

Evaluation of harvest timing and genotypic effect on bioactive composition and antioxidant activity of wheatgrass from traditional and modern wheat cultivars

Fatma Pehlivan Karakaş^{1*}

¹Bolu Abant İzzet Baysal University, Faculty of Science and Art, Department of Biology, Bolu, Türkiye

ARTICLE HISTORY

Received: May 20, 2025

Accepted: Oct. 16, 2025

KEYWORDS

Antioxidant activity,
Phytochemicals,
Triticum aestivum,
Triticum monococcum
ssp. *monococcum*,
Wheatgrass.

Abstract: Wheatgrass has gained increasing attention due to its high content of bioactive compounds and associated health benefits. This study aimed to evaluate the effects of harvest timing and wheat variety on plant growth, phytochemical composition—including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll content (TCC), total phenolic content (TPC), and total flavonoid content (TFC)—as well as antioxidant capacity in wheatgrass obtained from modern bread wheat (*Triticum aestivum* L.; cv. Tosunbey) and ancestral einkorn wheat (*Triticum monococcum* ssp. *monococcum*; cv. IZA). Seedlings were harvested at 10 different growth stages (5 to 15 days old). The results revealed that 9-day-old bread wheatgrass exhibited the highest Chl a (9.43 mg/g FW), Chl b (2.69 mg/g FW), and TCC (12.12 mg/g FW). In contrast, 9-day-old einkorn wheatgrass had the highest TPC (55.18 ± 0.29 mg FAE/g DW) and TFC (263.11 ± 1.92 mg QE/g DW). Notably, the strongest antioxidant activity (2.29 mg/L), comparable to ascorbic acid (2.26 mg/L), was observed in 5-day-old einkorn wheatgrass. The peak levels of TCC, TPC, and TFC were consistently detected on day 9 in both species. These findings highlight the critical role of harvest timing and genetic variation in optimizing the nutritional and functional properties of wheatgrass. While 9-day-old seedlings exhibited the highest phytochemical accumulation, the strongest antioxidant activity was observed in 5-day-old einkorn wheatgrass. Overall, einkorn wheatgrass demonstrated a more potent antioxidant system and higher phenolic and flavonoid content, whereas bread wheatgrass excelled in chlorophyll accumulation, emphasizing distinct compositional advantages between the two wheat varieties.

INTRODUCTION

Wheat, a strategic crop that fulfills a major portion of the global population's nutritional requirements, holds significant importance as a staple food. Given its essential role among the limited crop species extensively cultivated for staple food, the nutritional profile of wheat is particularly critical. In developed nations, a focus on health-conscious eating has increased demand for organic and traditional food products. Diploid *Triticum monococcum* ssp. *monococcum* (einkorn) (2n = 2x = 14, AA), one of the earliest cultivated hulled wheat species,

*Corresponding Author: Fatma PEHLİVAN KARAKAŞ ✉ fatmapehlivan karakas@gmail.com 📧 Bolu Abant İzzet Baysal University, Faculty of Science and Art, Department of Biology, Bolu, Türkiye

The copyright of the published article belongs to its author under CC BY 4.0 license. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>

e-ISSN: 2148-6905

represents an ancestral crop of considerable interest (Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). Genetic studies suggest that wild einkorn wheat was first domesticated in the Karacadağ region of Southeastern Anatolia (Özberk *et al.*, 2016). Currently, einkorn is primarily grown organically on mountainous terrains across Türkiye, Morocco, the Caucasus, Egypt, and various European countries (Watanabe, 2017). Compared to modern wheat varieties, hulled wheat species, including einkorn, are richer in essential minerals and vitamins (Watanabe, 2017; Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). However, the high yields and simplified harvesting processes of modern wheat have led to a reduction in ancestral einkorn cultivation. Nevertheless, einkorn cultivation has attracted renewed interest among farmers in developing countries, largely due to its high nutritional value and compatibility with organic farming practices (Watanabe, 2017; Pehlivan Karakas *et al.*, 2022). Bread wheat (*T. aestivum*, $2n = 6x = 42$, AABBDD), a hexaploid species, is extensively grown worldwide for producing wheatgrass (Özberk *et al.*, 2016; Skoczylas *et al.*, 2018; Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). The 5-15-day-old germinated sprouts of wheat are called “wheatgrass” (Akbas *et al.*, 2017; Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). Recently, especially in developed countries, the adoption of a healthy lifestyle and the development of natural plant-based eating habits, and the consumption of fresh grass juice have increased. Wheatgrass juice is one of the most widely consumed grass juices due to its remarkable nutritional value, powerful health benefits, and low acid content (Banerjee *et al.*, 2021; Garg *et al.*, 2021; Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022).

Chlorophyll is a vital pigment known for its strong antioxidant properties, which give plants their green color (Skoczylas *et al.*, 2018). Wheatgrass is often referred to as “green blood” due to its high chlorophyll content, comprising approximately 70% of its total chemical composition. The molecular structure of chlorophyll closely resembles that of hemoglobin, which is responsible for the red color of blood cells (Kumar *et al.*, 2016; Eissa *et al.*, 2020). Additionally, wheatgrass is recognized as one of nature’s most potent natural therapeutic agents (Kumar *et al.*, 2016; Pehlivan Karakas *et al.*, 2022). This designation is largely attributed to its reported properties in blood purification, liver detoxification, and colon cleansing within the human body (Kumar *et al.*, 2016; Eissa *et al.*, 2020). Wheatgrass is commonly available and consumed in various forms, including tablets, powder, capsules, and fresh juice (Banerjee *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). The endless health advantages of wheatgrass are based on its high contents of chlorophyll, vitamins (A, B, C, E, and K), minerals [iron (Fe), calcium (Ca), zinc (Zn), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), molybdenum (Mo), sulfur (S), aluminum (Al), copper (Cu), manganese (Mn), boron (B), selenium (Se), and chlorine (Cl)], phenolic molecules (rutin, gallic, ferulic, sinapic, *p*-coumaric, vanillic, caffeic, syringic, chlorogenic, *p*-hydroxybenzoic, and salicylic acids), bioflavonoids (apigenin, quercetin, and luteolin), active enzymes (amylase, protease, lipase, catalase, super oxide dismutase, cytochrome oxidase, and transhydrogenase) and amino acids (alanin, arginine, asparagine, aspartic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine) (Chauhan, 2014; Benincasa *et al.*, 2015; Kumar *et al.*, 2016; Shamloo *et al.*, 2017; Eissa *et al.*, 2020; Ahmed *et al.*, 2021; Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022).

Wheatgrass contains vitamin E isomers such as tocopherols [alpha-tocopherol (α -TP), beta-tocopherol (β -TP), gamma-tocopherol (γ -TP), delta-tocopherol (δ -TP)] and tocotrienols [α -TT, β -TT, gamma-tocotrienol (γ -TT), and delta-tocotrienol (δ -TT)], which have high antioxidant properties, and various B vitamins such as thiamine (B1), riboflavin (B2), niacin (B4), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9), and cyanocobalamin (B12), which plays an important role in many vital metabolic activities (Thakur *et al.*, 2019; Eissa *et al.*, 2020; Choi *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). The high amount of chlorophyll content of wheatgrass regulates body metabolism, increases the alkaline level in the blood, strengthens immunity, and detoxifies the body (Kumar *et al.*, 2016; Ghumman *et al.*, 2017;

Banerjee *et al.*, 2021). The important minerals contained in wheatgrass have functions that facilitate the normal functioning of the brain, heart, bones, and muscles (Kaur *et al.*, 2021). Wheatgrass also contains vital amino acids that repair cells, tissues, and organs and regulate the functioning of systems (Kaur *et al.*, 2021). Antioxidant substances such as vitamins and phenolics contained in wheatgrass prevent oxidative DNA damage, reducing oxidative stress and the resulting negative effects (Benincasa *et al.*, 2020; Pehlivan Karakas *et al.*, 2022). In general, biologically active molecules in wheatgrass contribute significantly to the improvement and prevention of cardiovascular diseases, liver damage, diabetes, cancer, and allergy (Garg *et al.*, 2021; Pehlivan Karakas *et al.*, 2021). These bioactive components make wheatgrass juice a valuable source of antioxidants, contributing to its positive health effects. Studies have demonstrated that wheatgrass juice exhibits antioxidant, antimutagenic, antimicrobial, antiallergenic, nephroprotective, anti-inflammatory, and diuretic properties (Özköse *et al.*, 2016; Avisar *et al.*, 2020; Hebbani *et al.*, 2020; Thakur *et al.*, 2020; Choi *et al.*, 2021; Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). At the same time, since it does not contain gluten, it is also safe for use by consumers with gluten intolerance and allergies (Qamar *et al.*, 2019).

