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Effects of Gibberellic Acid- and Water-Induced Germination on Bioactive Constituents, Phenolic Profile, Antioxidant Capacity, Fatty Acid Composition, and Mineral Content of Oat Sprouts

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ABSTRACT

Germination is a physiological process that substantially modifies the nutritional, biochemical and functional characteristics of cereal grains. Oat (*Avena sativa* L.) is particularly attractive for germination because its grain contains proteins, lipids, dietary fibre, minerals and a diverse range of phenolic compounds and avenanthramides. During germination, hydration and activation of hydrolytic and biosynthetic pathways can modify the distribution and availability of phenolic acids, flavonoids, antioxidants, lipids and minerals. In this study, the effects of gibberellic acid (GA) and water-induced germination on bioactive compounds, antioxidant capacity, phenolic and fatty acid composition, and mineral content in oat seed and oat sprouts were investigated. Oat sprouts germinated with gibberellic acid solution showed higher levels of moisture, total phenol, total flavonoid content, and antioxidant activity compared to oat samples germinated with water alone. Total phenolic and flavonoid amounts of the oat samples were characterized to be between 21.81 (control) and 93.69 mgGAE/100g (GA) to 3.33 (control) and 3.75 mgQE/100g (GA), respectively. Antioxidant activity values of nongerminated (control) and germinated oat sprouts were assessed to be between 2.62 (control) and 6.72 mmolTE/kg (GA). In general, the phenolic acid content (except caffeic acid) of oat sprouts germinated by water treatment was higher than the control. In addition, the phenolic acid content (gallic acid and caffeic acid) of oat samples germinated by water treatment was higher than that of those treated with GA. In general, the fatty acid composition (except palmitic and stearic acids) of oils extracted from oat sprouts germinated with GA solution was higher than that of the control group and oat sprouts germinated with water treatment. GA treatment was observed to cause a significant increase in K, Ca, and S content in oat seeds.

Keywords: *Avena sativa* L., oat seeds, giberellic acid, sprouting, bioactive properties, phenolics, fatty acids, minerals, HPLC.

INTRODUCTION

Oats (*Avena sativa* L.) are unique cereals in the Poaceae family, valued for their multifunctional components that provide important nutritional and health benefits (Butt et al., 2008). This herbaceous crop is widely cultivated in Europe and North America and thrives in cool, humid climates (Mert, 2020). Historically used in both livestock feed and human diets, mainly as oatmeal and rolled oats, the physiological benefits of oat products have only recently been recognized (Pirjo et al., 2003). Oats are among the major cereal crops worldwide and rank sixth in global grain production after wheat, corn, rice, barley, and sorghum (Kim et al., 2021; Martin-Diana et al., 2021). Oats are a nutrient-dense whole grain widely consumed as groats and valued for their high levels of soluble fiber, well-balanced proteins, vitamins, minerals, and antioxidants beneficial for human health (Peterson, 2001; Esposito et al., 2005). They also contain relatively high lipid content compared with other cereals, including essential unsaturated fatty acids such as linoleic, oleic, and linolenic acids (Ahmet et al., 2019; Culetu et al., 2020), along with saturated fatty acids like myristic, palmitic, and stearic acids. Oats provide bioactive compounds such as phenolics, amino acids, peptides, oligosaccharides, and

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short-chain fatty acids, which contribute antioxidant effects, especially in sprouts (Ahmed et al., 2024). These include polyphenols such as caffeic, coumaric, gallic, hydroxybenzoic, protocatechuic, syringic, and vanillic acids, along with antioxidants like beta-carotene, flavonoids, and chlorophyll (Kim et al., 2021; Martin-Diana et al., 2021).

Germination depends on an effective enzymatic system controlling synthesis and degradation processes, which is strongly influenced by plant hormones, especially gibberellic acid (GA3) (Carrera-Castano et al., 2020). Gibberellins play key roles in plant growth and development, including dormancy breaking, seed germination, seedling growth, organ elongation, flowering, and yield improvement, mainly by regulating enzyme synthesis required for germination (Gomez et al., 2016).

The optimum application concentration for GA_3 can vary depending on factors such as plant species, genotype, seed characteristics, and application time. However, in some species, a GA_3 concentration of 750 ppm has been reported to be effective in increasing germination. For example, in a study conducted on *Papaver rhoeas* L. seeds, the highest germination rate was obtained at a GA_3 concentration of 750 ppm after pre-treatment of the seeds with GA_3 for 48 hours. In the same study, it was determined that the application of 750 ppm GA_3 significantly increased germination in P. rhoeas seeds compared to the control group (Golmohammadzadeh et al., 2020).

