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Effect of growing conditions on proximate, mineral, amino acid, phenolic composition and antioxidant properties of wheatgrass from different wheat (*Triticum aestivum* L.) varieties

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ABSTRACT

Wheatgrass juice powder (WJP) from four wheat varieties grown using soil, coco-peat with nutrient solution (CNS) and water (soaked (8 h), germinated (36 h) and harvested on 10th day) were examined for proximate composition, mineral, amino acid, phenolic (free and bound) composition and antioxidant properties. The yield, ash and protein contents of WJP ranged between 4.88–7.87%, 5.18–15.93% and 38.75–50.17%, respectively. The total phenolic, flavonoid, chlorophyll content (TCC) and antioxidant activity varied from 12.02 to 17.44 mg GAE/g, 4.38–10.10 mg QE/g, 3.01–5.63 mg/g, and 13.54–17.33 µmol TE/g, respectively. HD-3086 grown using soil exhibited highest antioxidant properties, TCC and Mg content. WJP of C-306 grown using CNS had abundant essential amino acids (AAs). Phenolic acids (ferulic, syringic and sinapic acids) and flavonoids (catechin, rutin, vitexin and isovitexin) and minerals (K, P, Ca, Mg, Na and Fe) were predominant in WJP. The AAs and free phenolics were more in CNS and soil grown WJP, respectively.

1. Introduction

Wheat (*Triticum aestivum L.*) is the widely cultivated staple food crop for the majority of the world's population (Akbas, Kilercioglu, Onder, Koker, Soyler, & Oztop, 2017). The wheat grains are rich sources of carbohydrates, minerals, proteins, vitamins, dietary fibre and various phytochemicals that possess health-promoting activities (Zhu and Sang, 2017). Germination and sprouting have been known to elevate vitamins, minerals, phenolic compounds and antioxidant potential of wheat (Kulkarni, Acharya, Nair, Rajurkar, & Reddy, 2006; Fortună et al., 2018). The sprouts formed by germination of wheat grains over a period of 6–10 days are generally termed as "wheatgrass" (Akbas et al., 2017; Benincasa, Falcinelli, Lutts, Stagnari, & Galieni, 2019). Therapeutic potential of wheatgrass have been attributed to its rich nutrient contents including vitamins (A, B, C and E), minerals, flavonoids, phenolics (ferulic, gallic, sinapic, syringic, p-coumaric acids), amino acids, chlorophylls and active enzymes (Kulkarni, Acharya et al., 2006; Ghumman, Singh, & Kaur, 2017; Fortună et al., 2018). Wheatgrass is mainly used in the production of juice, which is consumed raw or dried into powder (Ghumman et al., 2017).

Wheatgrass juice is a good source of chlorophyll (70% of the total chemical constituents) and antioxidants known for numerous health benefits (Ghumman et al., 2017). Chlorophyll is structurally related to hemoglobin and bilirubin and has been proposed as an important

Abbreviations: WJP, Wheatgrass juice powder; CNS, Coco-peat with nutrient solution; MC, Moisture content; PC, Protein content; AC, Ash content; TAA, Total antioxidant activity; TFC, Total flavonoid content; TCC, Total chlorophyll content; TPC, Total phenolic content; AAs, Amino acids; EAAs, Essential amino acids; NEAAs, Non-essential amino acids; GABA, γ-aminobutyric acid; Ca, Calcium; K, Potassium; Fe, Iron; Mg, Magnesium; Zn, Zinc; Cu, Copper; Mn, Manganese; PCA, Protocatechuic acid; FRA, Ferulic acid; p-CA, p-Coumaric acid; GAC, Gallic acid; SYA, Syringic acid; CFA, Caffeic acid; CGA, Chlorogenic acid; SPA, Sinapic acid; CMA, Cinnamic acid

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dietary chemopreventive agent (Vaňková et al., 2018). The phenolic compounds represent an another interesting component of the wheatgrass that reverses the effect of oxidative stress and reduces the incidence of various degenerative diseases and disorders (Calzuola, Marsili, & Gianfranceschi, 2004). Consumption of wheatgrass juice decreases the requirement of medications during chemotherapy-induced hematological toxicity in breast cancer patients due to its antioxidant, anti-mutagenic, anti-inflammatory and apoptotic activities (Bar-Sela, Tsalic, Fried, & Goldberg, 2007). Furthermore, wheatgrass extract has been found to be an effective tonic for people suffering from thalassemia, diabetes, rheumatoid arthritis, inflammatory disorders, obesity, leucoderma, ulcerative colitis and hematological diseases (Bar-Sela et al., 2007, 2015).

