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COMPARATIVE ANALYSIS OF BIOACTIVE COMPOUNDS AND AMINO ACID PROFILES IN WHEAT AND WHEATGRASS (*TRITICUM AESTIVUM*)

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Abstract

This research offers an in-depth comparative examination of the amino acid profiles and bioactive substances in two different varieties of mature wheat grains (*Triticum aestivum*) and the young shoots of wheatgrass BARI gom 33. The amino acid profiles were analyzed using high-performance liquid chromatography (HPLC) after performing acid hydrolysis. Standardized methods were utilized to assess the bioactive compounds present. The results revealed significant variations in the amounts of both essential and non-essential amino acids, as well as bioactive compounds, between wheat and wheatgrass. Notably, wheatgrass exhibited higher concentrations of essential and non-essential amino acids, including histidine, lysine, phenylalanine, isoleucine, methionine, valine, threonine, serine, alanine, and tyrosine. This indicates that wheatgrass may have potential as a nutrient-dense dietary supplement. Wheatgrass exhibited significantly elevated levels of total phenols at 235 mg GAE/100g, total flavonoids at 166.3 mg QE/100g, and DPPH inhibition activity (70 %) compared with the mature wheat grain. Nonetheless, this research offers important information regarding the nutritional makeup of wheatgrass and mature wheat, emphasizing their unique amino acid and bioactive compound profiles. These results carry considerable implications for the possible health advantages related to wheatgrass consumption.

Keywords: wheat, wheatgrass, amino acid, HPLC, bioactive compounds

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Introduction

From the beginning of agriculture, wheat (*Triticum aestivum Linn.*) has been one of the most used grain-grass crops in the world. Taking into account that wheat is a staple crop, it is crucial to think about using the entire plant for nutrition by establishing new objectives in the hunt for variety in the nutritional value of the grain as well as the young wheat shoots or wheat-grass. Furthermore, a great deal of variation in the micronutrient and protein concentration of wheat grains has been observed in a number of studies, whereas there is little information available regarding the variability of the micronutrient and protein content of wheatgrass. From the perspective of the customer, the bioavailability of micronutrients is a crucial component of wheatgrass-based food supplements, in addition to their overall content. Wheatgrass (*Triticum aestivum* L.) refers to the young grass of the common wheat plant which belongs to the family Poaceae. It is known as the powerhouse of nutrients and vitamins. The bioactive components found in herbs that are natural or organic are now regarded as alternative medicines (Tian *et al*., 2022). Wheatgrass is gaining popularity as a functional food and as a study issue, and it is one of the herbal and natural active substances that is getting scientific recognition. Previous researchers reported that he nutritious value of two ounces of wheatgrass juice is equivalent to five pounds of the best unprocessed natural veggies. For example, wheatgrass contains twice as much Vitamin A as carrots and more Vitamin C than oranges. Along with all of the B complex vitamins, it also includes potassium, sodium, calcium, phosphorus, and magnesium in the proper amounts. Almost all of the essential amino acids are found in wheatgrass, making it a complete protein source. About 20 % of its total calories come from protein. The body uses these polypeptides, which are shorter, simpler chains of amino acids, more effectively as components of tissues and the circulatory system (Akram & Aftab, 2015; Ghani *et al*., 2015; Sofi *et al*., 2016; Amar *et al*., 2016 and Amanullah & Muhammad, 2015).

Amino acid content varies in wheat and wheatgrass. Some important amino acids, such as methionine, lysine, and threonine, are less prevalent in wheat grain. However, wheatgrass is regarded as a powerful source of chlorophylls and amino acids, including serine, arginine, aspartic acid, and glutamic acid. Wheatgrass contains seventeen distinct types of amino acids, eight of which are essential (Devi *et al.*, 2020 and Eissa *et al.*, 2020). A wide range of bioactive substances are present in wheat, which may enhance its antioxidant potential. These bioactive substances include carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acids, phytosterols, and flavonoids (Tian *et al*., 2022).