Plant growth regulators and micronutrients are commonly applied to increase crop yield and enhance nutrient accumulation (Naeem et al., 2020). In this study, germinated wheat samples were compared with non-germinated grains in terms of moisture content, phenolic and flavonoid profiles, antioxidant activity, and mineral composition. The study also aimed to investigate the effects of gibberellic acid (GA) and water-induced germination on bioactive compounds, antioxidant activity, phenolic and fatty acid composition, and mineral content in oat sprouts.

MATERIAL AND METHODS

Material

The cleaned oat seeds used in this study was sourced from Department of Crop Science, Faculty of Agriculture, Selçuk University in Konya in Türkiye in 2026.

Methods

Germination process

Cleaned oat seeds were immersed for 24 hours in either a 750 ppm gibberellic acid solution or distilled tap water. The GA dose (750 ppm) used for germination with GA was considered to be within the optimum range of previous studies and was used in the experiment (Golmohammadzadeh et al., 2020). After soaking, the grains were drained and evenly distributed in clean plastic trays to form a layer approximately 1 cm thick. During the 7-day germination period, the seeds treated with gibberellic acid were sprayed daily with the same solution to maintain moisture, while the water-soaked seeds received daily water spraying. Analytical measurements were performed after the completion of germination. The control group remained untreated throughout the experiment.

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. After pretreatments, the supernatant was evaporated at 40°C in a rotary evaporator under vacuum, and then residue was dissolved in 10 ml by methanol:water mixture (80/20, v/v).

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 Na_2CO_3 (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 results are shown as mg gallic acid equivalent (GAE)/100 g dry weight (dw).

Total flavonoid content

The extract (1 mL), 0.3 ml of $NaNO_2$, 0.3 ml of $AlCl_3$ and 2 ml of NaOH was used to determine total flavonoid contents of extracts according to Hogan et al. (2009).

DPPH free radical scavenging activity

The free radical scavenging activities of oat extracts were determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (1998). The extract was mixed with 2 mL methanolic solution of DPPH. After shaking vigorously, it was stored at room temperature for 30 min. The absorbance was recorded at 517 nm by using a spectrophotometer. 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 (5µm; 4.6 x 250mm) 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 µl. The elution programme was employed: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The total running time per sample was 60 min.

Fatty acid composition

Oat sample oils were extracted with petroleum benzine in Soxhlet Apparatus for 5 h and the solvent was removed with a rotary vacuum evaporator at 50ºC. Fatty acid methyl esters of oat oil was esterificated according to Multari et al. (2019) method, and gas chromatography (Shimadzu GC-2010, Kyoto, Japan) equipped with flame-ionization detector (FID) and capillary column was used to detect the fatty acid composition.

Mineral contents of oat seeds

After 0.5 g powdered oat samples were dried in an oven at 70ºC till fixed weight. Then, it was incinerated 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 analysis of variance (ANOVA) of the results of the chickpea extracts was carried out by A JMP version 9.0. The results are mean±standard deviation (MSTAT C) of independent germination types. The sample data were analyzed using analysis of variance based on the averaged values from three replicates. Three repeats represent independent biological repeats. Significant differences among germination types ($p < 0.05$) were determined using Duncan's Multiple Range.

RESULTS AND DISCUSSION

Chemical and bioactive properties of oat samples sprouted with GA and water

The moisture, total phenol, total flavonoid contents, and antioxidant activity values of oat sprouts germinated by treatment with gibberellic acid (GA) solution and water are given in Table 1.

Table 1. Bioactive properties of oat samples

Germination environment	Moisture content (%)	Total phenolic content (mgGAE/100 g)	Total flavonoid content (mgQE/100 g)	Antioxidant activity (mmol TE/kg)
Control	6.81 ± 0.07c	21.81 ± 2.07c	3.33 ± 1.43c	2.62 ± 0.01c
Gibberellic acid	63.24 ± 0.57a	93.69 ± 1.59a	3.75 ± 0.08a	6.72 ± 0.00a
Water	62.52 ± 0.61b	77.74 ± 1.31b	3.40 ± 0.11b	6.53 ± 0.02ab

