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Modulation of Bioactive Constituents, Phenolic Composition, Antioxidant Capacity, and Mineral Content in Wheat Sprouts through Gibberellic Acid and Water-Induced Germination

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ABSTRACT

In this study, the effects of gibberellic acid (GA) and water-induced germination on the bioactive compounds, antioxidant activity, phenolic composition, and mineral contents of wheat sprouts were investigated. Total phenolic and flavonoid amounts of the non-germinated and germinated wheat samples were characterized to be between 14.66 (control) and 49.74 mgGAE/100g (GA) to 3.30 (control) and 5.99 mg/100g (GA), respectively. Also, antioxidant capacity values of non-germinated and germinated wheat samples were assessed to be between 2.97 (control) and 6.52 mmolTE/kg (GA). The phenolic acid content of wheat samples germinated with GA application was higher than that of unsprouted and water-treated wheat samples (except for gallic acid and chlorogenic acids in water treatment). P and K amounts of the wheat seeds and sprouts were assessed to be between 37.61 (GA) and 3039.22 mg/kg (Water) to 0.06 (GA) and 2046.34 (Water), respectively. Fe and Zn amounts of the wheat samples were characterized to be between 0.001 (GA) and 0.446 mg/kg (Water) to 0.001 (GA) and 0.258 mg/kg (Water), respectively. The P, Ca, Ca, Mg, Fe, Zn, Cu, Mn, and B contents of wheat samples germinated by treatment with GA were significantly lower than those of control and water-treated wheat samples.

Keywords: wheat seeds, giberellic acid, bioactive properties, phenolics, minerals, HPLC.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops worldwide and serves as a staple food for a large proportion of the global population. However, wheat production is increasingly challenged by climate change, particularly through the occurrence of pre-harvest sprouting (Li et al., 2019). Sprouted wheat often loses its desirable baking characteristics and is therefore frequently discarded or diverted to alternative applications, including animal feed and industrial products such as starch, vital gluten, furfural, and ethanol (Olaerts and Courtin, 2018; Kringel et al., 2020). Despite these challenges, germination is also recognized as a valuable biological process that can enhance the nutritional, functional, and sensory qualities of grains while increasing their micronutrient content (Hefni and Witthöft, 2011). As a fundamental stage in the plant life cycle, successful germination determines seedling establishment and ultimately influences crop productivity (Zhu, 2001).

Germination may also be restricted by seed dormancy, a physiological mechanism that prevents viable seeds from germinating under unfavorable environmental conditions. During germination, stored seed reserves are mobilized and utilized for respiration and the synthesis of new cellular components, resulting in substantial biochemical and nutritional modifications. Studies have demonstrated increases in proteins, amino acids, soluble sugars, minerals, vitamins, dietary fiber, and antioxidant compounds such as phenolics, vitamin E, and vitamin C during this process (Sierra and Vidal-Valverde, 1999). Wheat grains were selected as a model organism for investigating strategies to stimulate germination and promote initial plant development (Šerá et al., 2025).

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Plant growth regulators (PGRs), whether naturally synthesized by plants or produced synthetically, are small organic molecules that act within plant cells to influence growth and development (Azuma et al., 1997; Sultana et al., 2025). Among these hormones, gibberellic acid (GA_3) plays a crucial role in breaking seed dormancy and promoting germination and early seedling growth (Finch-Savage and Leubner-Metzger, 2006). Applied at low concentrations, GA stimulates germination by activating hydrolytic enzymes such as amylases, which mobilize stored reserves to provide energy for rapid cell division and radicle elongation (Li et al., 2018). Plant growth regulators and micronutrients are also used to enhance yield and improve nutrient accumulation in crops (Naeem et al., 2020). Pre-harvest GA_3 priming of wheat seeds may provide a controlled approach to enhance germination while modulating the nutritional and bioactive properties of wheat sprouts. GA_3 can stimulate germination through enhanced α-amylase activity and endosperm mobilization, while germination itself may promote phenolic biosynthesis and antioxidant capacity. Therefore, GA_3 treatment could potentially alter phenolic profiles, antioxidant activity, bioactive compounds, and mineral accumulation, although these effects should be experimentally validated (Mohaddes Ardebili et al., 2019; Kanjevac et al., 2023). In this study, germinated wheat samples were evaluated against non-germinated grains for moisture, phenolic profiles, flavonoids, antioxidant activity, and mineral composition. The aim of this study was to evaluate the effects of gibberellic acid (GA) and water-induced germination on the bioactive compounds, antioxidant activity, phenolic composition, and mineral contents of wheat sprouts.