Wheatgrass, obtained from the early vegetative growth of wheat species, has gained attention in recent years not only for its nutritional profile but also for its potential applications in functional food development. While modern bread wheat (*T. aestivum*) dominates global cereal production, interest in ancient genotypes such as einkorn (*T. monococcum* ssp. *monococcum*) is resurging, especially in the context of sustainable agriculture and biodiversity preservation. Einkorn is recognized for its adaptability to marginal environments and its naturally enriched micronutrient and phytochemical composition (Pehlivan Karakas *et al.*, 2021; Pehlivan Karakas *et al.*, 2022).

The bioactive content of wheatgrass, including chlorophylls, phenolics, and flavonoids, is known to vary depending on both the wheat genotype and the developmental stage at which it is harvested. Despite this, comprehensive comparative evaluations of wheatgrass derived from ancient versus modern wheat species under controlled conditions remain limited. Identifying optimal harvest periods for maximizing bioactive compound accumulation may offer practical insights into breeding, crop management, and value-added product development.

This study was conducted to evaluate the phytochemical composition and antioxidant potential of wheatgrass obtained from einkorn and bread wheat cultivars harvested at ten distinct growth stages for the first time. By analyzing the temporal dynamics of key bioactive components, the findings aim to support genotype-based selection and harvest planning strategies for enhanced nutritional quality in wheatgrass production.

Recent research has been guided by the health utilities and phytochemical profile of wheatgrass from different varieties of wheat grown under different environmental conditions (Ghumman *et al.*, 2017; Avisar *et al.*, 2020; Banerjee *et al.*, 2021; Kaur *et al.*, 2021). However, there has been no comprehensive investigation comparing the bioactive properties and antioxidant activities of wheatgrass from different wheat species harvested at various germination stages. This study aims to address this gap by analyzing the chlorophyll content (Chl a, Chl b, TCC), phenolic content (TPC), flavonoid content (TFC), and antioxidant activity (DPPH free radical scavenging activity assay) of einkorn and bread wheatgrass harvested on 10 different days (5, 7, 8, 9, 10, 11, 12, 13, 14, 15-days old wheatgrass shoots). This study aims to evaluate the influence of harvest timing and wheat genotype on the phytochemical composition and antioxidant activity of wheatgrass produced from einkorn and bread wheat. The results will help identify optimal harvest periods for maximizing nutritional value, offering potential benefits for functional food development and sustainable agricultural practices.

MATERIAL and METHODS

Chemicals

Chemicals and consumables items were obtained from several vendors and suppliers. Ethanol (EtOH), sodium hydroxide (NaOH), aluminum chloride (AlCl₃), sodium nitrate (NaNO₃) and sodium carbonate (Na₂CO₃) were purchased from Merck (Merck, Darmstadt, Germany). Ascorbic acid, ferulic acid, quercetin, Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and N,N-Dimethylformamide (DMF) were procured from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Wheatgrass Preparation

The wheat seeds of einkorn (*T. monococcum* ssp. *monococcum*; Cv. IZA) were obtained from Bolu Quality and Feed Industry Corporation, Bolu, Turkey in 2023. Seeds of bread wheat (*T. aestivum*; Cv. Tosunbey) were supplied by the Central Research Institute for Agricultural Research, Ankara, Turkey in 2023. Fresh wheatgrasses of two different wheat species with 10 different germination times were obtained from their seeds under controlled laboratory conditions according to the method described by Pehlivan Karakas *et al.* (2021). Germination was performed under controlled conditions in a plant growth room, maintained at 22 ± 2 °C with a 16/8 h light/dark cycle and 55–65% relative humidity. Seeds were watered with tap water every other day throughout the germination period. The wheatgrass was harvested on the 5th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, and 15th day. Fresh wheatgrasses were packaged and labeled individually, and stored at -80 °C until analyzed.

Morphological Measurements

Shoot length was measured from 1 cm above the soil surface (above the coleoptile base) to the tip of the longest leaf using a ruler. For each wheatgrass cultivar, 15 seedlings were randomly selected per treatment for measurement, and the results were expressed in centimeters (cm). Leaf color was evaluated visually based on the overall greenness and vitality of the seedlings. Observations were made at each sampling point, and color differences among treatments were recorded descriptively. No color chart or instrument was used; the assessment was comparative and observational.

Extraction Procedure of Wheatgrass for Analysis

The 5, 7, 8, 9, 10, 11, 12, 13, 14, and 15-day-old fresh wheatgrasses (Figure 1) of two different wheat species (einkorn and bread wheat) were mashed and powdered with liquid nitrogen in a porcelain mortar. 500 mg of each powdered plant material was transported to a test tube with 10 mL of 80% EtOH. For the extraction process, they were placed in a shaking hot water bath at 37 ± 2 °C for 18 hours. After this process, all the test tubes were sonicated in an ultrasonic bath for 15 min. Then, all the extract samples were filtered separately with a 0.45 µm pore size Whatman syringe screen. The 80% EtOH extracts of wheatgrass harvested from two different species on 10 different germination days (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15th day) were stored at -20 °C for all subsequent analyses (Pehlivan Karakas *et al.*, 2022).

Determination of Chlorophyll Content

The amount of chlorophyll a, b, and total chlorophyll (TCC) was determined by employing a previously described method (Moran, 1982). Briefly, 100 mg of fresh leaves were finely ground separately for each test using a porcelain mortar and pestle with liquid nitrogen. Then, they were homogenized in 1 mL of 100% dimethylformamide (DMF) using a sonicator for 1 min. Later, the tubes were incubated in a refrigerator at 4°C for 15 min. Then, centrifugation was performed for 10 min. at 27.000 × g, and the supernatant was transferred to a new tube. Each sample absorbance was measured by the spectrophotometer (Hitachi U-1900, UV-VIS Spectrophotometer 200V, Japan) at 664.5, 647, and 603 nm. Chlorophyll a and chlorophyll b contents, and consequently TTC, were calculated by employing the following equations, and, results were expressed as mg/g fresh weight (FW).

$$\begin{aligned}\text{Chl a} &= (12.92 \times A_{664.5}) - (2.12 \times A_{647}) - (3.85 \times A_{603}) \\ \text{Chl b} &= (-4.67 \times A_{664.5}) + (26.09 \times A_{647}) - (12.79 \times A_{603}) \\ \text{Total Chl} &= (8.24 \times A_{664.5}) + (23.97 \times A_{647}) - (16.64 \times A_{603}).\end{aligned}$$

Determination of Total Phenolic Contents of Wheatgrass

The total phenolic content of wheatgrass extracts obtained from 10 different harvest times was determined using the Folin-Ciocalteu method, as described by Dewanto *et al.* (2002). Ferulic acid was used as the standard phenolic compound, and a calibration curve was prepared using ferulic acid solutions at concentrations of 12.5, 25, 50, 100, 125, 150, 175, and 200 mg/L ($R^2 = 0.994$). The assay was performed as follows: A volume of 125 μL of each standard solution, sample extract, or blank (80% ethanol) was added to separate tubes. To these, 500 μL of deionized water and 125 μL of Folin-Ciocalteu reagent were sequentially added and mixed thoroughly. After a 6-minute incubation, 125 μL of 10% sodium carbonate (Na_2CO_3) solution and 2125 μL of deionized water were added to the mixture, followed by thorough mixing. The solutions were then incubated in darkness for 90 minutes at $22 \pm 2^\circ\text{C}$. Absorbance was measured at 760 nm using a spectrophotometer against the blank. The total phenolic content in the wheatgrass extracts was calculated as milligrams of ferulic acid equivalents per gram of dry weight (mg FAE/g DW) using the standard calibration curve. All measurements were performed in triplicate to ensure accuracy and reproducibility.

