

Gibberellic Acid- and Water-Induced Germination Differentially Modulate Bioactive Compounds, Phenolic Profile, Antioxidant Capacity, and Mineral Composition in Barley Sprouts

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ABSTRACT

Total phenol and flavonoid amounts of the nongerminated seed and germinated barley sprouts were characterized to be between 30.07 (control) and 57.15 mgGAE/100g (Water) to 6.33 (control) and 8.88 mg/100g (Water), respectively. Antioxidant activity values of barley samples were assessed to be between 4.21 (control) and 8.37 mmolTE/kg (Water). The phenolic acid content of barley seeds (control) and barley sprouts germinated after treatment with GA solution and water was found to be at very low levels. Catechin and hesperidin were the predominant flavonoids of the barley samples, and their catechin and hesperidin contents were estimated to be between 0.33 (control) and 6.54 mg/100g (GA) to 0.35 (control) and 3.38 mg/100g (GA), respectively. In general, the phenolic acid content (except gallic acid and coumaric acid) of barley seeds germinated after treatment with gibberellic acid was higher than that of ungerminated (control) barley seeds and barley seeds germinated after treatment with water (except chlorogenic acid and coumaric acid). The P, K, and Ca contents of barley samples treated with GA were higher than those of control and water-treated barley samples. In addition, barley samples germinated by treatment with water had higher Fe, Zn, and Cu contents than barley samples germinated by treatment with control and GA solutions.

Keywords: barley sprouts, gibberellic acid, germination, bioactive properties, minerals, ICP-OES.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a member of the genus *Hordeum*, tribe Triticeae, and family Poaceae, and it is recognized as the fourth most extensively cultivated cereal crop worldwide, following wheat, rice, and maize. It is grown across diverse agro-ecological zones and contributes substantially to both human nutrition and animal feed production systems (Noreen et al., 2025). Owing to its high level of environmental adaptability, barley can thrive under a wide range of stressful conditions, particularly in arid and semi-arid regions where low temperature, drought, salinity, alkalinity, and nutrient-poor soils are prevalent (Gurel et al., 2016; Giraldo et al., 2019). Globally, a large proportion of barley production - including grain, straw, and green biomass - is primarily used as livestock feed, although it is also an important human food resource and a key raw

material in malting, brewing, starch extraction, and bioenergy industries (Langridge, 2018). Germination is widely regarded as an efficient and low-cost biotechnological method for improving the nutritional and functional quality of seeds. During germination, stored macromolecules are enzymatically mobilized to support respiration and embryonic development, resulting in substantial biochemical modifications. These metabolic changes enhance nutritional quality by increasing protein content, essential amino acids, soluble sugars, minerals, vitamins, and dietary fiber, thereby improving the overall value of plant-based foods without chemical intervention. Among the main biological regulators in this process, gibberellic acid (GA₃) acts as an essential endogenous plant hormone governing growth and development (Soad et al., 2010). In agricultural practice, GA₃ is applied in barley malting as a food

additive to improve germination and enzymatic activity (Hornsey, 2003). Germination is an eco-friendly method increasing bioactive compounds, improving nutrition, antioxidant capacity, and health benefits significantly (Zhu et al., 2021). These hormones play essential roles in dormancy release, seed germination enhancement, and seedling establishment, while also regulating elongation of roots, stems, and leaves. In addition, they are involved in flowering induction and seed formation, thereby contributing to improved crop productivity through the regulation of enzymes associated with germination (Gomez et al., 2016; Carrera-Castano et al., 2020). Consequently, gibberellic acid is widely recognized as an effective plant growth regulator with substantial agricultural benefits (Almubarak, 2009; Miceli et al., 2019). Barley sprouts are notably rich in bioactive constituents such as phenolic acids, flavonoids, lignans, vitamin E, sterols, and folates (Raj et al., 2023). Furthermore, it alters micronutrient and phytochemical content, including vitamins, minerals, enzyme inhibitors, and glucosinolates (Miyahira et al., 2021; Maqbool et al., 2024). Consequently, barley sprouts are increasingly regarded as functional foods with strong antioxidant and health-promoting potential (Castro et al., 2024; Oh et al., 2025). In recent years, barley inclusion in food products has risen significantly because of its documented health benefits, including glycemic control, cholesterol reduction, and antioxidant properties. Malting, a controlled germination technique, is the primary method for producing brewing malt. Barley is a widely consumed cereal grain used globally in food and beverage production, as well as in health-related applications such as barley-derived dietary fibers and probiotic products. The consumption of grains germinated with gibberellic acid, including barley, has been reported to be increasing, potentially due to their enhanced nutritional composition and improved functional properties. 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 barley sprouts.