MATERIAL AND METHODS

Material

The cleaned wheat seeds used in this study was sourced from a flour mill operating in Konya in Türkiye in 2026.

Methods

Germination process

The cleaned wheat grains were soaked separately in 1000 ppm gibberellic acid and tap water for 24 hours. Then, they were drained, and the soaked wheat was spread to a thickness of 1 cm in a clean plastic plate. To prevent the grains from drying out, gibberellic acid was sprayed onto them daily, and water was sprayed onto the other plate. After germination was achieved in 7 days, analyses were started. No treatment was applied to the control sample.

Moisture content

Moisture contents of samples were detected at 105°C using an oven (Nüve FN055 Ankara. Turkey) until a certain weight was determined.

Extraction procedure

The extracts were obtained based on a method informed by McDonald et al. (2020) with some modifications.

Total phenolic content

The total phenolic content of the extracts was quantified using the Folin-Ciocalteu (FC) assay following the method described by Yoo et al. (2004). The obtained values were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (dw) after treatment.

Total flavonoid content

For the determination of total flavonoid content, 1 mL of the extract was sequentially combined with 0.3 mL $NaNO_2$, 0.3 mL $AlCl_3$, and 2 mL NaOH. After pretreatments, the total flavonoid content was expressed as mg quercetin equivalents (QE) per 100 g dry weight (dw) according to the method described by Hogan et al. (2009).

DPPH free radical scavenging activity

The free radical scavenging capacity of the extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay following the method described by Lee et al. (1998). Following data processing, the results were expressed as mmol Trolox equivalents (TE) per kg of dry weight (dw).

Determination of phenolic compounds

The chromatographic separation of phenolic compounds was performed using an HPLC system (Shimadzu) fitted with a PDA detector and an Inertsil ODS-3 column (5 µm; 4.6 × 250 mm). A 20 µL injection volume was used for each sample. Detection of peaks was carried out at 280 nm with the PDA detector.

Mineral contents of wheat seeds

After 0.5 g powdered seeds were dried in an oven at 70ºC till fixed weight. Then, it was burned by using 5ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a microwave. The volume of samples was completed with 20 ml with distilled water. ICP-OES was used for element contents of raw (control) and germinated seeds.

Statistical Analyses

The sample data were analyzed using analysis of variance based on the averaged values from three replicates. Significant differences among germination types ($p < 0.05$) were determined using Duncan's Multiple Range Test. Results are presented as mean ± standard deviation for independent germination types, calculated using MSTAT C software.

RESULTS AND DISCUSSION

Moisture content, total phenol, total flavonoid content, and antioxidant activity values of wheat germinated by treatment with gibberellic acid (GA) solution and tap water are shown in Table 1.

Table 1. Moisture content and bioactive properties of non-germinated and germinated wheat samples

Germination environment	Moisture content (%)	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)	Antioxidant content (mmol/kg)
Control	5.87 ± 0.08c*	14.66 ± 1.69c	3.30 ± 0.14c	2.97 ± 0.03c
Gibberellic acid	58.04 ± 1.02a	49.74 ± 1.31a	5.99 ± 0.21a	6.52 ± 0.03a
Water	51.10 ± 0.75b	45.88 ± 1.43b	5.53 ± 0.16b	5.54 ± 0.02b

* values within each column followed by different letters are significantly different at $P < 0.05$.