Total Flavonoid Contents of Wheatgrass

The total flavonoid content of wheatgrass extracts, harvested at 10 different time points (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days), was determined using the aluminum chloride (AlCl_3) colorimetric assay, as described by Chang *et al.* (2002). Quercetin was used as the reference flavonoid, and a calibration curve was constructed using quercetin solutions at concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 $\mu\text{g/mL}$ ($R^2 = 0.9964$). The assay procedure was as follows: To 250 μL of each sample extract, standard solution, or blank (80% ethanol), 75 μL of 5% sodium nitrate (NaNO_3) was added in a test tube and mixed by vortexing. After a 5-minute incubation, 150 μL of 10% aluminum chloride (AlCl_3) was added, followed by 500 μL of 1 M sodium hydroxide (NaOH) and 1525 μL of distilled water, with thorough mixing after each addition. The absorbance of the resulting solutions was measured at 510 nm against a blank using a spectrophotometer. The total flavonoid content in the wheatgrass extracts was calculated and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW), based on the standard calibration curve. All analyses were performed in triplicate to ensure reproducibility and reliability.

Antioxidant Activity (DPPH Free Radical Scavenging Capacity) of Wheatgrass

The total antioxidant activity, or free radical scavenging capacity, of wheatgrass extracts harvested at 10 different time points from einkorn (cv. IZA) and bread wheat (cv. Tosunbey) species was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, based on the method of Brand-Williams *et al.* (1995), with minor modifications as described by Pehlivan Karakas *et al.* (2017). Ascorbic acid (vitamin C) was used as a positive control. The antioxidant activity was expressed as the percentage (%) inhibition of DPPH free radical using the following formula:

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Where;

A_{control} : Absorbance of the control solution (containing all reagents except the wheatgrass extract).

A_{sample} : Absorbance of the wheatgrass extract solution.

All measurements were conducted in triplicate to ensure reliability. Additionally, the results were expressed as IC_{50} values (mg/L), representing the concentration of wheatgrass extract required to reduce the absorbance of DPPH by 50%.

Statistical Analysis

The amounts of chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll content (TCC), total phenolic content (TPC), total flavonoid content (TFC), and IC₅₀ values of wheatgrass extracts obtained at 10 different harvest times (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days old seedlings) from two wheat species, *Triticum monococcum* ssp. *monococcum* (cv. IZA) and *Triticum aestivum* (cv. Tosunbey), were analyzed statistically using the Statistical Package for Social Sciences (SPSS) Version 15.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences among mean values was evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, with a significance level set at $P \leq 0.05$. Each assay was performed in triplicate, and results are presented as the mean $\pm$ standard deviation (SD) of three independent measurements. Significant differences between means are indicated by different lowercase letters (e.g., a, b, c, d, etc.).

RESULTS and DISCUSSION

The results of this study provide a comprehensive understanding of the morphological (length of wheatgrass), biochemical (chlorophyll a, chlorophyll b, total chlorophyll content, total phenolic content, and total flavonoid content), and antioxidant (DPPH free radical scavenging activity) properties of wheatgrass derived from *Triticum aestivum* (cv. Tosunbey) and *Triticum monococcum* ssp. *monococcum* (cv. IZA) across 10 different germination stages (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days old seedlings). Consistent with existing literature, these results highlight the critical influence of germination time and wheat species on the nutritional profile of wheatgrass.

Morphological Observations and Growth Parameters of Einkorn and Bread Wheatgrass

The morphological characteristics of wheatgrass differed significantly depending on species and germination day (Figure 1). Bread wheatgrass (cv. Tosunbey) exhibited a slightly darker, glossier green coloration, shorter stature, and thicker texture compared to einkorn wheatgrass (cv. IZA). These observations are consistent with previous studies indicating that morphological traits are influenced by both genetic background and environmental conditions (Sharma *et al.*, 2020).

Wheatgrass length increased linearly with germination duration in both species (Figure 2a). Across all sampling days, einkorn wheatgrass consistently exhibited significantly longer shoots than bread wheatgrass (Figure 2b). This greater shoot length may be attributed to the genetic predisposition of einkorn for more rapid early growth, as reported by Eissa *et al.* (2020), offering important agronomic insights for optimizing harvest time.

In terms of measured values, shoot lengths ranged from 4.27 ± 0.73 cm (day 5) to 19.81 ± 0.87 cm (day 15) in bread wheatgrass, and from 6.36 ± 1.08 cm (day 5) to 25.63 ± 0.96 cm (day 15) in einkorn (Figure 2b). No significant differences in shoot length were detected between days 12 and 13 in either species, or between days 14 and 15 in einkorn (Figure 2b). The most vibrant, bright, and healthy appearance was observed between days 7 and 10 (Figure 1). This is consistent with literature suggesting that wheatgrass harvested during this window exhibits optimal vitality and is therefore recommended for nutritional or therapeutic use (Eissa *et al.*, 2020; Pehlivan Karakas *et al.*, 2021; 2022).



Figure 1. Morphological appearance of wheatgrass from einkorn (*T. monococcum* subsp. *monococcum*, cv. IZA) and bread wheat (*T. aestivum*, cv. Tosunbey) cultivars at 10 different germination stages (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days).

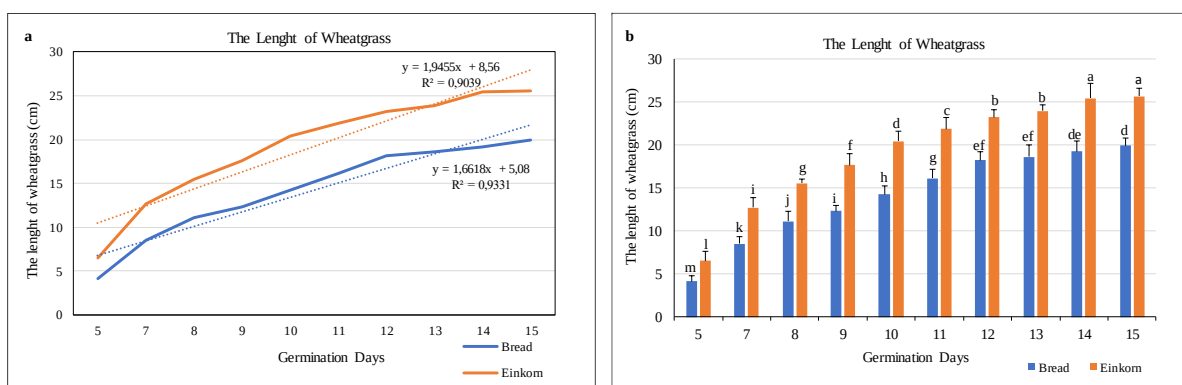


Figure 2. Length of wheatgrass from einkorn (*T. monococcum* subsp. *monococcum*, cv. IZA) and bread wheat (*T. aestivum*, cv. Tosunbey) cultivars measured at 10 different germination stages (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days).

Chlorophyll Content of Einkorn and Bread Wheatgrass

Chlorophyll content is a crucial factor in determining the nutritional quality and health benefits of wheatgrass. The germination period significantly influences chlorophyll synthesis, with specific days showing optimal accumulation. Studies indicate that chlorophyll content tends to peak at certain growth stages. For instance, it has been found that chl-a and chl-b levels are highest during the early stages of germination, typically around days 7 to 10 (Niroula *et al.*, 2019; Kaur *et al.*, 2021). This period corresponds with the plant's active growth phase, where photosynthetic activity is maximized, leading to increased chlorophyll production.