Different growing conditions (soil, tap water, soil with nutrients, tap water with nutrients, peat with or without fertilizer) have been used for wheatgrass cultivation (Kulkarni, Acharya et al., 2006; Özköse, Arslan, & Aysenur, 2016). In recent studies, attention has been directed toward using soil-less growth media for the production of microgreens (Verlinden, 2020; Di Gioia, Renna, & Santamaria, 2017; Muchjajib, Muchjajib, Suknikom, & Butsai, 2014). Soilless cultivation have been designed to overcome seasonal and agro-climatic limitations of a crop. The soilless media ascertains uniform growth under hygienic, pest and insect free growing conditions with judicious water and nutrient utilization. The microgreens growers have been exploring less expensive organic by-products as growing media. Coconut coir dust commercially known as coco-peat is a low cost, easily available and sustainable growing media for soil-less cultivation (Awang, Shaharom, Mohamad, & Selamat, 2009; Muchjajib et al., 2014). The supplemental application of nutrients can be used in soilless cultivation. The irrigation of the soilless medium with NPK solution provides necessary nutrients and enhances the growth of microgreen seedlings (Murphy & Pill, 2010).

The popularity of the wheatgrass juice has been increased over the past two decades due to growing awareness of its nutritional value and health-promoting activities. The previous studies have reported the effect of growing conditions on water-soluble dry matter, mineral, protein content and antioxidant potential of wheatgrass juice or extract (Kulkarni, Acharya et al., 2006; Özköse, Arslan, & Aysenur, 2016). However, the literature on amino-acid composition, mineral composition and phenolic composition (free and bound) of freeze-dried WJP from different wheat varieties is limited. It is interesting to explore how growing conditions affects antioxidant properties and the nutritional composition of wheatgrass. Therefore, the purpose of the present study was to investigate the effect of three different growing conditions (soil, coco-peat with nutrient solution and water) on the chemical composition, nutritional value and antioxidant properties of wheatgrass from different wheat varieties.

2. Materials and methods

2.1. Materials

Four wheat (*Triticum aestivum* L.) varieties, namely HD-3086 (hard wheat), C-306 (hard wheat), HD-2967 (medium-hard wheat) and HS-490 (soft wheat) were procured from the Indian Agricultural Research Institute (IARI), New Delhi. All chemicals and solvents used were purchased from Merck and standards from Sigma-Aldrich.