The raw and germinated wheat samples contained 5.87% (control), 58.04% (GA) and 51.10% (Water) moisture. Naturally, wheat samples germinated by treatment with water and GA were found to have significantly higher moisture content compared to unsprouted wheat grains. Germinating wheat grains treated with a 1000 ppm gibberellic acid (GA_3) solution exhibited higher moisture content than grains germinated only in water because gibberellic acid accelerates the physiological and biochemical events of germination. GA_3 stimulates the aleurone layer to produce hydrolytic enzymes, especially α-amylase, which hydrolyzes stored starch into soluble sugars. The accumulation of soluble sugars lowers the osmotic potential within the seed and enhances water uptake during imbibition. In addition, GA_3 promotes cell elongation and increases metabolic activity, thereby improving the seed's ability to absorb and retain water. Studies on wheat and cereal germination have shown that increased α-amylase activity is directly associated with higher moisture uptake and enhanced germination processes (Goswami et al., 1977). Total phenolic and flavonoid amounts of the non-germinated and germinated wheat samples were characterized to be between 14.66 (control) and 49.74 mgGAE/100g (GA) to 3.30 (control) and 5.99 mg/100g (GA), respectively. Also, antioxidant capacity values of non-germinated and germinated wheat samples were assessed to be between 2.97 (control) and 6.52 mmolTE/kg (GA). Wheat germinated after treatment with gibberellic acid (GA) had higher total phenol, total flavonoid content, and antioxidant activity values compared to control (non-germinated) and water-treated wheat samples. Ovelall, germination in gibberellic acid solution may enhance the synthesis and accumulation of phenolic and flavonoid compounds by stimulating metabolic activity and activating secondary metabolite pathways.

Consequently, the higher levels of these bioactive compounds could increase the antioxidant capacity of wheat seedlings compared with those germinated in water alone. Gibberellic acid may therefore promote antioxidant-related biochemical responses during germination.

Changes in the amounts of phenolic components (phenolic acid and flavonoid) in wheat samples germinated by treatment with gibberellic acid and water are given in Table 2.

Table 2. Phenolic compounds of non-germinated and germinated wheat samples

Phenolic acids (mg/100g)	Control	Gibberellic acid	Water
Gallic acid	0.49 ± 0.01a*	0.12 ± 0.00c	0.27 ± 0.02b
Protocatechuic acid	0.08 ± 0.03c	0.24 ± 0.02a	0.22 ± 0.01b
Chlorogenic acid	0.07 ± 0.01c	0.49 ± 0.04b	0.69 ± 0.04a
Caffeic acid	0.04 ± 0.01c	0.12 ± 0.02a	0.08 ± 0.02b
Coumaric acid	0.05 ± 0.03b	0.10 ± 0.01a	0.02 ± 0.01c
Ferulic acid	0.04 ± 0.01c	0.08 ± 0.01a	0.06 ± 0.01b
Cinnamic acid	0.05 ± 0.02a	0.05 ± 0.01a	0.03 ± 0.01b
Flavonoids (mg/100g)	Control	Gibberellic acid	Water
Catechin	0.15 ± 0.06c	1.17 ± 0.24a	0.24 ± 0.06b
Rutin	1.32 ± 0.39b	2.14 ± 0.23a	0.78 ± 0.09c
Hesperidin	0.09 ± 0.04b	0.08 ± 0.01bc	0.12 ± 0.02a
Quercetin	0.25 ± 0.10c	0.35 ± 0.10a	0.34 ± 0.06ab
Kaempferol	0.03 ± 0.01c	0.12 ± 0.01a	0.09 ± 0.02b

* values within each row followed by different letters are significantly different at $P < 0.05$.