The chlorophyll analysis revealed that the highest concentrations of chl a, chl b, and TCC in wheatgrasses were recorded on the 9th day of germination in both species (Figure 3 and 4). The

chlorophyll content peaked at 9 days of germination for both species, reinforcing the idea that this period is crucial for nutrient accumulation. The chl a content of all tested samples was higher than the chl b content in both species (Figure 3 and 4). Among the species, the highest chl a (9.43 mg/g FW), chl b (2.69 mg/g FW), and TCC (12.12 mg/g FW) were observed in 9-day-old wheatgrass of bread wheat cultivar (Tosunbey) (Figure 3). TCC values ranged from 5.90 to 12.12 mg/g FW in wheatgrass of the bread wheat cultivar (Tosunbey) (Figure 3) and from 4.13 to 8.94 mg/g FW in wheatgrass of einkorn cultivar (IZA) (Figure 4). While the amount of chlorophyll in wheatgrass of both species increases significantly from the 5th to the 9th day, it also decreases significantly from the 9th to the 15th day (Figure 3, and 4). These findings corroborate the work of Niroula *et al.* (2019) and Kaur *et al.* (2021), who noted similar peaks in chlorophyll content during this growth period.

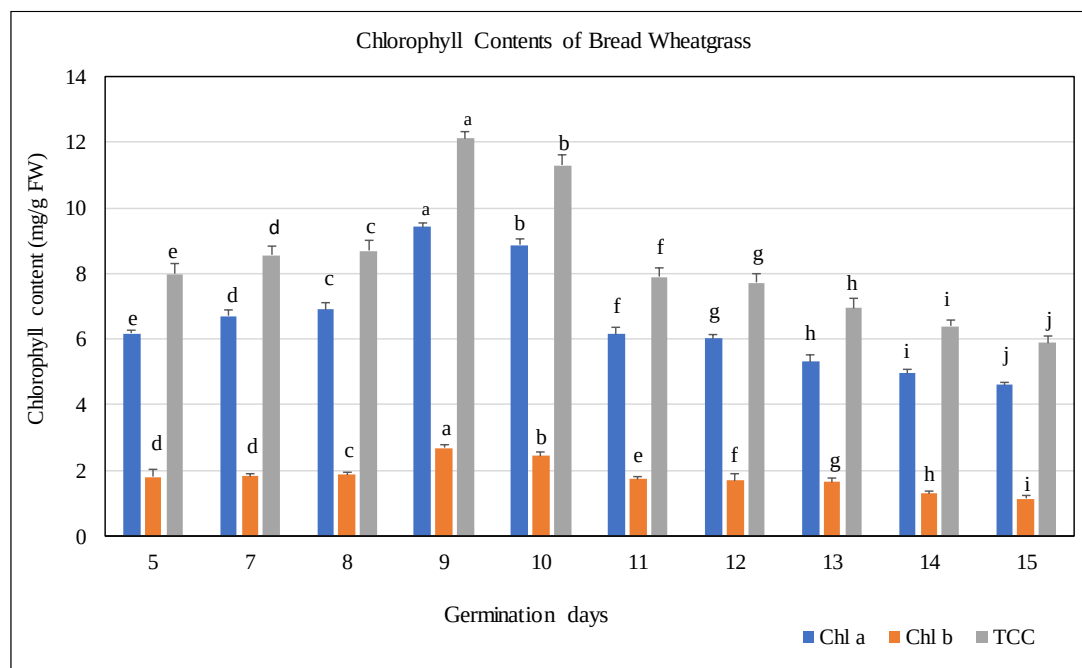


Figure 3. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (TCC) contents of wheatgrass from bread wheat (*T. aestivum*, cv. Tosunbey) harvested at 10 different germination stages (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days).

Similar to our study results, Adhikari *et al.* (2022) showed that the chlorophyll content of wheatgrass harvested at 9 days was significantly higher than that at later stages, such as 14 or 15 days. This aligns with findings from Wangcharoen and Phimphilai (2016), who stated that chlorophyll concentration decreases as the plant matures and shifts resources toward reproduction rather than photosynthesis. The observed increase in chlorophyll a, chlorophyll b, and TCC from day 5 to day 11, followed by a decrease at day 13 and 15, may be attributed to the senescence process or oxidative degradation, as supported by earlier reports that describe chlorophyll breakdown in later growth stages (Zhang *et al.*, 2021).

Some studies have reported that chlorophyll can comprise up to 70% of the total pigment content in wheatgrass (Wangcharoen and Phimphilai, 2016), although this figure likely refers to relative pigment composition rather than absolute values on a fresh weight basis. In the present study, chlorophyll content was quantified in wheatgrass from two wheat species harvested at ten different time points, with values ranging from 0.413% to 1.212% on a fresh weight basis—more precise and physiologically meaningful measurements. Our results are consistent with previous findings by Skoczylas *et al.* (2018), who reported chlorophyll levels in wheatgrass of approximately 0.6%, significantly higher than those found in commonly consumed vegetables such as broccoli (0.008%), kale (0.187%), and spinach (0.125%) (Ferruzzi & Blakeslee, 2007). These comparisons highlight the exceptional chlorophyll content and nutritional potential of wheatgrass.

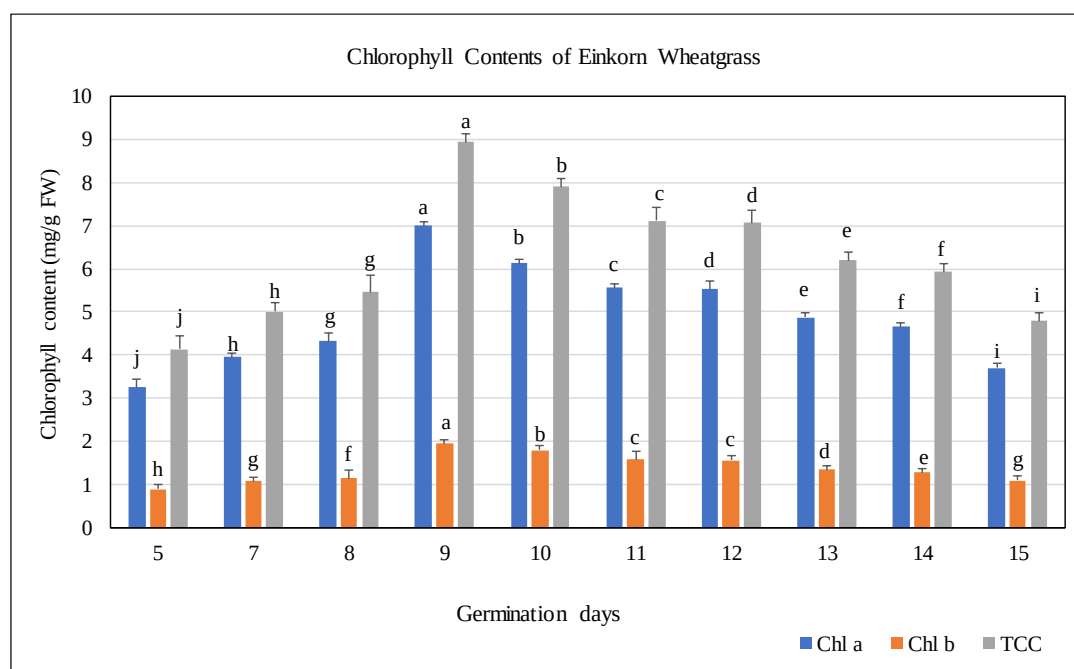


Figure 4. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (TTC) contents of wheatgrass from einkorn (*T. monococcum* subsp. *monococcum*, cv. IZA) harvested at 10 different germination stages (5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 days).