Additionally, wheatgrass possesses potent activity against many diseases due to bioactive compounds like vitamins, minerals, antioxidants, bioflavonoids, etc. The effectiveness of wheatgrass is duly attributed to the existence of these bioactive and

nutritionally active ingredients. It has anti-fungal, anti-bacterial, anti-ulcer, anti-diabetic, anti-inflammatory, anti-arthritic, antioxidant, anti-leukemic, anti-hypertensive, and anti-microbial properties. Wheatgrass helps with wound healing, digestion, and general detoxification of the body (Al-Awaida *et al.,* 2020; Banerjee *et al*., 2021; Dasari *et al*., 2021; Sahoo *et al.,* 2019). It also acts as an adjuvant therapy in hemolytic anemia and helps blood-building activity in thalassemia. It is suggested to include wheatgrass in the daily diet (powder, tablet, juice, bread, cake, biscuit or cookies and papad) (Devi *et al.,* 2020) as it contains chlorophyll, which is almost similar to haemoglobin and strengthens the body's immune system (Minocha *et al*., 2022).

However, in comparison with wheat, wheatgrass appears to be a very prospective herbal medication, and further investigation is required to examine its potential for medicinal use in an assortment of illnesses. Increasing the shelf life of wheatgrass products can extend their lifetime. By creating unique herbal preparations, it is possible to optimize the potential of wheatgrass by incorporating herbs with therapeutic qualities. Therefore, the present study set out to determine and compare the essential and non-essential amino acid profiles, bioactive compounds and antioxidant activity in both wheat and wheatgrass to understand their nutritional content.

Materials and methods

Procurement of Raw Material

To maintain consistency and eliminate any potential differences in variety, a singular batch of "BARI gom 33" wheat was procured from the Bangladesh Wheat & Maize Research Institute (BWMRI) located in Dinajpur.

Cultivation of Wheatgrass

The land for cultivation was meticulously prepared. Land is allowed to rest for 2-3 days before sowing the seeds. Before planting the seeds were washed and rinsed with regular tap water. They were rinsed once more before gently distribution them across the prepared land. A light covering of dry soil is then applied by hand, followed by a gentle sprinkle of water. The sprout was monitored and provided additional watering, if necessary, after 4-5 days to maintain optimal moisture level.

Harvesting of Wheatgrass

They were prepared to harvest after 9 to 10 days when the wheatgrass was probably between 16 and 26 cm tall. We cut the wheatgrass with a pair of scissors just above the soil line. Wheatgrass loses output with each harvest even if it will re-shoot two or three times. They underwent three stages of cultivation and harvest.

Drying of Wheatgrass

After harvesting the wheatgrass, it was thoroughly rinsed with clean tap water and spread out on a white towel on a tabletop to air dry under a ceiling fan. The samples were subsequently dried in a hot air oven at a controlled temperature of 60 ± 5 °C. Once fully dried, the samples were carefully ground using a blender and sifted through a sieve with a mesh size of 1 mm (sieve no. 18). The resulting powder was then stored in high-density polyethylene bags at room temperature, approximately 25 ± 5 °C, in preparation for future studies (Figure 1).

Figure 1. Different stages of wheat seed to wheatgrass powder preparation

Chemicals and Reference Solutions

The subsequent analytical grade reagents were obtained for various experiments, including Gallic acid, Folin-Ciocalteau reagent, Sodium Carbonate, Aluminum chloride, Sodium hydroxide, DPPH, Phenol, Methanol, Ethanol, Sodium nitrite, Hydrochloric Acid, Nitric acid, Potassium, Sodium, Magnesium, Phosphorus, Iron, Zinc, and Calcium standard solution (1000 mg/L) were sourced from Merck (Darmstadt, Germany).

Preparation of Wheatgrass Powder Extracts

In a 250 mL conical flask, 1 g of sample (wheatgrass and wheat grain powder) (Triticum aestivum L.) was combined with 100 mL of methanol and subjected to agitation on an orbital shaker (RIS -24 Plus, Remi, India) for a duration of 48 hours at a controlled temperature of 37 °C. Following this extraction period, the resulting

supernatant was filtered using Whatman filter paper no. 1 to remove any solid residues. The filtered extract was then stored in an airtight container to ensure its stability and prevent degradation prior to subsequent analyses.