MATERIAL AND METHODS

Material

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

Methods

Germination process

Barley seeds were soaked for 24 hours in a solution at a concentration of 750 ppm and in regular tap water, then placed in a plastic tray to moisten to saturation. The trays were kept at room temperature (21-24°C) and 70% relative humidity for 7 days. Analysis of unsprouted barley samples and germinated sprouts was started immediately. 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 Folin-Ciocalteu (FC) reagent was used to determine total phenolic contents of extracts according to Yoo et al. (2004). FC (1 ml) and Na₂CO₃ (10 ml) were added to extract and mixed with vortex. The deionised water was added until the final volume was 25 ml, and kept at dark for 1 h. The absorbance was measured at 750 nm in a spectrophotometer. The results are shown as mg gallic acid equivalent (GAE)/100 g dry weight (dw).

Total flavonoid content

Total flavonoid contents of extracts were carried out according to the study stated by Hogan et al. (2009). The extract (1 mL) was mixed with 0.3 ml of NaNO₂, 0.3 ml of AlCl₃

and 2 ml of NaOH, respectively, and kept in dark for 15 min. The absorbance of mixture was measured at 510 nm using spectrophotometer. The results are given as mg quercetin (QE)/100 g dw.

DPPH free radical scavenging activity

The free radical scavenging activities of extracts were determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (1998). After preprocessing, the results were given as mmol trolox (TE)/kg dw.

Determination of phenolic compounds

Chromatographic separation of phenolic compounds was determined by HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 (5 μ m; 4.6 x 250 mm) column. The mobile phase was a mixture of 0.05% acetic acid in water (A) and acetonitrile (B) with the flow rate of 1 ml/min at 30°C. The injection volume was 20 μ l. The peaks were taken at 280 using a PDA detector. The total running time per sample was 60 min.

Mineral contents of wheat seeds

After 0.5 g powdered barley were dried in

an oven at 70°C till fixed weight. Then, it was incinerated by using 5ml of 65% HNO₃ and 2 ml of 35% H₂O₂ in a microwave. The volume of samples was completed with 20 ml with distilled water. ICP-OES (Agilent-5110) 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 ($p < 0.05$ and $p < 0.01$) were determined using Duncan's Multiple Range Test. Results are presented as mean $\pm$ standard deviation for independent germination types, calculated using MSTAT C software. The nature of experimental replications was given as n:3.

RESULTS AND DISCUSSION

Changes in moisture, total phenol, total flavonoid content, and antioxidant activity values of barley samples germinated by treatment with gibberellic acid (GA) solution and water are presented in Table 1.

Table 1. Moisture content and bioactive properties of barley samples

Germination environment	Moisture content (%)	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)	Antioxidant content (mmol/kg)
Control	5.73 $\pm$ 0.12c*	30.07 $\pm$ 1.39c	6.33 $\pm$ 0.26c	4.21 $\pm$ 0.00c
Gibberellic acid	61.09 $\pm$ 1.70b	55.55 $\pm$ 1.30b	6.73 $\pm$ 0.25b	7.50 $\pm$ 0.03b
Water	62.05 $\pm$ 1.27a	57.15 $\pm$ 2.04a	8.88 $\pm$ 0.04a	8.37 $\pm$ 0.02a

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

The moisture contents of nongerminated barley seed (control) and germinated barley sprouts were found to be between 5.73% (control) and 62.05% (water). It appears that the moisture content of barley sprouts subjected to the soaking process is significantly higher than that of non-germinated seeds. Total phenol and flavonoid amounts of the nongerminated seed and germinated barley sprouts were characterized to be between 30.07 (control) and 57.15 mgGAE/100g (Water) to 6.33 (control) and 8.88 mg/100g (Water), respectively. Antioxidant activity values of barley samples

were assessed to be between 4.21 (control) and 8.37 mmolTE/kg (Water). As shown in Table 1, the moisture, total phenol, total flavonoid content, and antioxidant activity values of barley sprouts germinated by water treatment were higher than those of barley samples treated with gibberellic acid solution and barley seeds.