Both bread wheat (cv. Tosunbey) and einkorn wheatgrass (cv. IZA) exhibited their highest chlorophyll concentrations on the 9th day of germination, with bread wheatgrass consistently showing significantly higher levels (Figure 3 and 4). This disparity likely reflects physiological differences between the species and suggests that bread wheatgrass may be more suitable for applications that require high chlorophyll content, such as dietary supplements and health-oriented food products.

In addition to its role as a photosynthetic pigment, chlorophyll contributes to the antioxidant properties of plants by scavenging free radicals and supporting cellular defense mechanisms (Ferruzzi & Blakeslee, 2007). It is also associated with the presence of essential micronutrients, further enhancing the nutritional value of wheatgrass. The high chlorophyll concentration observed in bread wheatgrass, especially at day 9, underscores its potential advantage in functional food applications and supports its classification as a nutrient-dense “superfood.”

Total Phenolic, Flavonoid Contents and Antioxidant Activity of Wheatgrass Samples

Table 1 presents key findings regarding the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (IC_{50} values) of wheatgrass derived from einkorn and bread wheat, assessed across ten germination stages (days 5–15). Statistically significant differences were observed among harvest times and between species, emphasizing the combined influence of genetic background and developmental stage on the bioactive composition of wheatgrass.

Total phenolic content and total flavonoid content

Both wheatgrass species exhibited dynamic changes in TPC and TFC throughout the germination period (Table 1, Figure 5). The highest levels of TPC (55.18 ± 0.29 mg FAE/g DW) and TFC (263.11 ± 1.92 mg QE/g DW) were recorded in einkorn wheatgrass at day 9, followed closely by bread wheatgrass (TPC: 50.67 ± 0.33 mg FAE/g DW; TFC: 254.78 ± 1.92 mg QE/g DW) at the same stage.

For einkorn, mean TPC values ranged from 31.65 ± 0.33 (day 15) to 55.18 ± 0.29 mg FAE/g DW (day 9), while for bread wheat they varied from 17.53 ± 0.33 (day 15) to 50.67 ± 0.33 mg FAE/g DW (day 9). After day 9, a significant decline in TPC was observed in both species (Table 1, Figure 5a). No statistically significant differences were found between the TPC values on days 13 and 14 in einkorn, or on days 5 and 7, and days 12 and 13 in bread wheatgrass.

TFC followed a similar trend. In einkorn, values ranged from 138.67 ± 3.33 (day 5) to 263.11 ± 1.92 mg QE/g DW (day 9); in bread wheatgrass, from 122.56 ± 1.90 (day 15) to 254.78 ± 1.92 mg QE/g DW (day 9). Post day 9, TFC levels declined significantly in both species (Table 1, Figure 5b). Interestingly, on day 7, the TFC levels of einkorn and bread wheatgrass were nearly identical (179.78 vs. 179.22 mg QE/g DW, respectively), showing no significant difference. On other days, however, notable differences were found both within and between species. These patterns indicate that both the germination stage and wheat genotype substantially influence the accumulation of phenolic and flavonoid compounds, aligning with previous findings (Eissa *et al.*, 2020; Pehlivan Karakas *et al.*, 2022).

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and IC₅₀ values of wheatgrass from einkorn (*Triticum monococcum* subsp. *monococcum*, cv. IZA) and bread wheat (*Triticum aestivum*, cv. Tosunbey) cultivars harvested at 10 different growth stages.

Wheats	Harvest time of sprout (days)	TPC	TFC	IC ₅₀ mg/L
		mg FAE/g DW	mg QE/g DW	
Einkorn wheat (cv. IZA)	5	49.29 ± 0.29^c	138.67 ± 0.33^n	2.29 ± 0.005^a
	7	46.35 ± 0.29^d	179.78 ± 1.92^k	5.62 ± 0.005^e
	8	44.00 ± 0.59^e	232.56 ± 5.09^c	6.61 ± 0.005^h
	9	55.18 ± 0.29^a	263.11 ± 1.92^a	3.43 ± 0.011^d
	10	43.08 ± 0.33^f	229.22 ± 1.92^d	5.92 ± 0.010^f
	11	42.24 ± 0.33^g	213.11 ± 1.92^f	10.18 ± 0.005^j
	12	40.80 ± 0.58^h	199.78 ± 3.84^g	10.66 ± 0.011^k
	13	34.92 ± 0.33^k	188.67 ± 1.92^j	10.83 ± 0.010^l
	14	34.07 ± 0.29^k	178.67 ± 1.92^k	11.32 ± 0.005^m
	15	31.65 ± 0.33^l	170.89 ± 1.92^l	32.97 ± 0.010^s
Bread wheat (cv. Tosunbey)	5	38.71 ± 0.29^i	152.56 ± 1.92^m	2.44 ± 0.005^b
	7	38.31 ± 0.29^i	179.22 ± 1.92^k	5.96 ± 0.010^f
	8	37.33 ± 0.33^j	217.56 ± 3.84^e	2.58 ± 0.005^c
	9	50.67 ± 0.33^b	254.78 ± 1.92^b	6.49 ± 0.005^g
	10	30.08 ± 0.58^m	195.89 ± 1.92^h	8.81 ± 0.005^i
	11	27.33 ± 0.33^n	193.11 ± 5.09^i	13.82 ± 0.010^n
	12	24.20 ± 0.29^o	173.11 ± 1.92^l	14.67 ± 0.015^o
	13	24.00 ± 0.29^o	139.78 ± 1.92^n	15.41 ± 0.010^p
	14	18.71 ± 0.33^p	125.33 ± 3.33^o	16.50 ± 0.010^r
	15	17.53 ± 0.33^r	122.56 ± 1.90^p	40.61 ± 0.005^t
Ascorbic acid (positive control)		-	-	2.26 ± 0.005^a

Values are presented as means $\pm$ standard deviation (SD) of three independent measurements. Different lowercase letters indicate statistically significant differences among the mean values ($p \leq 0.005$).

These results are consistent with those of Eissa *et al.* (2020), who found that phenolic and flavonoid levels peak at specific growth stages. During early wheat germination, secondary metabolites such as phenolic acids and flavonoids increase as part of the plant's development and defense mechanisms. Typically, these levels follow a bell-shaped pattern—rising until about days 7–10, then declining. This decrease is especially apparent in tissues like the embryonic axis and roots, which are crucial in early seedling growth (Szablinska-Piernik and Lahuta, 2023). Similar fluctuations have been observed in cereals, reflecting the regulation of plant metabolism during early development. As seedlings mature, the accumulation and function of these compounds shift, affecting both stress response and overall growth (Reshi *et al.*, 2023).

In agreement with our findings, Eissa *et al.* (2020) and Kaur *et al.* (2021) reported that total phenolic (TPC) and flavonoid content (TFC) varied among wheat varieties. Like chlorophyll content, TPC and TFC peaked on the 9th day in both species (Table 1, Figure 5), aligning with Sharma *et al.* (2020), who observed maximum phenolic accumulation between days 9 and 11. This increase likely reflects a defense-related response driven by secondary metabolism (Kaur *et al.*, 2021). The decrease after day 10 may be related to the utilization of these compounds in managing oxidative stress during later growth stages. Einkorn wheatgrass (IZA) contained higher phenolic and flavonoid levels than bread wheatgrass (Tosunbey) (Table 1), supporting the view that ancient wheats possess stronger antioxidant profiles due to their genetic background (Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2021; 2022).