Method for Analyzing Amino Acid Profile by HPLC

The amino acid (AA) composition of wheatgrass powder was examined with a Shimadzu LC-30 AD HPLC system, equipped with a fluorescence detector and a C18 column, following established protocols (Kaur *et al.*, 2021). In conclusion, 100 mg of wheatgrass powder underwent digestion with 6N HCl for 24 hours at 110 °C in an anaerobic setting. After digestion, the amino acids were derivatized with mercaptopropionic acid, o-phthalaldehyde, and 9-fluorenyl methyl chloroformate. A gradient mobile phase was employed, consisting of (I) 20 mM phosphate buffer and (II) a blend of water, acetonitrile, and methanol in a 15:45:40 (v/v/v) proportion. The pump was calibrated to a flow rate of 1 mL/min, while the oven temperature was kept steady at 40 °C. The gradient elution settings were defined as follows: from 0 to 2 minutes, the mixture consisted of 88% I and 12% II; from 2 to 4 minutes, it changed to 83% I and 17% II; from 5 to 8.5 minutes, it adjusted to 69% I and 31% II; from 8.5 to 14 minutes, it was comprised of 67.5% I and 32.5% II; from 14 to 16 minutes, the ratio shifted to 53.5% I and 46.5% II; from 16 to 19 minutes, it became 45% I and 55% II; and, finally, from 19 to 25 minutes, the composition was 0% I and 100% II. The peaks were identified at 254 nm and examined using LAB Solutions 5.54SP 5 software, with amino acid levels measured in mg per 100 g, as previously outlined (Mefleh *et al*., 2022).

Determination of Mineral Composition

The analysis of mineral content in powdered samples, including potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), and sodium (Na), was conducted using established methodologies. Approximately 0.5 g of each sample, along with a blank control, was placed into digestion tubes. Each tube received 5 mL of 68% nitric acid, which was mixed thoroughly and allowed to stand overnight. The tubes were then placed in a digester, covered with a depleted manifold to seal the openings. Once boiling began, the samples were digested at a temperature of 125°C for a duration of 4 hours. After cooling, the digestion mixture was transferred to a volumetric flask and diluted to a total volume of 100 mL. The resulting solutions were filtered through a dry filter and stored in sealed containers for subsequent analysis. For the subsequent analysis, 20 mL of the filtered solution was pipetted into a 100 mL volumetric flask, which was then filled to the mark with distilled water and mixed thoroughly. Next, 20 mL of this diluted solution was transferred to a 50 mL volumetric flask, and 5 mL of an $AlCl_3$ solution was added. This mixture was also brought to volume with water and mixed well. The

concentrations of calcium, magnesium, iron, phosphorus, and zinc were measured using an Atomic Absorption Spectrometer (AAS) (AA-7000, Shimadzu, Japan), which was calibrated with a standard solution prior to the analysis of the digested samples. Potassium levels were determined using a flame photometer (PFP7, Jenway Ltd., UK), while phosphorus content in the digested samples was assessed using a UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan). The spectrometer was operated at specific wavelengths of 422.7 nm, 248.3 nm, 285.7 nm, 213.9 nm, 890 nm, and 589 nm, with a spectral band pass of 0.7 nm for the determination of Ca, Fe, Mg, Zn, P, and Na, respectively.

Determination of Total Phenolic Content (TPC)

The method described by Bibi *et al*. (2022) was adapted with minor adjustments to evaluate the total phenolic content of the extract samples. Initially, 0.5 mL of the sample was combined with the Folin-Ciocalteu reagent. Following this, 1 mL of saturated sodium carbonate and 8 mL of distilled water were added, and the mixture was thoroughly vortexed. The samples were then protected from direct sunlight and allowed to incubate at room temperature for thirty-five minutes. After incubation, the tubes were centrifuged for 10 minutes at 4000 g. The transmittance of the resulting supernatant was measured using a UV-1800 UV/VIS spectrophotometer (Shimadzu, Japan) at a wavelength of 725 nm.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using a modified colorimetric method based on the approach outlined by Lin *et al*. (2011). First, 1 mL of the sample was combined with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite. This mixture was allowed to stand for 5 minutes. Next, 0.3 mL of 10% aluminum chloride was incorporated, and the solution was left to rest for an additional minute. After centrifuging the mixture at 4000 g for 5 minutes, 2 mL of 1 M sodium hydroxide and 2.4 mL of distilled water were added and kept in the dark at room temperature for 15 minutes. The absorbance of the resulting solution was then measured at 510 nm using a UV/VIS spectrophotometer (UV-1800, Shimadzu, Japan), with a blank control prepared by substituting the sample mixture with methanol. The results were reported as milligrams of quercetin equivalent per gram of dry sample (mg QE/g).

Determination of DPPH Activity

The antioxidant properties of the samples were evaluated using a free radical scavenging assay that utilized DPPH as the source of free radicals (Chen *et al*., 2016). DPPH easily engages with different antioxidants, enabling them to counteract the radicals. The degree of DPPH reduction was assessed by quantifying the reduction in absorbance at a designated wavelength during a constant reaction period

of 30 minutes. A total of 1.9 mL of DPPH solution was added to a cuvette, and the absorbance was measured at 515 nm with a UV/VIS spectrophotometer (UV-1800, Shimadzu, Japan). The scavenging capacity was determined with this formula: DPPH scavenging capacity (%) = (A control – A sample) / A control × 100, where A indicates the absorbance recorded at 515 nm.