2.2. Wheatgrass cultivation

The seeds of wheat varieties were washed with distilled water, soaked for 8 h and drained. The soaked grains were germinated for 36 h in dark and sown in trays (40 × 30 × 10 cm) containing (i) soil, (ii) coco-peat with nutrient solution and (iii) water. For each growing condition, a total of 150 g of wheat grains were allocated and three trays (each containing 50 g of wheat grains) were cultivated. In the first growing condition, the germinated grains were spread on soil trays sprinkled with water and covered with dry soil. The clay loam soil containing Ca (71.82 mg/g), Mg (259.80 mg/g), Fe (9.80 mg/g), N (0.38 mg/g), P (0.57 mg/g), K (0.71 mg/g), Na (0.39 mg/g), Mn (0.21 mg/g), Zn (0.024 mg/g), Cu (0.011 mg/g) and pH of 7.94 was used for wheatgrass cultivation. In the second growing condition, coco-peat with nutrient solution (CNS) was used for wheatgrass cultivation. The nutrient solution was prepared by adding NPK solution (6 ml/l) in water. The composition of NPK solution was nitrogen (80.3 mg/ml), calcium (78.2 mg/ml), zinc (1.4 mg/ml), phosphate (80.5 mg/ml), iron (1.6 mg/ml), boron (0.8 mg/ml), potassium (80 mg/ml), sulphur (45.2 mg/ml) and magnesium (32.8 mg/ml). The trays containing cocopeat were provided with nutrient solution twice a day. In the third growing condition, germinated grains were spread in plastic trays, covered with wet soaking paper and watered every 3 h to maintain moisture. The trays were uncovered (paper removed) on the second day and wheat plants were watered twice a day throughout the growing period. The potable water containing Ca (52.05 mg/l), Na (48.37 mg/l), Mg (28.12 mg/l), K (6.92 mg/l), nitrate (2.61 mg/l), phosphate (0.024 mg/l), Cu (0.12 mg/l), Zn (0.05 mg/l), total dissolved solids (318 mg/l) and pH of 7.29 was used for all the growing conditions. All trays were placed under natural sunlight conditions (average daylight and sunshine of 10.5 and 7.6 h, respectively) with daily minimum and maximum air temperature ranged from 9 to 12 °C and from 21 to 26 °C, respectively. The relative humidity ranged between a minimum of 32% and a maximum of 73% during the growth of wheat plants.

2.3. Production of wheatgrass juice powder

The wheatgrass was harvested on the 10th day of germination, washed with distilled water and the juice was squeezed by a manual screw juicer. The juice obtained was freeze-dried and the percentage yield (%, w/w) of wheatgrass juice powder (WJP) was calculated using the following equation.

$$\text{Yield (\%)} = W_p/W_J \times 100$$

where W_p is the weight of freeze-dried WJP and W_J is the weight of wheatgrass juice. The freeze-dried WJP was stored at −30 °C for further analysis.

2.4. Proximate composition

The moisture content (MC) of WJP was determined by oven-drying at 130 °C as per method 44-19 and ash content (AC) was estimated according to method 08–01 by burning of organic matter in a muffle furnace at 550 °C (AACC, 2000). Protein content (PC) of WJP was measured by the standard Kjeldahl method and was estimated by multiplying nitrogen content with protein conversion factor of 6.25 (AACC, 2000).

2.5. Total chlorophyll content (TCC)

DMSO (Dimethyl sulfoxide) method described by Hiscox and Israelstam (1979) was used for the determination of TCC in WJP. Briefly, 100 mg of WJP mixed in 1 ml of DMSO was incubated at 65 °C for 15 min, centrifuged at 8000 rpm for 10 min to collect the supernatant. The leftover sample was again extracted with 1 ml DMSO and the supernatant collected was made up to a final volume of 10 ml with DMSO. The absorbance of the chlorophyll extract was recorded at 645 nm and 663 nm using a Carry-60 UV–VIS spectrophotometer (Agilent Technologies, USA). The following equations were used to calculate chlorophyll *a* and b content (mg/g) in WJP and TCC was measured by adding values of these two equations.

$$\text{Chlorophyll } a = [(12.7 \times A_{663})\text{-}(2.69 \times A_{645})] \times \text{ml DMSO/mg sample}$$

$$\text{Chlorophyll } b = [(22.9 \times A_{645})\text{-}(4.68 \times A_{663})] \times \text{ml DMSO/mg sample}$$

2.6. Mineral composition

The mineral composition of WJP was estimated using iCE™ 3400 Atomic Absorption Spectrometer (Agilent Technologies, USA) as described previously (Bhinder, Kaur, Singh, Yadav, & Singh, 2020). Briefly, WJP (1 g) taken in a crucible was charred and burned at 600 °C in a muffle furnace. The ash obtained was dissolved in 2.5 ml of nitric acid (1 N) solution, filtered and diluted with Milli-Q water. The diluted filtrate was analyzed for Zn, Ca, Fe, Cu, K, Mg, Mn, Na and P contents using Atomic Absorption Spectrometer. The instrument was calibrated with standard mineral stock solutions.