In general, the phenolic acid content of both unsprouted (control) and germinated wheat samples was found to be below 0.69 mg/100g. However, the phenolic acid content of wheat samples germinated with GA application was higher than that of unsprouted and water-treated wheat samples (except for gallic acid and chlorogenic acids in water treatment). In general, while an increase was observed in some phenolic acid contents of wheat sprouts with germination, a decrease was observed in some phenolic acid contents (coumaric acid and cinnamic acid). The phenolic acids that increased in quantity the most through germination were gallic acid, protocatechuic acid, and chlorogenic acid. Gallic and protocatechuic acid amounts of non-germinated (control) wheat seed and germinated wheat sprouts were characterized to be between 0.12 mg/100g (GA) and 0.49 mg/100g (control) to 0.08 (control) and 0.24 mg/100g (GA), respectively. Also, chlorogenic acid contents of the wheat samples varied between 0.07 (control) and 0.69 mg/100g (water). The higher levels of gallic acid and chlorogenic acid observed in wheat sprouts germinated in water compared with those treated with gibberellic acid (GA_3) may be attributed to differences in carbon allocation and phenylpropanoid metabolism during germination. As a result, phenolic compounds, including gallic acid and chlorogenic acid, may be consumed at a faster rate, diluted as biomass increases, or redirected toward other metabolic pathways. In contrast, sprouts germinated solely in water may exhibit higher levels of phenolic compounds, as normal germination activates the phenylpropanoid pathway without hormonal stimulation that promotes rapid tissue growth. Therefore, the elevated gallic acid and chlorogenic acid contents in water-germinated wheat sprouts likely reflect a higher investment in phenolic biosynthesis and/or lower dilution of these compounds by growth processes (Park et al., 2017). The higher flavonoid content observed in wheat samples germinated under gibberellic acid (GA_3) treatment compared with control and water-treated samples can be attributed to the stimulatory effect of GA_3 on seed metabolism and secondary metabolite biosynthesis. Gibberellic acid enhances germination by promoting enzyme activation, reserve mobilization, and cellular growth, which increases metabolic activity during sprouting.

This enhanced metabolism can induce the phenylpropanoid pathway, the primary biosynthetic route for flavonoid production. GA_3-treated wheat sprouts likely accumulated higher levels of flavonoids because gibberellic acid intensified germination-related metabolic processes and enhanced the biosynthetic pathways responsible for flavonoid production (Chen et al., 2017; Kim et al., 2018). Catechin and rutin amounts of the non-germinated and germinated wheat samples were identified to be between 0.15 (control) and 1.17 mg/100g (GA) to 0.78 (Water) and 2.14 mg/100g (GA), respectively. In addition, quercetin and kaempferol contents of the wheat samples were established to be between 0.25 (control) and 0.35 mg/100g (GA) to 0.03 (control) and 0.12 mg/100g (GA), respectively. Also, hesperidin amounts of the non-germinated and germinated what samples were assayed to be between 0.08 (control) and 0.12 mg/100g (Water). The higher phenolic content observed in wheat grains germinated with gibberellic acid may be attributed to its stimulatory effects on germination and metabolic activity. Gibberellic acid can enhance enzyme activity and accelerate mobilization of stored nutrients, potentially promoting phenolic biosynthesis and increasing the release of bound phenolic compounds.

The macro and microelement contents of wheat samples germinated by treatment with gibberellic acid and water are illustrated in Table 3.

Table 3. Macro and micro element contents of non-germinated and germinated wheat samples (mg/kg)

Samples	P	K	Ca	Mg	S
Control	2312.98±1.20 B*	1284.82±2.74 B	429.58±1.55 B	128.28±1.11 B	0.38±0.001 B
Giberellic acid	37.61±1.05 C	0.06±0.02 C	0.02±0.001 C	0.01±0.001 C	0.02±0.001 C
Water	3039.22±3.14 A	2046.34±1.54 A	1044.17±1.77 A	323.29±1.35 A	0.58±0.003 A
Samples	Fe	Zn	Cu	Mn	B
Control	0.180±0.002 B	0.097±0.002 B	0.0122±0.001 B	0.0062±0.0001 B	0.0113±0.00001 B
Giberellic acid	0.001±0.0001 C	0.001±0.0001 C	0.0001±0.0001 C	0.0001±0.0001 C	0.0001±0.00001 C
Water	0.446±0.004 A	0.258±0.003 A	0.0237±0.001 A	0.0107±0.0001 A	0.0187±0.00001 A

* values within each column followed by different letters are significantly different at $P < 0.05$.