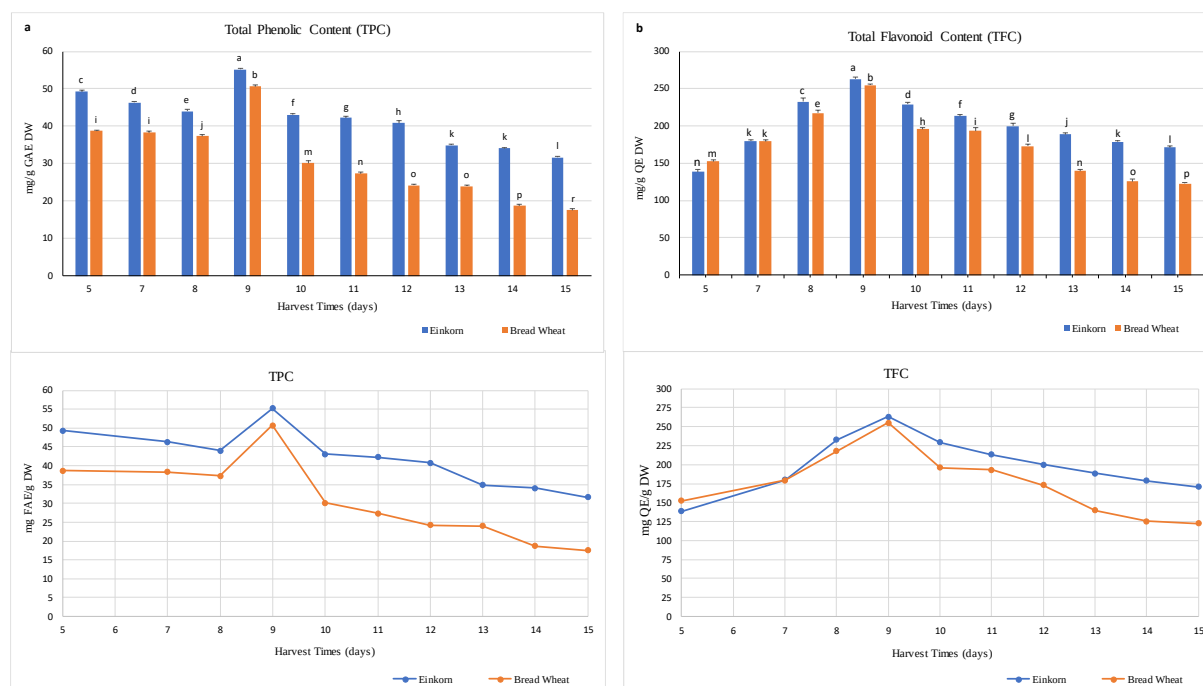


Figure 5. (a) Total phenolic content (TPC), (b) total flavonoid content (TFC) of wheatgrass from einkorn (*Triticum monococcum* subsp. *monococcum*, cv. IZA) and bread wheat (*Triticum aestivum*, cv. Tosunbey) cultivars harvested at 10 different growth stages.

Overall, both TPC and TFC reached their highest values on day 9, with einkorn wheatgrass showing significantly higher concentrations. These results underline the importance of genetic variation in antioxidant capacity and highlight einkorn wheatgrass as a promising source for functional food and nutraceutical use (Eissa *et al.*, 2020; Kaur *et al.*, 2021).

Free radical scavenging activity (DPPH assay)

Free radical scavenging activity, evaluated by IC₅₀ values (where lower values denote stronger antioxidant capacity), varied significantly depending on germination time and wheat species (Table 1). IC₅₀ values ranged from 2.29 mg/L (5th day) to 32.97 mg/L (15th day) for einkorn wheatgrass, and from 2.44 mg/L to 40.61 mg/L for bread wheatgrass. Remarkably, the antioxidant activity of 5-day-old einkorn wheatgrass (2.29 mg/L) was comparable to pure ascorbic acid (2.26 mg/L), highlighting its strong antioxidant potential. Overall, wheatgrass harvested between days 5 and 9 exhibited the highest antioxidant activities, which declined significantly thereafter. These findings are consistent with previous reports indicating early growth stages as optimal for antioxidant potential (Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). It is well known that wheat species, genotype, environmental factors, and growth conditions influence antioxidant activity (Kaur *et al.*, 2021; Pehlivan Karakas *et al.*, 2022). Wheatgrass is a recognized source of antioxidants due to its rich content of vitamins, minerals,

amino acids, phenolics, and flavonoids (Pehlivan Karakas *et al.*, 2021; 2022; Skoczylas *et al.*, 2018).

Phenolic compound levels also varied with the germination stage. The biosynthesis and accumulation of secondary metabolites like phenolics depend on multiple factors, including genotype, growth conditions, drying and storage methods, and extraction techniques (Li *et al.*, 2008; Pehlivan Karakas *et al.*, 2021; Tekin *et al.*, 2018; Verma *et al.*, 2009; Wang *et al.*, 2013; Ward *et al.*, 2008). No clear correlation was observed between TPC, TFC, and antioxidant activity in einkorn (cv. IZA) and bread wheatgrass (cv. Tosunbey). As noted by Pehlivan Karakas and Turker (2013), phenolic and flavonoid contents alone may not fully explain antioxidant activity. Other antioxidant molecules, including vitamins (A, C, E), carotenoids (β -carotene, lycopene, lutein, zeaxanthin), chlorophylls, essential oils, and minerals (selenium, copper, manganese, zinc), also significantly contribute (Fernandes de Oliveira *et al.*, 2012; Pehlivan Karakas & Turker, 2016; Pehlivan Karakas *et al.*, 2021; 2022; Skoczylas *et al.*, 2018). This perspective is supported by Tullo and Abera (2023), who emphasized that the complex interplay among phytochemicals enhances wheatgrass's health benefits. The strong antioxidant capacity observed—especially in 5-day-old einkorn wheatgrass—underscores its functional potential.

This study revealed clear differences between *Triticum monococcum* ssp. *monococcum* (IZA) and *Triticum aestivum* (Tosunbey) wheatgrass in terms of growth characteristics, phytochemical profiles, and antioxidant activities during germination. Measurements of shoot length, TPC, TFC, Chl a, Chl b, TCC, and IC₅₀ at ten germination stages demonstrated the significant effects of species, genotype, and harvest timing on wheatgrass quality. These results support earlier findings that germination enhances chlorophylls, phenolic compounds, and antioxidant activity in cereal sprouts (Falcinelli *et al.*, 2017; Kathuria *et al.*, 2024).

Einkorn (IZA) wheatgrass consistently showed higher TPC and TFC and stronger antioxidant activity than bread wheat (Tosunbey), confirming the idea that ancient wheat species preserve richer phytochemical profiles due to their genetic background (Gawlik-Dziki *et al.*, 2012; Pehlivan Karakas *et al.*, 2022). These findings highlight einkorn wheatgrass as a promising functional food ingredient. Notably, the 9th day of germination was optimal for maximizing bioactive compound accumulation in both cultivars. This pattern aligns with studies suggesting that intermediate germination stages are linked to higher enzymatic activity and secondary metabolite synthesis (Zielińska-Dawidziak, 2021). Given the well-established role of dietary phenolics in mitigating oxidative stress-related diseases (Pandey & Rizvi, 2009), optimizing harvest timing may significantly enhance wheatgrass's nutritional value.

In conclusion, both genetic background and germination timing play a crucial role in determining the nutritional and functional properties of wheatgrass. Further studies should investigate the molecular mechanisms underlying these effects and assess how environmental factors such as light, temperature, and nutrients influence phytochemical profiles.

CONCLUSION

This study highlights that the optimization of wheatgrass as a functional food is strongly influenced by wheat genotype and harvest timing. Among the germination stages evaluated, wheatgrass harvested on day 9 exhibited superior growth performance, higher phenolic and flavonoid contents, and increased chlorophyll accumulation. The strongest antioxidant activity was observed in 5-day-old wheatgrass, particularly in the einkorn (IZA) cultivar, which consistently outperformed bread wheat (Tosunbey) in terms of phytochemical and functional quality. These findings emphasize the superior nutritional and therapeutic potential of einkorn wheatgrass as a rich source of natural antioxidants and bioactive compounds. Its elevated phenolic and flavonoid levels suggest promising applications in preventing oxidative stress-related disorders, including cardiovascular and metabolic diseases. Harvesting at day 9 appears optimal for producing wheatgrass-based products with maximized functional properties.