2.7. Amino acid (AA) profile

The AA profile of WJP was determined by Shimadzu LC-30 AD HPLC system fitted with a fluorescence detector and C18 column as previously reported (Bhinder et al., 2020). Briefly, 100 mg of WJP was digested with 6 N HCl for 24 h at 110 °C under anaerobic conditions. The derivatization of amino acids was carried out with mercaptopropionic acid, *o*-phthaladehyde and 9-fluorenyl methyl chloroformate. The pump applied a gradient of mobile phase containing (I) 20 mM/L phosphate buffer and (II) water: acetonitrile: methanol (15:45:40, v/v/v) with a flow rate of 1 ml/min at oven temperature of 40 °C. The gradient elution conditions were: I (88%) and II (12%) from 0 to 2 min; I (83%) and II (17%) from 2 to 4 min; I (69%) and II (31%) from 5 to 8.5 min; I (67.5%) and II (32.5%) from 8.5 to 14 min; I (53.5%) and II (46.5%) from 14 to 16 min; I (45%) and II (55%) from 16 to 19 min; I (0%) and II (100%) from 19 to 25 min. The peaks detected at 254 nm were analyzed using LAB Solutions 5.54SP 5 software and AA contents in mg/100 g were established as described in previous reports (Kumari, Bhinder, Singh, Kaur, & Singh, 2020; Bhinder et al., 2020).

2.8. Phenolic content and composition

The free and bound phenolics were extracted from WJP using method described elsewhere with minor modifications (Guo, Guo, Li, Fu, & Liu, 2017). For free phenolics, WJP (0.5 g) was extracted with 30 ml of 80% methanol, homogenized and centrifuged at 5000 rpm for 5 min. The residue was resuspended in 80% methanol (30 ml) and extraction was repeated twice. The supernatant collected was dried at 45 °C using rotavapor under reduced pressure. The residue left was further utilized for the extraction of bound phenolics by treating with 20 ml of NaOH (4 M) for 2 h. The pH of the mixture was adjusted to 2 and the mixture was centrifuged at 3000 rpm for 5 min. Then, the supernatant containing bound phenolics was extracted with 20 ml of ethyl acetate and diethyl ether mixture (1:1, v/v) for three times. The organic layer was collected and dried at 45 °C under reduced pressure. The residue was re-dissolved in 1 ml of methanol/water (80:20, v/v), filtered through 0.45 µm filter and the filtrate was analysed for bound phenolics. The extracts were used for the estimation of free and bound phenolics, flavonoids, antioxidant activity and phenolic composition.

2.8.1. Total phenolic content (TPC)

TPC of WJP was estimated using the method given by Singleton, Orthofer, and Lamuela-Raventós (1999) with minor modifications as described elsewhere (Singh, Kaur, Shevkani, & Singh, 2015). Briefly, WJP extract (100 µl) was diluted with 4.8 ml of distilled water and Folin-Ciocalteu reagent (300 µl) was added. After 8 min of incubation, 20% sodium carbonate (900 µl) was added with continuous vortex-mixing and the solution was incubated at 40 °C for 30 min. Then, the absorbance of the solution was recorded at 765 nm on UV–VIS spectrophotometer. The free, bound and total phenolics were reported as mg gallic acid equivalents (GAE)/g dry weight basis (DWB).

2.8.2. Total flavonoid content (TFC)

The TFC was quantified using the method published by Heimler, Pieroni, Cimato, Galardi, and Romani (2002) with minor modification as described in our previous report (Singh et al., 2015). Briefly, 250 µl of WJP extract was mixed with 1.25 ml of deionized water and 5% sodium nitrite (75 µl) solution was added. After 6 min of incubation, 150 µl of aluminum chloride hydrate solution (10%) was added and the solution was allowed to react for 5 min. Then, 1 M NaOH solution (500 µl) and ethanol (275 µl) were added. The volume of solution was raised to 2.5 ml with deionized water and absorbance was measured at 510 nm using a UV–VIS spectrophotometer. The free, bound and total flavonoids were expressed as mg quercetin equivalents (QE)/g on DWB.