The phenolic components (phenolic acid and flavonoid) of barley seeds (control) and barley sprouts germinated after treatment with water and gibberellic acid solutions are shown in Table 2.

Table 2. Phenolic compounds of barley samples

Phenolic acids (mg/100 g)	Control			Gibberellic acid			Water		
Gallic acid	0.59	±	0.20b*	0.10	±	0.01c	1.11	±	0.23a
Protocatechuic acid	0.25	±	0.12c	2.62	±	0.06a	2.42	±	0.14b
Chlorogenic acid	0.10	±	0.03c	0.15	±	0.01b	0.24	±	0.03a
Caffeic acid	0.06	±	0.02b	0.09	±	0.01a	0.05	±	0.01bc
Coumaric acid	0.29	±	0.01a	0.03	±	0.01c	0.10	±	0.02b
Ferulic acid	0.04	±	0.01c	0.08	±	0.01a	0.07	±	0.01ab
Cinnamic acid	0.06	±	0.03c	0.17	±	0.06a	0.09	±	0.02b
Flavonoids (mg/100 g)	Control			Gibberellic acid			Water		
Catechin	0.33	±	0.25c	6.54	±	1.44a	0.61	±	0.04b
Rutin	0.50	±	0.10b	0.86	±	0.05a	0.51	±	0.05b
Hesperidin	0.35	±	0.11c	3.38	±	0.09a	0.97	±	0.13b
Quercetin	0.24	±	0.08b	0.26	±	0.05a	0.14	±	0.02c
Kaempferol	0.06	±	0.02c	0.27	±	0.02a	0.21	±	0.01b

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

In general, the phenolic acid content of barley seeds (control) and barley sprouts germinated after treatment with GA solution and water was found to be very low. The phenolic acid contents of barley sprouts obtained from untreated seeds (control) as well as seeds treated with water or gibberellic acid (GA) solution were found to be at very low levels. This observation may be attributed to the early stage of germination, during which the biosynthesis and accumulation of phenolic acids have not yet reached their maximum levels, as metabolic activity is primarily directed toward reserve mobilization and seedling development rather than secondary metabolite production (Gan et al., 2017). Moreover, most phenolic acids in barley grains are present in bound forms esterified to cell wall polysaccharides, which limits their extractability and results in relatively low concentrations of free phenolic acids during the initial stages of germination (Adom and Liu, 2002). Previous studies have also demonstrated that substantial increases in phenolic acids generally occur after longer germination periods or following elicitor or stress treatments rather than during the early stages of sprouting (Gan et al., 2017). The highest phenolic acid concentration in barley samples was protocatechuic acid, with amounts ranging from 0.25 (control) to 2.62 mg/100g (GA). In addition, gallic acid amounts of the barley samples were characterized to be between 0.10 (GA) and 1.11 mg/100g (Water). Overall, barley seeds germinated after treatment with gibberellic

acid had higher phenolic acid content (except gallic acid and coumaric acid) than ungerminated (control) barley seeds and barley seeds germinated after treatment with water (except chlorogenic acid and coumaric acid). Additionally, the flavonoid component content of the barley samples was higher than their phenolic acid content. Catechin and hesperidin were the predominant flavonoids in barley samples, and catechin and hesperidin contents of the barley samples were assayed to be between 0.33 (control) and 6.54 mg/100g (GA) to 0.35 (control) and 3.38 mg/100g (GA), respectively. Also, rutin and quercetin amounts of the barley samples were identified to be between 0.50 (control) and 0.86 mg/100g (GA) to 0.14 (Water) and 0.26 mg/100g (GA), respectively. The highest levels of kaempferol GA were detected in barley samples germinated after treatment with kaempferol GA (0.27 mg/100g), followed in decreasing order by barley samples germinated after treatment with water (0.21 mg/100g) and the control group (non-germinated) (0.06 mg/100g). Barley samples germinated after treatment with gibberellic acid had higher flavonoid content compared to ungerminated (control) and water-treated barley samples. The higher flavonoid content observed in barley grains germinated with gibberellic acid (GA₃) compared with both ungerminated (control) grains and water-germinated grains can be attributed to the stimulatory effect of GA₃ on secondary metabolism during germination. Gibberellic acid enhances seed metabolic