Analysis of Statistics

All analytical measurements were carried out in triplicate. The average values and standard deviations were computed using Microsoft Excel. The results are presented as mean ± standard deviation based on the three trials. For the statistical analysis, paired sample t-tests were conducted using SPSS for Windows (Version 22.0) to evaluate the means, with a significance level set at $P \leq 0.05$.

Results and discussion

Amino Acid (AA) Profile by HPLC

Table 1 presents the amino acid composition of wheatgrass and wheat grain derived from the BARI gom 33 variety. The analysis, conducted using HPLC, identified a total of fifteen amino acids. Among these, eight are categorized as essential amino acids: isoleucine, histidine, lysine, leucine, phenylalanine, methionine, threonine, and valine. The remaining seven amino acids are classified as non-essential, which include aspartic acid, serine, glutamic acid, alanine, cysteine, tyrosine, and proline. Notably, the amino acid profile of wheatgrass juice powder shows a significant similarity to that of wheat sprouts, as indicated in previous studies (Benincasa *et al.*, 2019). Table 1 also presented that among all amino acids that are present in both wheat & Wheatgrass, Glutamic acid was present in the highest amount in wheat and its content was 6.059 µg/100g. This acid influences metabolic processes in the brain and participates in a large number of metabolic reactions. A source of glucose, glutamic acid maintains a normal blood glucose level. It keeps the blood and tissues' acid-base balance in check (Bollenbecke**r** *et al*., 2022).

Wheatgrass is noted for its elevated concentrations of various amino acids, both essential and non-essential, particularly when compared to wheat grain. In addition to glutamic acid, it contains significant amounts of aspartic acid, threonine, serine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and proline. The specific content of essential amino acids in wheatgrass includes 0.789 µg/100g for threonine, 0.284 µg/100g for methionine, 2.440 µg/100g for leucine, 0.625 µg/100g for isoleucine, 1.833 µg/100g for lysine, 1.120 µg/100g for phenylalanine, 1.110 µg/100g for valine, and 5.330 µg/100g for histidine. These values are reported to be lower than those found in previous research (Thakur & Nanda, 2018). The acquired result also demonstrated that, of all the

essential amino acids, histidine was the most abundant and methionine was the least prevalent in wheatgrass. It's important to note that while wheatgrass may be lower in methionine, it can still be a valuable part of a balanced diet. Methionine is a crucial amino acid, indicating that the human body cannot produce it and must acquire it through food intake. Animal-derived protein sources such as meat, fish, eggs, and dairy are generally richer in methionine than their plant-derived counterparts. Furthermore, wheatgrass exhibits the largest level of methionine when compared to wheat. However, tryptophan (35.32 mg/100g) and histidine (36.44 mg/100g) were discovered to be the smallest and maximum amounts of essential amino acids, respectively, by Thakur *et al.* (2022), with leucine (120.39 mg/100g) being the highest. The essential amino acids leucine, lysine, and histidine were highly concentrated in wheatgrass which closely resembles the research findings of Thakur *et al*. (2022). Valine, phenylalanine, and histidine were found in wheat grains, though. Conversely, wheatgrass had greater concentrations of all the essential amino acids than wheat grain.

Table 1. Concentration of specific amino acid content of wheat and wheat-grass obtained from HPLC analysis.

Amino acid (µg/100)	Wheat	Wheatgrass	t- statistics	*p* (2-tailed)
Asp	0.963±0.003	2.580±0.023	-1.966	0.106
Thr	0.516±0.001	0.789±0.008	5.213	0.003
Ser	0.909±0.002	0.951±0.005	2.661	0.045
Glu	6.059±0.006	2.870±0.009	-3.165	0.025
Ala	0.841±0.001	1.850±0.007	55.957	0.000
Cys	0.939±0.003	2.860±0.009	-1.940	0.110
Val	0.550±0.002	1.110±0.002	6.803	0.001
Met	0.123±0.002	0.284±0.003	6.909	0.001
Ile	0.021±0.001	0.625±0.010	13.291	0.000
Leu	0.363±0.004	2.440±0.014	0.409	0.699
Tyr	0.370±0.003	0.607±0.003	5.925	0.002
Phe	0.754±0.007	1.120±0.007	3.971	0.011
Lys	0.458±0.004	1.833±0.004	4.229	0.008
His	4.503±0.007	5.330±0.007	-88.126	0.000
Pro	0.693±0.003	4.107±0.003	-1.667	0.156