2.8.3. Phenolic composition

The phenolic composition of WJP extracts was estimated with Agilent 1260 Infinity HPLC system equipped with binary pump, Diode Array type detector (DAD-PDA), C18 column and rapid separation autosampler. The binary elution phase consist of (I) trifluoroacetic acid (0.1%) in water and (II) acetonitrile (50%), water (49.8%) and trifluoroacetic acid (0.2%) with a flow rate of 1 ml/min. The gradient elution conditions were : I (95%) and II (5%) from 0 to 5 min; I (95–75%) and II (5–25%) from 5 to 40 min; I (75–62%) and II (25–38%) from 40 to 47 min; I (62–55%) and II (38–45%) from 47 to 49 min; I (55%) and II (45%) from 49 to 51 min; I (55–20%) and II (45–80%) from 51 to 70 min; I (20–5%) and II (80–95%) from 70 to 75 min; I (5–95%) and II (95–5%) from 75 to 77 min; I (95–5%) and II (5–95%) from 77 to 90 min. The peaks of phenolic compounds were detected at different wavelengths (280 nm, 320 nm, 350 nm, and 367 nm) with a C18 column maintained at 35 °C. The results were calculated through area normalization and expressed as µg/100 g on DWB.

2.9. Total antioxidant activity (TAA)

The TAA of free and bound extracts of WJP was estimated using DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay as described previously (Bhinder et al., 2019). Briefly, WJP extract (100 µl) was reacted with 3.9 ml of freshly prepared DPPH solution (6×10^{-9} mol l^{-1}) and incubated at 25 °C for 30 min. The absorbance of the solution was measured at 515 nm using a UV-VS spectrophotometer. TAA of extracts was determined as µmol Trolox equivalents (TE)/g on DWB.

2.10. Statistical analysis

Experiments were carried out in triplicates and the data were reported as mean values ± standard deviation. Two-way analysis of variance (ANOVA) was performed on data to determine the difference between mean values. Principal component (PCA) and Pearson correlation analysis were employed on the data to determine the relation among various parameters. Minitab software (Minitab, version 14.12.0, U.S.A.) was used for statistical evaluation.

3. Result and discussion

3.1. Wheatgrass yield

The WJP yield of four wheat varieties grown using soil, CNS and water varied from 4.88 to 7.87% (Table 1). The wheat varieties grown using CNS showed higher yield (6.89–7.87%) than those grown using soil (6.36–6.76%) and water (4.88–6.09%). The higher yield in CNS could be due to the use of NPK fertilizer solution. Similar results were noted by Seadh, Abido, and Ghazy (2017) who revealed that the application of NPK fertilizer significantly increases the yield and growth characters of wheat plants. NPK solution increases plant growth due to the vital contribution of nitrogen in several biochemical processes that stimulates meristematic activity and increases the vegetative growth of plants. Phosphorus is an important component of high energy

Table 1
Effect of growing conditions on proximate composition, chlorophyll content and mineral composition of WJP from different wheat varieties.

