.2a               | 17.8 ± 2.9a                 |
| Gp-3      | 20.62 ± 2.6c            | 4.26 ± 0.1c5              | 15.36 ± 4.05c               |
| Gp-4      | 21.03 ± 2.9b            | 5.2 ± 2.1b                | 16.83 ± 0.8b                |
| Parameter | Phosphorus | Potassium | Calcium | Magnesium | Sulfur | Iron | Zinc | Manganese | Boron | Copper |
|-----------|------------|-----------|---------|-----------|--------|------|------|-----------|-------|--------|
| Gp-1      | 2.8 ± 0.05 | 4.51 ± 0.09| 0.32 ± 0.07| 1.23 ± 0.1| 1.45 ± 0.09| 0.06 ± 0.02| 0.031 ± 0.01| 0.37 ± 0.08| 0.02 ± 0.01| 0.03 ± 0.01 |
| Gp-2      | 2.86 ± 0.1 | 4.57 ± 0.06| 0.37 ± 0.09| 1.31 ± 0.1| 1.48 ± 0.05| 0.067 ± 0.01| 0.044 ± 0.03| 0.38 ± 0.1| 0.023 ± 0.01| 0.04 ± 0.002 |
| Gp-3      | 2.83 ± 0.07| 4.55 ± 0.08| 0.35 ± 0.09| 1.23 ± 0.15| 1.47 ± 0.07| 0.05 ± 0.02| 0.036 ± 0.02| 0.34 ± 0.1| 0.017 ± 0.02| 0.01 ± 0.1 |
| Gp-4      | 2.85 ± 0.06| 4.54 ± 0.07| 0.34 ± 0.08| 1.22 ± 0.1| 1.43 ± 0.06| 0.054 ± 0.02| 0.024 ± 0.01| 0.3 ± 0.07| 0.01 ± 0.2| 0.02 ± 0.2 |

Table 3. Mineral content in rye flour (mg/g).
The results are in accordance with the study of Bağcı et al.\textsuperscript{32} reported a similar trend in the mineral composition while determining the significance of the differences in the fatty acid compositions, mineral, and protein contents among several rye types growing in Konya (Sarayönü) in Turkey. Another author, Gartaula et al.\textsuperscript{31} also mentioned the similar ranges in the mineral profile of the rye during the study of the chemical constituents of different genotypes.

### Antioxidant activity of the rye flour

The means regarding the phytochemical profile of the rye variants are shown in Table 4. The results presented that the average total phenolic contents (TPC) were 1.05 to 2.73 mg/g. The maximum TPC (2.73 mg/g) was observed in the Gp-2, while the minimum TPC was observed in the Gp-4 (1.05 mg/g). The average DPPH was between 1.05 and 4.25 mg/g. The highest DPPH (4.25 mg/g) was observed in the Gp-2, while the lowest DPPH was observed in the Gp-4 (1.05 mg/g). The average FRAP was between 1.017 and 1.25 mg/g. The maximum FRAP (1.25 mg/g) was observed in the Gp-2, while the minimum FRAP was observed in the Gp-4 (1.017 mg/g). However, the average ABTS was between 1.02 and 1.92 mg/g. The maximum ABTS (1.92 mg/g) was observed in Gp-2, while the minimum ABTS was observed in the Gp-4 (1.02 mg/g). The average of total flavonoids was between 6.51 and 12.31 mg/g. The highest total flavonoids (12.31 mg/g) was observed in Gp-2, while the lowest total flavonoids were observed in the Gp-4 (6.51 mg/g).

### Qualitative phytochemical screening of rye flour

The qualitative phytochemical and anti-nutrient screening of the rye flour is presented in Table 5. The results regarding the anti-nutrients in the rye flour showed that the saponin was not detected in the Gp-1 and Gp-2 as these showed no foam formation, whereas the rest of the flours, i.e. Gp-3 and Gp-4, showed the positive test and formation of foam was observed. The terpenoid contents indicated the reddish-brown color. The results showed that the terpenoids were not detected in the Gp-1, Gp-2, whereas the Gp-3 and Gp-4 showed the reddish-brown color, which means that the terpenoid contents were present in the samples. Tannin is an anti-nutritional component that is mostly present in grains, beans, legumes, and nuts. None of the samples showed a blue-black color, indicating that tannin content was not present in the rye. Tannin is the most critical anti-nutrient as it binds many important minerals like Zn, Ca, and Fe and makes them unavailable for human body utilization.

On the other hand, tannins have been considered to be cardio-protective, anti-inflammatory, anti-carcinogenic, and antimutagenic, among others. The phytochemical screening showed that the flavonoid contents were not present in the Gp-3, while Gp-1, Gp-2, and Gp-4 showed color change, which means that flavonoids were present in the flour. Meanwhile, the phenols were not present in the Gp-4, whereas Gp-1, Gp-2, and Gp-3 showed blue color, indicating that the phenols were present.

Table 4. Antioxidant activity of the rye flour.

| Parameter | TPC (GAE mg/100 g) | DPPH (Trolox mg/g) | FRAP (GAE mg/g) | ABTS (μM TE/g) | Total flavonoids (mg RE/100 g) |
|-----------|---------------------|-------------------|-----------------|----------------|-----------------------------|
| Gp-1      | 1.59 ± 0.02         | 3.13 ± 0.01b      | 1.106 ± 0.02    | 1.25 ± 0.01    | 10.27 ± 0.037b              |
| Gp-2      | 2.73 ± 0.02         | 4.25 ± 1a         | 1.25 ± 0.01     | 1.92 ± 0.01    | 12.31 ± 0.01a               |
| Gp-3      | 1.22 ± 0.02         | 2.02 ± 0.01c      | 1.054 ± 0.01    | 1.04 ± 0.07    | 8.24 ± 0.04d                |
| Gp-4      | 1.05 ± 0.01         | 1.05 ± 0.01 cd    | 1.017 ± 0.01    | 1.02 ± 0.01    | 6.51 ± 0.03c                |

Table 5. Qualitative phytochemical screening of rye flour.

| Parameter | Saponin | Tannin | Terpenoid | Flavonoid | Phenols |
|-----------|---------|--------|-----------|-----------|---------|
| Gp-1      | -       |        |           | +         | +       |
| Gp-2      | -       | -      |           | +         | +       |
| Gp-3      | +       | -      |           | -         | +       |
| Gp-4      | +       | -      |           | +         | -       |
Conclusion

Among the four rye variants (GP-1, Gp-2, Gp-3, and Gp-4), the Gp-2 rye variant is more nutritious as it contains high phytochemical contents as compared to other rye variants. GP2 was found to be superior in terms of flavonoid and phenolic contents. Furthermore, no anti-nutrients (tannin, saponin, and terpenoids) were detected in GP-2.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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List of abbreviations

| Acronym | Description |
|---------|-------------|
| TPC     | Total Phenolic Compounds |
| DPPH    | 2,2-diphenyl-1-picrylhydrazyl Activity |
| ABTS    | 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid Activity |
| FRAP    | Ferric Reducing Antioxidant Power |

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