activity by promoting the synthesis of hydrolytic enzymes and facilitating reserve mobilization, thereby creating favorable conditions for the biosynthesis of secondary metabolites, including flavonoids (Yamauchi, 2001). Germination itself induces oxidative stress due to enhanced respiration and rapid cellular growth, and flavonoids are synthesized as important antioxidant compounds that protect plant tissues against reactive oxygen species (Dixon and Paiva, 1995). Therefore, the exogenous application

of GA₃ further amplifies these metabolic and antioxidant responses, leading to significantly higher flavonoid concentrations than those observed in water-germinated grains, whereas both germinated treatments contain higher flavonoid levels than ungerminated barley because germination activates secondary metabolism (Ali et al., 2021; Jan et al., 2021).

The macro and microelement contents of barley sprouts treated with gibberellic solution and water are illustrated in Table 3.

Table 3. Macro and micro element contents of barley samples (mg/kg)

Samples	P	K	Ca	Mg	S
Control	1393.02±1.91 C	271.98±0.31 C	103.59±0.11 C	50.28±0.10 C	0.26±0.02 C
Gibberellic acid	1655.82±2.05 A*	437.63±0.55 A	269.14±0.24 A	118.83±0.30 B	0.32±0.03 B
Water	1583.09±3.13 B	394.59±0.41 B	250.75±0.30 B	168.43±0.24 A	0.40±0.03 A
Samples	Fe	Zn	Cu	Mn	B
Control	0.054±0.010 C	0.075±0.018 C	0.008±0.0001 B	0.007±0.0001 C	0.003±0.0002 B
Gibberellic acid	0.082±0.016 B	0.091±0.020 B	0.010±0.0001 A	0.021±0.0004 A	0.011±0.0001 A
Water	0.104±0.035 A	0.096±0.022 A	0.011±0.0002 A	0.014±0.0001 B	0.011±0.0001 A

* values within each row followed by different letters are significantly different at $P < 0.01$.

P and K amounts of the barley samples were characterized to be between 1393.02 (control) and 1655.82 mg/kg (GA) to 271.98 (control) and 437.63 mg/kg (GA), respectively. Also, Ca and Mg contents of the barley samples were assessed to be between 103.59 (control) and 269.14 mg/kg (GA) to 50.28 (control) and 168.43 mg/kg (Water), respectively. Also, S amounts of the barley samples were found to be between 0.26 (control) and 0.40 mg/kg (Water). The microelement content of the barley samples was found to be at very low levels. Fe and Zn contents of the nongerminated barley seed and germinated barley sprouts were established to be between 0.054 (control) and 0.104 mg/kg (water) to 0.75 (control) and 0.096 mg/kg (water), respectively. In addition, while Cu contents of the barley samples vary between 0.008 (control) and 0.011 mg/kg (Water), Mn amounts of the barley samples were characterised to be between 0.007 (control) and 0.021 mg/kg (GA). In barley samples, the highest levels of B were determined in samples treated with gibberellic acid solution and water, followed in decreasing order by the control group. The

P, K, and Ca contents of barley samples treated with GA were higher than those of barley samples germinated after treatment with control and water. In addition, the Fe, Zn, and Cu contents of barley samples germinated after treatment with water were higher than those of barley samples germinated after treatment with control and GA solution. The higher P, K, and Ca contents observed in GA₃-treated barley samples compared with the control and water-treated germinated barley can be explained by the stimulatory effect of gibberellic acid on seed germination and reserve mobilization. GA₃ induces the synthesis of hydrolytic enzymes, particularly α -amylase and phosphatases, in the aleurone layer, which accelerates the degradation of stored starch and phosphorus-containing compounds and enhances the translocation of nutrients from the endosperm to the developing embryo (Jones and Carbonell, 1984; An and Lin, 2011). In contrast, the higher Fe, Zn, and Cu contents in water-treated germinated barley may be attributed to the dilution effect associated with the enhanced biomass accumulation induced by