	Wheat Varieties					
	C306			HD3086		
	Soil	CNS	Water	Soil	CNS	Water
Yield (%)	6.52 ± 0.03^{d}	6.89 ± 0.05^{d}	4.88 ± 0.06^{a}	6.71 ± 0.05^{d}	7.23 ± 0.11^{e}	5.41 ± 0.03^{b}
MC (%)	12.30 ± 0.05^{d}	9.40 ± 0.04^{c}	14.05 ± 0.06^{f}	7.38 ± 0.20^{a}	8.80 ± 0.01^{b}	9.36 ± 0.08^{c}
AC (%)	12.87 ± 0.04^{e}	11.58 ± 0.07^{d}	5.18 ± 0.02^{a}	15.93 ± 0.07^{g}	14.61 ± 0.05^{f}	5.95 ± 0.03^{b}
PC (%)	46.83 ± 0.41^{e}	50.17 ± 0.05^{g}	45.50 ± 0.24^{d}	44.26 ± 0.52^{c}	48.44 ± 0.50^{f}	44.70 ± 0.15^{c}
TCC (mg/g)	5.38 ± 0.23^{e}	4.91 ± 0.06^{d}	4.88 ± 0.06^{d}	5.63 ± 0.06^{f}	5.49 ± 0.08^{e}	4.39 ± 0.66^{c}
K (mg/kg)	8154.66 ± 43.52^{e}	6080.00 ± 19.51^{c}	3474.46 ± 25.50^{a}	9571.66 ± 27.53^{f}	7842.22 ± 10.00^{e}	5530.00 ± 17.32^{b}
P (mg/kg)	3839.86 ± 29.00^{c}	5058.26 ± 39.02^{f}	3443.40 ± 18.88^{b}	4876.46 ± 75.51^{e}	6243.66 ± 44.52^{g}	4550.33 ± 50.01^{e}
Ca (mg/kg)	2990.13 ± 10.00^{e}	847.40 ± 12.50^{b}	441.53 ± 13.50^{a}	2996.40 ± 17.03^{e}	1845.00 ± 45.00^{d}	1120.00 ± 20.00^{c}
Mg (mg/kg)	1086.46 ± 4.50^{d}	869.73 ± 10.01^{b}	1032.66 ± 33.00^{c}	1645.66 ± 4.50^{f}	1452.66 ± 14.01^{e}	1120.66 ± 34.00^{d}
Na (mg/kg)	727.40 ± 7.50^{b}	1098.06 ± 42.51^{i}	1048.26 ± 27.00^{h}	905.73 ± 40.02^{f}	1150.00 ± 50.00^{j}	882.00 ± 7.54^{e}
Fe (mg/kg)	495.33 ± 11.06^{a}	520.13 ± 20.00^{a}	484.93 ± 15.00^{a}	1157.60 ± 12.04^{b}	1568.33 ± 10.40^{e}	1010.33 ± 10.50^{d}
Zn (mg/kg)	54.33 ± 5.50^{b}	52.33 ± 2.51^{b}	42.39 ± 4.39^{a}	50.00 ± 5.00^{b}	62.33 ± 7.50^{c}	41.66 ± 4.04^{a}
Mn (mg/kg)	48.85 ± 2.33^{c}	37.70 ± 2.25^{b}	23.28 ± 0.32^{a}	97.33 ± 7.50^{f}	88.60 ± 1.50^{e}	65.00 ± 10.00^{d}
Cu (mg/kg)	1.20 ± 0.20^{a}	1.66 ± 0.30^{a}	1.26 ± 0.11^{a}	4.40 ± 0.40^{d}	5.16 ± 0.76^{e}	3.16 ± 0.28^{c}