All data are presented as mean ± standard deviation. The paired t-test (p) was conducted to compare the amino acid profiles of the wheat and wheatgrass samples, with a significance level set at $p \leq 0.05$. The amino acids analyzed include Isoleucine (Ile), Histidine (His), Lysine (Lys), Leucine (Leu), Phenylalanine (Phe), Methionine (Met), Threonine (Thr), Valine (Val), Aspartic Acid (Asp), Serine (Ser), Glutamic Acid (Glu), Alanine (Ala), Cysteine (Cys), Tyrosine (Tyr), and Proline (Pro).

Except glutamic acid, Table 1 also showed that wheatgrass had a higher concentration of non-essential amino acids than wheat grain. In wheatgrass, the high-content non-essential amino acids were proline (4.107 µg/100g), glutamic acid (2.870 µg/100g), cystine (2.860 µg/100g), aspartic acid (2.580 µg/100g), serine (0.951µg/100g), alanine (1.850 µg/100g), tyrosine (0.607 µg/100g) respectively. The data presented in the table indicates a notable difference in amino acid content between wheat and wheatgrass, with a significance level of $p < 0.05$. Aside from glutamic acid, the findings demonstrate that both essential and non-essential amino acids are found in considerably greater amounts in wheatgrass compared to wheat grain.

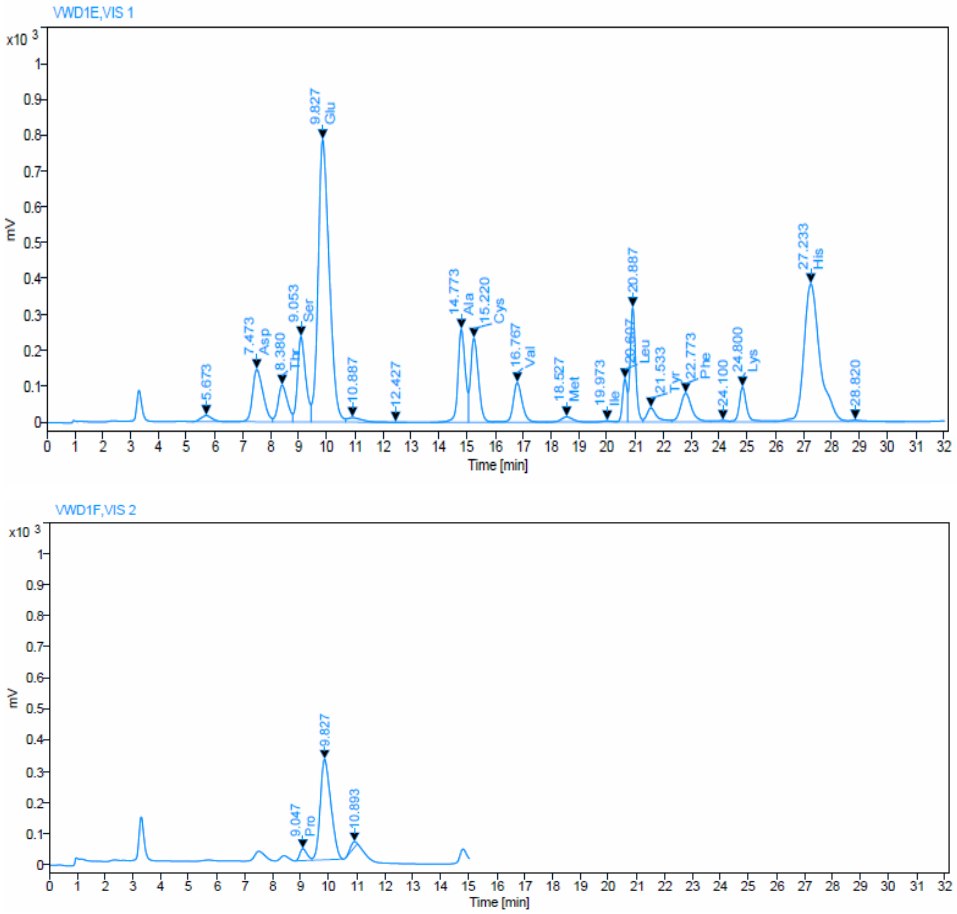

Figure 2. HPLC chromatogram showing the profile of amino acids present in wheat seeds. The identified amino acids include essential amino acids: Isoleucine (Ile), Histidine (His), Lysine (Lys), Leucine (Leu), Phenylalanine (Phe), Methionine (Met), Threonine (Thr), and Valine (Val); and non-essential amino acids: Aspartic Acid (Asp), Serine (Ser), Glutamic Acid (Glu), Alanine (Ala), Cysteine (Cys), Tyrosine (Tyr), and Proline (Pro).