Overall, this study provides valuable insights for the strategic development of wheatgrass as a functional food and offers a foundation for future breeding efforts to enhance the bioactive compound profile of cereal sprouts for the health food industry.

Acknowledgments

The author would like to thank Alper Karakas Laboratory for laboratory equipment support in the Department of Biology, Bolu Abant İzzet Baysal University, Bolu, Türkiye. The author would like to thank her students, Aylin Akyüz and Sarah Anjam, for their assistance in participating in the preliminary tests of the studies as part of the Independent Study course.

Data Availability Statement

Research data is available and can be sent with e-mail upon request.

Declaration of Conflicting Interests and Ethics

The authors declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Orcid

Fatma Pehlivan Karakaş  <https://orcid.org/0000-001-5245-6294>

REFERENCES

- Adhikari, B., Joshi, S., & Maskey, B. (2022). Effects of harvesting period on phytochemicals of wheatgrass (*Triticum aestivum*, WK 1204 variety). *Caraka Tani: Journal of Sustainable Agriculture*, 37(1), 36–47. <http://dx.doi.org/10.20961/carakatani.v37i1.52076>
- Ahmed, Z., Faisal Manzoor, M., Hussain, A., Hanif, M., & Zia-ud-Din Zeng, X.A. (2021). Study the impact of ultra-sonication and pulsed electric field on the quality of wheat plantlet juice through FTIR and SERS. *Ultrasonics Sonochemistry*, 76, 105648. <https://doi.org/10.1016/j.ultsonch.2021.105648>
- Akbas, E., Kilercioglu, M., Onder, O.N., Koker, A., & Soyler, B., Oztop, M.H. (2017). Wheatgrass juice to wheat grass powder: Encapsulation, physical and chemical characterization. *Journal of Functional Foods*, 28, 19–27. <https://doi.org/10.1016/j.jff.2016.11.010>
- Avisar, A., Cohen, M., Katz, R., Shentzer Kutiel, T., Aharon, A., & Bar-Sela, G. (2020). Wheatgrass juice administration and immune measures during adjuvant chemotherapy in colon cancer patients: Preliminary results. *Pharmaceuticals*, 13(6), 129. <https://doi.org/10.3390/ph13060129>
- Banerjee, S., Katiyar, P., Kumar, V., Waghmode, B., Nathani, S., Krishnan, V., Sircar, D., & Roy, P. (2021). Wheatgrass inhibits the lipopolysaccharide-stimulated inflammatory effect in RAW 264.7 macrophages. *Current Research in Toxicology*, 2, 116-127. <https://doi.org/10.1016/j.crtox.2021.02.005>
- Benincasa, P., Galieni, A., Manetta, A. C., Pace, R., Guiducci, M., Pisante, M., & Stagnari, F. (2015). Phenolic compounds in grains, sprouts and wheatgrass of hulled and nonhulled wheat species. *Journal of the Science of Food and Agriculture*, 95, 1795–1803. <https://doi.org/10.1002/jsfa.6877>
- Benincasa, P., Tosti, G., Farneselli, M., Maranghi, S., Bravi, E., Marconi, O., Falcinelli, B., & Guiducci, M. (2020). Phenolic content and antioxidant activity of einkorn and emmer sprouts and wheatgrass obtained under different radiation wavelengths. *Annals of Agricultural Sciences*, 65, 68–76. <https://doi.org/10.1016/j.aos.2020.02.001>
- Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Chang, C.C., Yang, M.H., Wen, H.M., & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182. <https://doi.org/10.38212/2224-6614.2748>
- Choi, M.H., Lee, M., Yang, S.H., Shin, H.J. & Jeon, Y. (2021). Hydrophobic fractions of *Triticum aestivum* L. extracts contain polyphenols and alleviate inflammation by regulating nuclear factor-kappa B. *Biotechnology and Bioprocess Engineering*, 26, 93–106. <https://doi.org/10.1007/s12257-020-0352-7>
- Chauhan, M. (2014). A pilot study on wheat grass juice for its phytochemical, nutritional and therapeutic potential on chronic diseases. *International Journal of Chemical Studies*, 2(4), 27–34.

- Dewanto, V., Wu, X., Adom, K.K., & Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50, 3010–3014. <https://doi.org/10.1021/jf0115589>
- Eissa, H.A., Mohamed, S.S., & Hussein, A.M.S. (2020). Nutritional value and impact of wheatgrass juice (Green Blood Therapy) on increasing fertility in male albino rats. *Bulletin of the National Research Centre*, 44(1), 30. <https://doi.org/10.1186/s42269-020-0272-x>
- Falcinelli, B., Benincasa, P., Calzuola, I., Gigliarelli, L., Lutts, S., & Marsili, V. (2017). Phenolic content and antioxidant activity in raw and denatured aqueous extracts from sprouts and wheatgrass of einkorn and emmer obtained under salinity. *Molecules*, 22(12), 2132. <https://doi.org/10.3390/molecules22122132>
- Fernandes de Oliveira, A.M., Sousa Pinheiro, L., Souto Pereira, C.K., Neves Matias, W., Albuquerque Gomes, R., Souza Chaves, O., Vanderlei de Souza, M.F., Nobrega de Almeida, R., & Simoes de Assis, T. (2012). Total phenolic content and antioxidant activity of some Malvaceae family species. *Antioxidants (Basel)*, 1, 33–43. <https://doi.org/10.3390/antiox1010033>
- Ferruzzi, M.G., & Blakeslee, J. (2007). Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research*, 27(1), 1-12. <https://doi.org/10.1016/j.nutres.2006.12.003>
- Garg, M., Sharma, A., Vats, S., Tiwari, V., Kumari, A., Mishra, V., & Krishania, M. (2021). Vitamins in cereals: A critical review of content, health effects, processing losses, bioaccessibility, fortification, and biofortification strategies for their improvement. *Frontiers in Nutrition*, 8, 254. <https://doi.org/10.3389/fnut.2021.586815>
- Gawlik-Dziki, U., Świeca, M., & Dziki, D. (2012). Comparison of phenolic acids profile and antioxidant potential of six varieties of spelt (*Triticum spelta* L.). *Journal of Agricultural and Food Chemistry*, 60(18), 4603–4612. <https://doi.org/10.1021/jf3011239>
- Ghumman, A., Singh, N., & Kaur, A. (2017). Chemical, nutritional and phenolic composition of wheatgrass and pulse shoots. *International Journal of Food Science and Technology*, 52(10), 2191–2200. <https://doi.org/10.1111/ijfs.13498>
- Hebbani, A.V., Saradamma, B., Kanu, V.R., Malini, A.B., Reddy, V.D., & Chakravarthula, N.V. (2020). Nephroprotective activity of wheatgrass juice against alcohol-induced oxidative damage in rats. *Toxicology Mechanisms and Methods*, 30(9), 679-685. <https://doi.org/10.1080/15376516.2020.1810837>
- Kathuria, D., Hamid, H., Chavan, H.P., Jaiswal, A.K., Thakur, A., & Dhiman, A.K. (2024). A comprehensive review on sprouted seeds bioactives, the impact of novel processing techniques and health benefits. *Food Reviews International*, 40(1), 370-398. <https://doi.org/10.1080/87559129.2023.2169453>
- Kaur, N., Singh, B., Kaur, A., Yadav, M.P., Singh, N., Ahlawat, A.K., & Singh, A.M. (2021). Effect of growing conditions on proximate, mineral, amino acid, phenolic composition and antioxidant properties of wheatgrass from different wheat (*Triticum aestivum* L.) varieties. *Food Chemistry*, 341, 128201. <https://doi.org/10.1016/j.foodchem.2020.128201>
- Kumar, N.S., Murali, M., Nair, A.M., & Nair, A.S. (2016). Green blood therapy of wheat grass – nature’s finest medicine: A literature review. *Journal of Pharmaceutical and Biological Sciences*, 11, 57–64. <https://doi.org/10.9790/3008-1102045764>
- Li, L., Shewry, P.R., & Ward, J.L. (2008). Phenolic acids in wheat varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, 56, 9732-9739. <https://doi.org/10.1021/jf801069s>
- Moran, R. (1982). Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. *Plant Physiology*, 69, 1376–1381. <https://doi.org/10.1104/pp.69.6.1376>
- Niroula, A., Khatri, S., Timilsina, R., Khadka, D., Khadka, A., & Ojha, P. (2019). Profile of chlorophylls and carotenoids of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) microgreens. *Journal of Food Science and Technology*, 56(5), 2758–2763. <https://doi.org/10.1007/s13197-019-03768-9>
- Özberk, İ., Atay, S., Cabi, E.E., Özkan, H., & Atlı, A.A. (2016). *Türkiye’nin buğday atlası* [Wheat Atlas of Turkey]. İstanbul, WWF-Türkiye.
- Özköse, A., Arslan, D., & Acar, A. (2016). The comparison of the chemical composition, sensory, phenolic and antioxidant properties of juices from different wheatgrass and turfgrass species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 44(2), 499-507. <https://doi.org/10.15835/nbha44210405>