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

Moisture content of oat samples is given as fresh weight (except control), and the moisture contents of oat seed and sprouts were determined to be between 6.81 (control (nongerminated seed) and 63.24% (GA). Total phenolic and flavonoid amounts of the oat samples were characterized to be between 21.81 (control) and 93.69 mgGAE/100g (GA) to 3.33 (control) and 3.75 mgQE/100g (GA), respectively. Antioxidant activity values of nongerminated (control) and germinated oat sprouts were assessed to be between 2.62 (control) and 6.72 mmolTE/kg (GA). Oat sprouts germinated after treatment with gibberellic acid solution had higher moisture, total phenol, total flavonoid content, and antioxidant activity values than oat samples germinated after treatment with water alone. GA_3 treatment improves nutritional and functional qualities of oat sprouts by enhancing metabolic activity during germination processes. GA_3 also increases water uptake and tissue hydration during germination, raising moisture content relative to controls. Overall, GA_3-treated sprouts show bioactive and antioxidant activity (Carrera-Castano et al., 2020). Among the oat genotypes evaluated by Brindzová et al. (2008), the black oat cultivar Jostrain exhibited the greatest antioxidant capacity, recording 17.8 mg Trolox/g dry weight in the DPPH assay. The total phenolic contents of the naked oat samples analyzed in the present study were comparable to those previously reported for five oat cultivars by Emmons and Peterson (1999) (238-278 μg GAE/g), as well as for oat grains (300 μg GAE/g). Holasova et al. (2002) reported a considerably higher total phenolic concentration (1138 μg GAE/g) in an unspecified oat cultivar.

Phenolic compounds of oat samples sprouted with GA and water

The phenolic components of oat samples germinated by treatment with gibberellic acid (GA) and water are shown in Table 2.

Table 2. Phenolic compounds of oat samples (mg/100g)

Phenolic acids	Control			Gibberellic acid			Water		
Gallic acid	1.34	±	0.58a*	1.12	±	0.21b	0.71	±	0.04c
Protocatechuic acid	0.06	±	0.01c	0.14	±	0.02b	0.27	±	0.04a
Chlorogenic acid	0.04	±	0.02c	0.08	±	0.01b	0.19	±	0.03a
Caffeic acid	0.26	±	0.03a	0.08	±	0.01b	0.07	±	0.01bc
Coumaric acid	0.04	±	0.02	0.04	±	0.00	0.04	±	0.00
Ferulic acid	0.03	±	0.01c	0.29	±	0.04b	0.49	±	0.02a
Cinnamic acid	0.05	±	0.03b	0.04	±	0.00bc	0.15	±	0.02a
Flavonoids	Control			Gibberellic acid			Water		
Catechin	0.38	±	0.28c	1.02	±	0.10b	2.47	±	0.21a
Rutin	4.69	±	0.25a	0.79	±	0.04b	0.60	±	0.09c
Hesperidin	0.14	±	0.06	0.45	±	0.06	0.31	±	0.06
Quercetin	0.91	±	0.49b	0.95	±	0.16a	0.51	±	0.05c
Kaempferol	0.13	±	0.03c	0.19	±	0.01b	0.55	±	0.12a

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

In general, the phenolic acid content (except caffeic acid) of oat sprouts germinated by water treatment was higher than the control. In addition, the phenolic acid content (gallic acid and caffeic acid) of oat samples germinated by water treatment was higher than that of those treated with GA. The increased phenolic acids in water-treated oat sprouts versus controls (excluding caffeic acid) result from germination-induced activation of endogenous metabolism. Water hydration supports these natural responses without external hormonal influence, whereas gibberellic acid favors rapid growth and reserve use, reducing secondary metabolism and causing dilution. Caffeic acid shows distinct regulation within the pathway during germination and sprouting processes (Gan et al., 2016; Ding et al., 2018; Shah et al., 2023). The dominant phenolic acid in the oat samples was gallic acid, and the gallic acid content of the oat samples ranged from 0.71