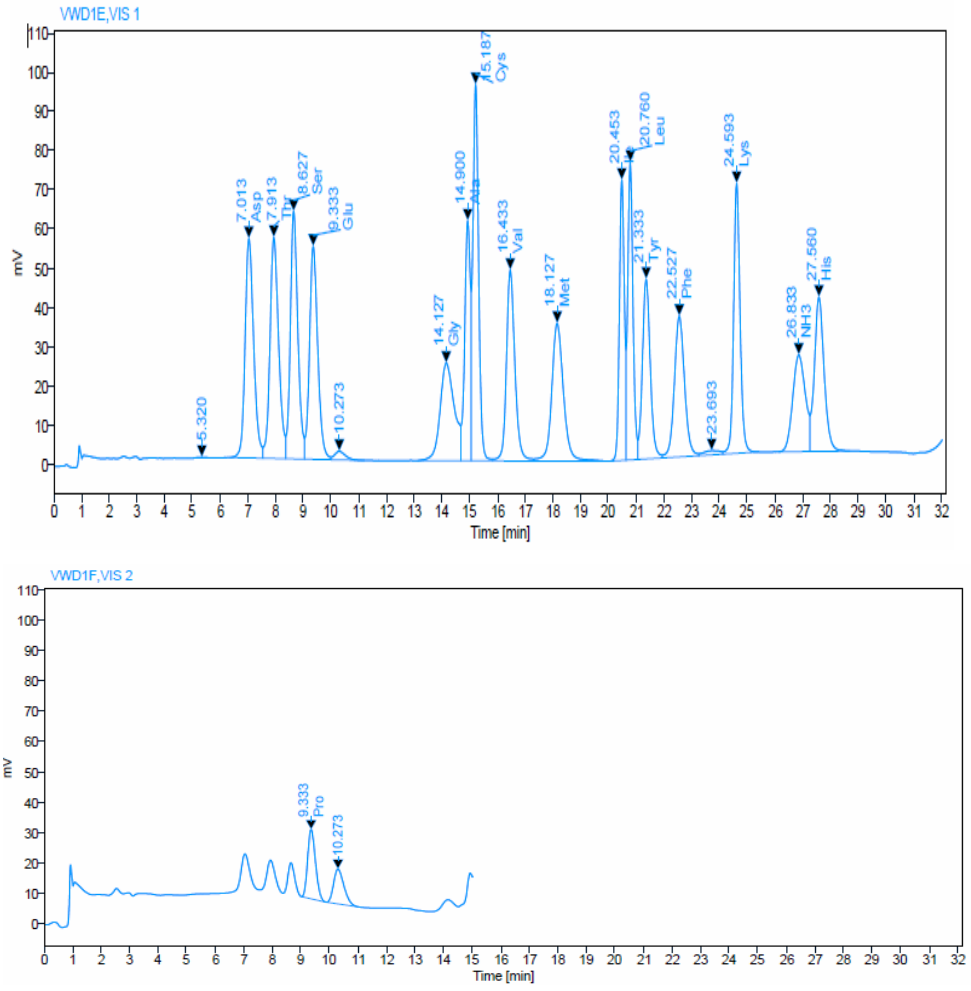

Figure 3. HPLC chromatogram showing the profile of amino acids present in wheatgrass. The identified amino acids include essential amino acids: Isoleucine (Ile), Histidine (His), Lysine (Lys), Leucine (Leu), Phenylalanine (Phe), Methionine (Met), Threonine (Thr), and Valine (Val); and non-essential amino acids: Aspartic Acid (Asp), Serine (Ser), Glutamic Acid (Glu), Alanine (Ala), Cysteine (Cys), Tyrosine (Tyr), and Proline (Pro).