- Qamar, A., Saeed, F., Nadeem, M.T., Hussain, A.I., Khan, M.A., & Niaz, B. (2019). Probing the storage stability and sensorial characteristics of wheat and barley grasses juice. *Food Science and Nutrition*, 7(2), 554–562. <https://doi.org/10.1002/fsn3.841>
- Pandey, K.B., & Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2, 897484. <https://doi.org/10.4161/oxim.2.5.9498>
- Pehlivan Karakas, F., & Turker, A.U. (2013). An efficient in vitro regeneration system for *Bellis perennis* L. and comparison of phenolic contents of field-grown and in vitro-grown leaves by LC-MS/MS. *Industrial Crops and Products*, 48, 162–170. <https://doi.org/10.1016/j.indcrop.2013.04.008>
- Pehlivan Karakas, F., & Turker, A.U. (2016). Improvement of shoot proliferation and comparison of secondary metabolites in shoot and callus cultures of *Phlomis armeniaca* by LC-ESI-MS/MS analysis. *In Vitro Cellular and Developmental Biology – Plant*, 52, 608-618. <https://doi.org/10.1007/s11627-016-9792-3>
- Pehlivan Karakas, F., Keskin, C.N., Agil, F., & Zencirci, N. (2021). Profiles of vitamin B and E in wheat grass and grain of einkorn (*Triticum monococcum* spp. *monococcum*), emmer (*Triticum dicoccum* ssp. *dicoccum* Schrank.), durum (*Triticum durum* Desf.), and bread wheat (*Triticum aestivum* L.) cultivars by LC-ESI-MS/MS analysis. *Journal of Cereal Science*, 98, 103177. <https://doi.org/10.1016/j.jcs.2021.103177>
- Pehlivan Karakas, F., Ucar Turker, A., Karakas, A., Mshvildadze, V., Pichette, A., & Legault, J. (2017). In vitro cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers of common daisy—A medicinal plant. *Journal of Herbal Medicine*, 8, 31-39. <https://doi.org/10.1016/j.hermed.2016.11.003>
- Pehlivan Karakas, F., Keskin, C.N., Agil, F., & Zencirci, N. (2022). Phenolic composition and antioxidant potential in Turkish einkorn, emmer, durum, and bread wheat grain and grass. *South African Journal of Botany*, 149, 407–415. <https://doi.org/10.1016/j.sajb.2022.06.022>
- Reshi, Z.A., Ahmad, W., Lukatkin, A.S., & Javed, S.B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites*, 13(8), 895. <https://doi.org/10.3390/metabo13080895>
- Shamloo, M., Babawale, E.A., Furtado, A., Henry, R.J., Eck, P.K., & Jones, P.J.H. (2017). Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. *Scientific Reports*, 7, 9133. <https://doi.org/10.1038/s41598-017-09681-5>
- Sharma, H., Bhandawat, A., Kumar, P., Rahim, M.S., Parveen, A., Kumar, P., Madhawan, A., Rishi, V., & Roy, J. (2020). Development and characterization of bZIP transcription factor based SSRs in wheat. *Gene*, 756, 144912. <https://doi.org/10.1016/j.gene.2020.144912>
- Skoczylas, Ł., Korus, A., Tabaszewska, M., Gędoś, K., & Szczepańska, E. (2018). Evaluation of the quality of fresh and frozen wheatgrass juices depending on the time of grass harvest. *Journal of Food Processing and Preservation*, 42, e13401. <https://doi.org/10.1111/jfpp.13401>
- Szablińska-Piernik, J. & Lahuta, Ł. B. (2023). Changes in polar metabolites during seed germination and early seedling development of pea, cucumber, and wheat. *Agriculture*, 13(12), 2278. <https://doi.org/10.3390/agriculture13122278>
- Thakur, N., Dhaliwal, H.S., & Sharma, V. (2019). Chemical composition, minerals and vitamins analysis of lyophilized wheatgrass juice powder. *International Journal on Emerging Technologies*, 10(4), 137–144.
- Thakur, N., Dhaliwal, H., & Sharma, V. (2020). Qualitative and quantitative RP-HPLC-PDA method of analysis of polyphenols in lyophilized wheat seedling juice powder. *International Journal on Emerging Technologies*, 11(2), 36–43.
- Tekin, M., Cengiz, M.F., Abbasov, M., Aksoy, A., Canci, H., & Akar, T. (2018). Comparison of some mineral nutrients and vitamins in advanced hulled wheat lines. *Cereal Chemistry*, 95, 436–444. <https://doi.org/10.1002/cche.10045>
- Tullo, A., & Abera, S. (2023). Review on nutrient contents and health benefits of wheatgrass juice. *International Journal of Smart Agriculture*, 1, 32–39. <https://doi.org/10.54536/ijsa.v1i1.2193>
- Verma, B., Hucl, P., & Chibbar, R.N. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, 116, 947-954. <https://doi.org/10.1016/j.foodchem.2009.03.060>

- Wang, L., Yao, Y., He, Z., Wang, D., Liu, A., & Zhang, Y. (2013). Determination of phenolic acid concentrations in wheat flours produced at different extraction rates. *Journal of Cereal Science*, 57, 67–72. <https://doi.org/10.1016/j.jcs.2012.09.013>
- Wangcharoen, W., & Phimphilai, S. (2016). Chlorophyll and total phenolic contents, antioxidant activities and consumer acceptance test of processed grass drinks. *Journal of Food Science and Technology*, 53(12), 4135–4140. <https://doi.org/10.1007/s13197-016-2380-z>
- Ward, J.L., Poutanen, K., Gebruers, K., Piironen, V., Lampi, A.M., Nyström, L., Andersson, A.A.M., Åman, P., Boros, D., Rakszegi, M., Bedő, Z., & Shewry, P.R. (2008). The HEALTHGRAIN cereal diversity screen: Concept, results, and prospects. *Journal of Agricultural and Food Chemistry*, 56, 9699–9709. <https://doi.org/10.1021/jf8009574>
- Watanabe, N. (2017). Breeding opportunities for early, free-threshing and semi-dwarf *Triticum monococcum* L. *Euphytica*, 213, 201. <https://doi.org/10.1007/s10681-017-1987-0>
- Zhang, Y.M., Guo, P., Xia, X., Guo, H., & Li, Z. (2021). Multiple layers of regulation on leaf senescence: New advances and perspectives. *Frontiers in Plant Science*, 12, 788996. <https://doi.org/10.3389/fpls.2021.788996>
- Zielińska-Dawidziak, M. (2021). Influence of stress conditions on the quality of obtained sprouts. *Quality Assurance and Safety of Crops & Foods*, 13(2), 1-12. <https://doi.org/10.15586/qas.v13i2.836>