(Water) to 1.34 mg/100g (control). The amounts of other phenolic acids were found to be below 0.49 mg/100g. In general, the flavonoid content of oat sprouts germinated after treatment with GA was significantly increased compared to the control group (except rutin). In addition, while the catechin, hesperidin, and kaempferol contents of oat samples germinated by water treatment increased compared to the control (non-germinated) sample, the rutin and quercetin contents of the oat samples were slightly decreased compared to the control. Catechin and rutin amounts of the nongerminated and germinated oat samples were characterized to be between 0.38 (control) and 2.47 mg/100g (Water) to 0.60 (Water) and 4.69 mg/100g (control), respectively. Also, quercetin amounts of the oat samples changed between 0.51 (Water) and 0.95 mg/100g (GA). The levels of hesperidin and kaempferol were detected as below 0.55 mg/100g. Consequently, the routine content of oat sprouts germinated after treatment with GA and water was reduced by approximately 83% and 87% compared to the control, respectively. In addition to the common phenolic aldehydes and acids previously reported in oat - namely protocatechuic acid, *p*-hydroxybenzaldehyde, vanillin, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid - protocatechuic aldehyde and syringic acid were likewise detected in the analyzed samples (Varga et al., 2018). Metabolic and physiological changes occurring during oat (*Avena sativa* L.) germination can significantly affect the nutritional and functional properties of the grain. In this process, avenanthramides (AVNs) are of particular importance due to their oat-specific phenolic amide properties and strong antioxidant and anti-inflammatory activities. Current studies show that germination significantly stimulates avenanthramide biosynthesis, and this increase depends on germination time, genotype, and environmental conditions (Jágr et al. 2024).

Fatty acid composition of the oils extracted oat samples sprouted with GA and water

The fatty acid compositions of oils extracted from oat sprouts germinated by treatment with GA solution and water, and from ungerminated seeds, are given in Table 3.

Table 3. Fatty acid composition of oat samples

Fatty acids (%)	Control			Gibberellic acid			Water		
Palmitic	26.78	±	0.13b*	26.32	±	1.62c	36.22	±	0.14a
Stearic	5.75	±	0.06b	3.98	±	0.17c	10.06	±	0.01a
Oleic	40.31	±	0.03b	44.22	±	0.63a	35.62	±	0.09c
Linoleic	26.24	±	0.02a	24.54	±	0.49b	17.31	±	0.07c
Linolenic	0.71	±	0.01b	0.73	±	0.03a	0.55	±	0.00c
Behenic	0.21	±	0.03c	0.40	±	0.02a	0.25	±	0.01b

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

The oil content of the oat samples was calculated on a dry matter basis. Palmitic and stearic acid amounts of the oat oils were identified to be between 26.32 (GA) and 36.22 (Water) to 3.98 (GA) and 10.06% (Water), respectively. In addition, oleic and linoleic acid contents of the oat oils were determined to be between 35.62 (Water) and 44.22% (GA) and 17.31 (Water) and 26.24% (Control), respectively. Linolenic and behenic acid amounts of the oat oils were established to be between 0.55 (Water) and 0.73 (GA) to 0.21 (Water) and 0.40% (GA), respectively. Overall, the fatty acid composition (excluding palmitic and stearic acids) of oils extracted from oat sprouts germinated with GA solution was found to be higher than that of the control group and oat sprouts germinated with water. In contrast, the highest amounts of palmitic and stearic acids were determined in oat seed oil germinated in water. Oil from oat sprouts germinated with GA_3 shows higher fatty acid levels (except palmitic and stearic acids) than

control and water-germinated sprouts, likely due to GA_3 stimulating seed metabolism during germination. As a result, nutritionally important unsaturated fatty acids as oleic, linoleic, and α-linolenic acids increase, while saturated fatty acids remain unchanged or decrease as they are substrates for further modification. GA_3 supports membrane biogenesis requiring unsaturated fatty acids for fluidity and function (Nonogaki, 2019; Zhou et al., 2023).The major fatty acids identified in oils extracted from oat cultivars include palmitic, stearic, oleic, linoleic, and linolenic acids, with concentrations ranging from 15.5-17.3%, 1.3-2.2%, 33.4-42.2%, 34.4-42.7%, and 1.1-1.9%, respectively (Brindzová et al., 2008). The lipid composition of foods is considered an important nutritional attribute, and the fatty acid profile may significantly influence the stability and shelf life of cereal-based products (Brindzová et al., 2008). Compared with hulled oat, hulless oat seed exhibited significantly higher levels of oleic acid (C18:1 n-9; 44.6% vs. 39.1%), stearic acid (C18:0; 2.255% vs. 1.523%), and arachidic acid (C20:0; 0.187% vs. 0.153%). Conversely, the concentrations of myristic acid (C14:0; 0.260% vs. 0.369%), palmitoleic acid (C16:1 n-7; 0.255% vs. 0.297%), and vaccenic acid (C18:1 n-7; 0.997% vs. 1.165%) were significantly lower in hulless oat than in hulled oat (Wojtacki et al., 2024).