Mineral composition of wheat and wheatgrass

Interactions between minerals can increase or decrease the body's ability to absorb specific micronutrients, affecting how beneficial minerals are in diets. Therefore, knowing about these interactions is helpful when choosing ingredients that may contribute to meeting particular dietary requirements for enhancing micronutrient status (Gemede, 2020). One of the staple crops that is grown most widely in the world is wheat. Wheat grains are highly vital for utilizing their nutrients in a variety

of food items, such as bread, pasta, and other bakery products, since they may be processed into semolina, flours, and other products. In addition to its high levels of energy and carbohydrates, wheat provides noteworthy quantities of other components that are essential or beneficial for overall health, including protein, vitamins (particularly B vitamins), dietary fiber, and micronutrients. Moreover, wheat grain contains certain elements (Ca, Zn, Fe, Mg, and P) essential to our biological processes (Kiran *et al.,* 2021). The mineral contents of the wheat and wheatgrass displayed in Table 2. Wheatgrass had higher Ca, Fe, Zn, Mg, Na, P and K concentrations than wheat. Sowjanya et al. (2015); Tullo et al. (2022); Tullo & Abera (2023) and Akbas *et al*. (2017) found 24 29 mg/100 g of calcium, 0.61 mg/100g of Fe, 363 mg/100 g of K and 0.32 mg/100g of Zn contents in the wheatgrass, respectively. Nonetheless, the discrepancies in the findings of the current study may stem from differences in the rice bran types, the extraction techniques used in each research, or the solvents applied. Calcium (Ca) is the primary driver of essential functions. It aids in treating conditions such as bleeding, bloating of the body, sluggish movements, chilliness, and varicose veins. Iron (Fe) is a vital component of existence. A deficiency of iron results in inadequate hemoglobin levels in the bloodstream. It supports pregnancy, addresses excessive sweating, pale complexion, tiredness and lethargy, along with insomnia. Inorganic iron often leads to constipation, whereas the iron present in wheatgrass does not cause any adverse effects (Mujoriya & Bodla, 2011).

Table 2. Mineral composition of wheat and wheatgrass

Minerals (mg/100g)	Wheat	Wheatgrass	t- statistics	*p* (2-tailed)
Magnesium (Mg)	22±0.5	26±0.4	0.893	0.600
Potassium (K)	107±0.1	150±1	0.271	0.114
Phosphorous (P)	34.6±1	75.5±0.2	-1.000	0.110
Sodium (Na)	2.0±0.0	12.3±0.7	-0.124	0.332
Iron (Fe)	1.2±0.0	11.15 ± 2.0	0.531	0.333
Zinc (Zn)	0.7±0.1	0.41±1.2	0.732	0.001
Calcium (Ca)	15±1.5	28.4±1.6	0.412	0.481

All data are expressed as mean ± standard deviation. The paired t-test (p) was used to compare the mineral composition of the wheat and wheatgrass samples, with a significance level set at $p \leq 0.05$.

Phytochemical Composition of Wheat and Wheatgrass

Naturally occurring antioxidants, and phenolic chemicals enhance the oxidative stability of food and are very probable due to their positive health effects. Food products originating from plants are abundant in polyphenols, which include antioxidant and nutraceutical qualities. Table 3 shows that wheatgrass has a greater total phenolic content (TPC) than wheat grain, with wheatgrass having a TPC of 234.932 mg GAE/100g compared to wheat grain's 11.418 mg GAE/100g. Furthermore, freeze-dried wheatgrass powder was found to have a lower TPC (6.73 mg GAE/g) in the study by Akbas *et al*. (2017). A higher TPC is generally indicative of an increased presence of natural antioxidants. Lower TPC recorded in wheat grain powder was 0.50 mg GAE/g (Hebbani *et al.,* 2020; Rexhepi & Renata, 2015). The results of this study are consistent with the findings of Suppakul *et al*. (2018), who reported a phenolic content of 10.7±1.0 mg GAE/g in wheat grain powder. Additionally, Karakas *et al*. (2022) indicated that the total phenolic content in wheatgrass was greater than that found in wheat grain, further supporting our findings. Our Observation shows that Wheatgrass generally contains a higher level of total phenolic content compared to wheat grain. Phenolic compounds are more concentrated in the young shoots of plants like wheatgrass. These compounds contribute to the antioxidant properties of the plant. Notably, the phenolic profiles of grain and grass derived from different growth stages of wheat exhibited significant variation. The biosynthesis and accumulation of secondary metabolites, including phenolic compounds, are influenced by a range of factors, such as genotype, year of cultivation, geographical location, environmental conditions, soil structure (whether organic or conventional), as well as post-harvest practices including drying, storage, milling methods, and the type of solvent and extraction techniques utilized (Karakas *et al.,* 2022; Thamkaew *et al.,* 2021; Tekin *et al.,* 2018).