GA₃, whereby faster seedling growth decreases the concentration of these micronutrients on a dry-weight basis despite efficient reserve utilization. Moreover, Fe, Zn, and Cu are less mobile than macronutrients during germination and are tightly regulated by specific metal transport systems; therefore, under slower growth conditions in water-treated seedlings, these micronutrients remain more concentrated in the tissue (Amri et al., 2016; Betts et al., 2020). The moisture contents of barley varieties were assessed to be between 10.23 to 10.75% (Noreen et al. 2025). The total phenolic content (TPC) and total flavonoid content (TFC) increased significantly throughout the germination process, reaching their highest levels after 60 hours of germination. In addition, the inclusion of gibberellic acid in the soaking solution significantly enhanced the accumulation of both TPC and TFC (Lien et al., 2016). The increase in total antioxidant capacity may be attributed to the elevated phenolic content observed in both seeds and seedlings (Vignesh et al., 2012). Total phenol (TPC) and flavonoid contents (TFC) of seeds of barley varieties were characterized to be between 35.52 and 43.83 mgGAE/100g to 11.36 and 13.65 mgQE/100g, respectively (Noreen et al., 2025). Total phenol, flavonoid and antioxidant activity (DPPH assay) values of Barley sprouts were 12.64 ± 0.04 mg of gallic acid equivalent (GAE)/g, 5.99 ± 0.09 mg of rutin equivalent (RE)/g and 21.04 mg/mL, respectively (Oh et al. 2025). A 7-day germination period was found to be optimal for the extraction of total phenolic compounds and flavonoids from Esmeralda barley. In the Perla variety, the maximum total phenolic content and flavonoid levels were recorded at 5 and 7 days of germination, respectively. Overall, the highest total phenolic concentration was observed in Perla samples, reaching 13.60 mg GAE/g, whereas the greatest total flavonoid content was detected in Esmeralda barley at 1.73 mg QE/g (Garcia-Castro et al., 2023). The findings demonstrated pronounced temporal variations in total flavonoid and total phenolic contents throughout germination. Total flavonoids showed an initial substantial

decline of 32.59% during the early phase (0-12 h), decreasing from 2.64 to 1.78 mg/g, followed by a modest recovery of 15.34% by 72 h. Similarly, total phenolic content decreased by 36.35% in the initial stage (0-12 h), from 6.52 to 4.15 μ mol/g, but subsequently exhibited a significant increase of 44.73% during the later stage (60-72 h), reaching 6.13 μ mol/g (Hu et al., 2025). The overall trajectory of phenolic acid metabolites paralleled that of dibutyl phthalate and related compounds; however, total phenolic acid levels remained statistically unchanged across the three developmental stages (Hu et al., 2025). In a previous study, the application of GA₃ at the first and second stages significantly enhanced the phenolic compound content in *C. officinalis* (61.96 and 62.74 mg gallic acid/100 g dry weight, respectively), whereas a reduction in phenolic compounds was observed at the third stage. Across all spraying stages, increasing GA₃ concentration led to a corresponding increase in phenolic content in *C. officinalis* extracts (Sardoei et al., 2014). Similarly, Sharaf-Eldin et al. (2007) reported that although GA₃ increased phenolic compounds in leaves, it resulted in a decrease in the flower receptacles of globe artichoke. Mineral content in different varieties of barley were 2.32-3.07 K, 0.03-0.08 Ca, 0.01-0.04 Fe, 0.01-0.02 Zn, 0.01-3.09 P and 0.09-1.02 mg/100g Mg (dw) (Noreen et al., 2025). The increase in the bioactive properties and mineral content of barley sprouts germinated by applying gibberellic acid solution was similar to the results of previous studies.

CONCLUSIONS

This study demonstrated that germination can improve the phytochemical, antioxidant, and mineral properties of barley sprouts. Germination increased the total phenolic and flavonoid contents and enhanced antioxidant activity compared with nongerminated barley seeds, highlighting the potential of germination to improve the functional value of barley. Although phenolic acids were detected at relatively low levels, gibberellic acid treatment generally promoted their

accumulation and modified the phenolic profile of germinated barley. Catechin and hesperidin were identified as the predominant flavonoids, with their contents increasing following germination, particularly under gibberellic acid treatment. Germination conditions also affected the mineral composition of the samples. Gibberellic acid treatment enhanced phosphorus, potassium, and calcium contents, whereas water-induced germination resulted in higher levels of iron, zinc, and copper. Overall, the findings indicate that controlled germination, particularly with gibberellic acid, is a promising strategy for enhancing the nutritional and functional properties of barley sprouts and their potential application in the development of value-added functional foods.

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