As seen in Table 3, the P, Ca, Ca, Mg, Fe, Zn, Cu, Mn, and B contents of wheat samples germinated by treatment with GA were significantly lower than those of control and water-treated wheat samples. A possible explanation for the significantly lower P, Ca, K, Mg, Fe, Zn, Cu, Mn, and B contents in wheat grains germinated after gibberellic acid (GA_3) treatment, compared with the control and water-germinated samples, is that GA_3 strongly stimulates germination metabolism, and seedling growth. This accelerated growth increases the utilization and redistribution of mineral nutrients from the seed reserves to newly developing tissues. In addition, tap water may contribute small amounts of minerals such as Ca, Mg, Na, and K during wheat germination, unlike distilled water. Evidence shows that the mineral composition of irrigation water can alter mineral concentrations in wheat sprouts, although the magnitude depends on water quality and germination conditions (Pongrac et al., 2016). Therefore, tap water could provide a minor mineral contribution and should be considered when interpreting mineral-content results. P and K amounts of the wheat seeds and sprouts were assessed to be between 37.61 (GA) and 3039.22 mg/kg (Water) to 0.06 (GA) and 2046.34 (Water), respectively. Ca and Mg contents of the non-germinated and germinated wheat samples were assayed to be between 0.02 (GA) and 1044.17 mg/kg (Water) to 0.01 (GA) and 323.29 mg/kg (Water), respectively. S content in wheat and germinated wheat samples was found to be at very low levels. Fe and Zn amounts of the wheat samples were characterized to be between 0.001 (GA) and 0.446 mg/kg (Water) to 0.001 (GA) and 0.258 mg/kg (Water), respectively. While Cu amounts of the wheat samples vary between 0.0001 (GA)

and 0.0237 mg/kg (Water), Mn amounts of the wheat samples were established to be between 0.0001 (GA) and 0.0107 mg/kg (Water). Also, B amounts of the non-germinated and germinated wheat samples were determined to be between 0.0001 (GA) and 0.0187 mg/kg (Water). Wheat samples treated with water showed higher levels of macro and microelement content compared to wheat samples treated with GA. The reason why macro and microelement contents in wheat sprouts treated with water are found to be higher compared to those treated with gibberellic acid (GA_3) is mainly that GA_3 promotes rapid growth and cell elongation in plants. With gibberellic acid application, the biomass of the sprouts increases rapidly, but mineral uptake may not occur at the same rate. This situation is referred to as the "dilution effect", which leads to a decrease in mineral concentration per unit weight as the elements are distributed within a larger biomass. In addition, GA_3 accelerates metabolic activity by increasing the activity of enzymes that break down starch and storage compounds during germination. During this increased metabolic activity, some macro and microelements may be used more in enzymatic reactions or transported to different tissues within the plant. It is an expected result that macro and microelement contents are higher in wheat sprouts treated with water (Taiz et al., 2015). The lower phenolic content observed at the third developmental stage compared with other stages may be attributed to differences in plant developmental status, the timing of GA_3 application, and complex metabolic changes associated with the transition from vegetative to reproductive growth (Sharaf-Eldin et al., 2007). The exogenous application of plant growth regulators (PGRs) and micronutrients has been widely recognized as an effective approach for enhancing yield, improving crop quality, and regulating the uptake and accumulation of mineral nutrients in plants (Naeem et al., 2020; Sofy et al., 2020). As a results, gibberellic acid may accelerate germination and metabolic activity, increasing mineral mobilization and utilization, thereby reducing the measured mineral concentrations compared with water-germinated wheat.

CONCLUSIONS

In conclusion, germination enhanced the bioactive properties of wheat, with more pronounced effects in gibberellic acid (GA)-treated sprouts than in water-treated and non-germinated controls. Germination increased moisture content due to water uptake and metabolic activation. GA treatment significantly elevated total phenolic and flavonoid contents and antioxidant activity, indicating stimulation of antioxidant biosynthesis. Phenolic profiles showed increases in compounds such as gallic acid, chlorogenic acid, catechin, and rutin, likely due to activated phenylpropanoid metabolism. However, water-treated sprouts retained higher levels of some acids. Mineral contents decreased in GA-treated sprouts, likely due to intensive metabolic use. Overall, GA germination improved wheat's functional and nutritional value.

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