Flavonoids are one of the primary categories of phytochemicals recognized for their capacity to neutralize free radicals and act as antioxidants (Saeed *et al.,* 2022). Furthermore, a number of studies have shown that the wheatgrass is a substantial natural source of phytochemicals (Halim *et al.,* 2024). Wheatgrass powder is categorized as a functional food or nutraceutical because it is rich in nutrients. Nutraceuticals are food-based products that provide health advantages, whereas functional foods offer extra benefits beyond fundamental nutrition. Wheatgrass belongs to both categories because of its multiple health benefits and medicinal applications (Rodriguez *et al.,* 2022). Total flavonoid contents of wheat grain and wheatgrass were 125.902 and 166.29 mg QE/100g, respectively (Table 3). According to the results, wheatgrass has a higher TFC content than wheat grain. It ranges from 4.38 to 10.10 mg QE/100g, which is likewise greater than the results of Thakur *et al*. (2022).

DPPH assay is commonly employed to assess the ability of antioxidants to neutralize free radicals. Sharma *et al.* (2020) indicate that the DPPH radical's capacity to neutralize free radicals arises from its tendency to lose color when antioxidants are present. The DPPH radical scavenging activity assay was conducted on both wheat and wheatgrass powder. The findings are presented in Table 3. Wheatgrass powder demonstrated a notably greater % inhibition of DPPH scavenging activity compared to wheat grain. Reports indicate that a significant relationship exists between total phenols and antioxidant activity across numerous plant species (Yoon *et al*., 2024). The present research likewise demonstrates that elevated polyphenol activity enhances scavenging activity. Increased TPC will lead to enhanced DPPH scavenging activity, as multiple studies indicate that this is probably due to the effects of phenolic compounds at varying concentrations and their strong hydrogen atom-donating capabilities. Kaushal (2017) also noted that DPPH scavenging activity increased with time as TFC rose, reaching a maximum of 9 days of growth. Again, for wheat grain extracts % inhibition was lower than wheatgrass extracts because as the plant matures into wheat grain, it redirects its energy towards seed production, resulting in a different nutrient composition. This leads to a lower concentration of certain antioxidants compared to wheatgrass. Zendehbad *et al.* (2014) reported that wheatgrass had a significant concentration of antioxidant properties. Consequently, the results demonstrated wheatgrass's extremely strong radical scavenging ability.

Table 3. Phytochemical composition of wheat and wheatgrass

Bioactive components and Anti-oxidant activity	Wheat	Wheatgrass	t- statistics	*p* (2-tailed)
TPC (mg GAE/100g)	11.418±0.75	234.932±7.83	-2.443	0.058
TFC (mg QE/100g)	125.902±5.03	166.291±6.13	-15.991	0.000
DPPH (% inhibition)	26.710±2.13	69.662±4.23	-4.935	0.004

All data are reported as mean ± standard deviation. The paired t-test (p) was utilized to compare the bioactive compounds and antioxidant activity of the wheat and wheatgrass samples, with a significance threshold established at $p \leq 0.05$.

Conclusions

The study aimed to provide a comprehensive comparative analysis of the amino acid composition and bioactive components in both wheat and wheatgrass. Wheat and wheatgrass are vital components of human nutrition, with wheat being a main grain

and wheatgrass gaining popularity as a health supplement. The research found significant differences in the amino acid profiles of wheat and wheatgrass. Wheatgrass demonstrated a higher level of both essential and non-essential amino acids. This suggests that wheatgrass could be a vital source of essential amino acids for individuals with specific dietary needs. Moreover, the study investigated the bioactive components present in wheat and wheatgrass. Regarding phytochemicals, a significant difference was noted in both wheat and wheatgrass. As a result, wheatgrass exhibited a greater concentration of antioxidants, including flavonoids and phenolic compounds. These findings highlight the potential health benefits of consuming wheatgrass, particularly its antioxidant and immune-boosting properties. In conclusion, this comparative research emphasizes the distinctive nutritional features of wheat and wheatgrass. While wheat remains a crucial ingredient in global diets, wheatgrass seems to be a possible source of essential amino acids and bioactive compounds. Adding wheatgrass to dietary plans may offer unique health benefits, particularly for individuals looking to enhance their nutrient intake and boost their immune system. Further research aims to explore the full spectrum of potential health advantages associated with wheatgrass consumption.

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