Mineral contents of oat samples sprouted with GA and water

The macroelement contents of oat sprouts germinated by treatment with GA solution and water, and of ungerminated oat seeds, are presented in Table 4. Mineral contents of oat samples is given as dry matter, and the macroelement detected in the highest amounts in oat samples was P, followed in decreasing order by K, Ca, Mg, and S.

Table 4. Macro and micro element contents of oat samples (mg/kg; dry weight)

Samples	P	K	Ca	Mg	S
Control	1432.51±1.53 B*	609.89±0.90 B	584.32±0.67 B	109.45±0.098 C	0.92±0.06 B
Gibberellic acid	1434.94±1.54 B	664.55±0.66 A	643.64±0.77 A	117.21±0.112 B	1.31±0.09 A
Water	1955.13±1.37 A*	444.41±0.54 C	341.80±0.45 C	232.17±0.127 A	0.41±0.02 C
Samples	Fe	Zn	Cu	Mn	B
Control	0.047±0.011 C	0.022±0.001 C	0.004±0.0001 C	0.004±0.001 C	0.009±0.005B
Bibberellic acid	0.071±0.022 B	0.032±0.005 B	0.005±0.0001 B	0.005±0.001 B	0.006±0.001C
Water	0.179±0.043 A	0.122±0.034 A	0.008±0.0001 A	0.024±0.001 A	0.095±0.007 A

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

P and K amounts of oat samples were characterized to be between 1432.51 (control) and 1955.13 mg/kg (Water) to 444.41 (Water) and 664.55 mg/kg (GA), respectively. In addition, Ca and Mg contents of the oat samples were assessed to be between 341.80 (Water) and 643.64 mg/kg (GA) to 109.45 (control) and 232.17 mg/kg (Water), respectively. The highest sulfur (S) content was detected in the oat sample treated with GA solution (1.31 mg/kg), followed in decreasing order by control (0.92 mg/kg) and the water-treated sample (0.41 mg/kg). A significant increase in the K, Ca, and S content of oat seeds was observed with GA application. Overall, the GA-treated oat sample and the control group had higher K, Ca, and S content compared to the water-treated sample. Gibberellic acid may increase nutrient accumulation by stimulating plant growth, root development, ion uptake, and nutrient translocation. Studies have reported enhanced K and S accumulation following GA_3 application, while GA_3 can also modify Ca uptake and cation selectivity. Thus, the higher K, Ca, and S may reflect enhanced nutrient acquisition and transport (Montague et al., 1973; Castro and Oliveira, 1982). Fe and Zn amounts of the oat samples were assayed to be between 0.047 (Control) and 0.179 mg/kg (Water) to 0.022 (control) and 0.122 mg/kg (Water), respectively. In addition, Cu and Mn amounts of oat samples were characterized to be between 0.004

(control) and 0.008 mg/kg (Water) to 0.004 (Control) and 0.024 mg/kg (Water), respectively. The microelement levels in oats germinated following gibberellic acid treatment were lower than those observed in water-germinated samples, yet remained higher than those of the control group, with sulfur (S) being the only exception. Gibberellic acid (GA_3) may accelerate germination, reserve mobilization, and seedling growth, increasing nutrient utilization and causing a dilution effect in rapidly growing tissues; therefore, micronutrient concentrations may be lower than in water-treated seeds. Nevertheless, enhanced mineral mobilization during germination can maintain higher levels than the ungerminated control, except for S (Chauhan et al., 2019). Compared with the water-treated sprouts, gibberellic acid (GA_3)-treated oats showed lower micronutrient concentrations, likely because GA_3 promoted faster growth, causing a dilution effect as biomass increased more rapidly than mineral accumulation (Nonogaki et al., 2019). The germ portion of cereal seeds is a valuable source of nutrients, including minerals such as phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), and sulfur (S), in addition to dietary fiber (Sidhu et al., 2007).

CONCLUSIONS

This research shows that germination improves the nutritional and functional characteristics of oat sprouts, with gibberellic acid producing the greatest benefits. It increases antioxidant activity, moisture content, beneficial phenolic and flavonoid compounds, essential minerals, and favorable fatty acids while altering phenolic acid composition. These findings support its use to enhance oats for functional food development.

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