	Wheat Varieties					
	HD2967			HS490		
	Soil	CNS	Water	Soil	CNS	Water
Yield (%)	6.36 ± 0.06^{c}	7.87 ± 0.05^{f}	6.09 ± 0.06^{c}	6.76 ± 0.03^{d}	7.11 ± 0.11^{e}	5.16 ± 0.07^{b}
MC (%)	9.30 ± 0.05^{c}	8.12 ± 0.10^{b}	13.52 ± 0.06^{e}	8.39 ± 0.07^{b}	8.81 ± 0.10^{b}	9.40 ± 0.05^{c}
AC (%)	14.76 ± 0.09^{f}	15.79 ± 0.05^{g}	6.06 ± 0.05^{b}	15.08 ± 0.06^{f}	15.05 ± 0.01^{f}	8.34 ± 0.06^{c}
PC (%)	41.45 ± 0.15^{b}	44.60 ± 0.35^{c}	38.75 ± 0.12^{a}	44.27 ± 0.33^{c}	46.30 ± 0.30^{d}	43.31 ± 0.61^{c}
TCC (mg/g)	4.70 ± 0.12^{d}	4.12 ± 0.38^{c}	4.28 ± 0.46^{c}	4.23 ± 0.10^{c}	3.73 ± 0.38^{b}	3.01 ± 0.005^{a}
K (mg/kg)	8386.00 ± 44.67^{e}	7601.00 ± 43.50^{d}	3221.00 ± 11.00^{a}	5384.40 ± 5.10^{b}	3097.00 ± 11.53^{a}	3596.66 ± 8.02^{a}
P (mg/kg)	3994.60 ± 20.55^{c}	5100.00 ± 20.0^{f}	3552.66 ± 21.00^{c}	2795.20 ± 29.07^{b}	4219.20 ± 19.00^{d}	2608.60 ± 41.63^{a}
Ca (mg/kg)	2565.60 ± 56.83^{e}	1862.33 ± 17.50^{d}	1842.33 ± 17.50^{d}	1338.00 ± 12.53^{c}	679.73 ± 9.01^{b}	1117.00 ± 17.52^{c}
Mg (mg/kg)	1127.80 ± 17.50^{d}	1012.00 ± 14.52^{c}	800.00 ± 10.00^{b}	1027.06 ± 23.00^{c}	858.33 ± 8.50^{b}	710.66 ± 8.02^{a}
Na (mg/kg)	782.93 ± 17.80^{c}	886.00 ± 12.52^{e}	655.33 ± 10.50^{a}	895.73 ± 8.01^{e}	830.13 ± 14.00^{d}	861.86 ± 8.00^{e}
Fe (mg/kg)	772.80 ± 7.00^{b}	882.00 ± 8.00^{c}	530.00 ± 6.00^{a}	577.73 ± 7.015^{a}	817.53 ± 17.50^{c}	450.63 ± 9.55^{a}
Zn (mg/kg)	60.66 ± 6.02^{c}	71.66 ± 4.04^{d}	48.33 ± 1.52^{b}	70.80 ± 2.83^{d}	88.00 ± 3.00^{e}	66.33 ± 3.51^{d}
Mn (mg/kg)	65.33 ± 5.50^{d}	54.86 ± 3.11^{c}	42.333 ± 6.50^{b}	53.60 ± 1.39^{c}	42.53 ± 2.39^{b}	27.26 ± 0.30^{a}
Cu (mg/kg)	2.33 ± 0.11^{b}	2.16 ± 0.28^{b}	1.02 ± 0.064^{a}	2.05 ± 0.12^{b}	1.60 ± 0.40^{a}	1.20 ± 0.19^{a}

Values with similar superscripts in a row do not differ significantly ($p < 0.05$). CNS, Cocopeat with nutrient solution; MC, Moisture content; AC, Ash content; PC, Protein content; TCC, Total chlorophyll content; K, Potassium; P, Phosphorus; Ca, Calcium; Mg, Magnesium; Na, Sodium; Fe, Iron; Zn, Zinc; Mn, Manganese; Cu, Copper.

compounds and is involved in activating the enzyme and osmotic regulation, thus enhances the growth of plants (Seadh et al., 2017). The results of higher yield of wheatgrass grown using NPK solution are in good agreement with those reported for the pumpkin (Oloyede, 2012) and *Amaranthus* species (Oyedeji, Animasaun, Bello, & Agboola, 2014). Another study by Murphy and Pill (2010) also reported that the supplementation of soilless medium with NPK solution enhances the growth of Arugula seedlings. HD-2967 wheat variety grown using CNS showed the highest yield of WJP. The yield of WJP showed higher significant variation ($P \leq 0.005$) among growing conditions than the wheat varieties (Table S1). Pearson correlation showed a highly significant positive correlation of WJP yield with AC ($r = 0.839$, $p \leq 0.005$) and significant positive correlation with P content ($r = 0.596$, $p \leq 0.05$) as shown in Table 5.

3.2. Proximate composition

MC, AC and PC of WJP varied from 7.38 to 14.05%, 5.18 to 15.93% and 38.75 to 50.17%, respectively for wheat varieties grown using soil, CNS and water (Table 1). The study conducted by Devi, Bains, and Kaur (2019) reported lower MC (1.55%) and PC (30.04%) while comparable AC (8.27%) in freeze-dried WJP. Ghumman et al. (2017) also reported lower PC (22.01% to 25.77%) and comparable AC (7.55 to 18.51%) in freeze-dried WJP and shoot powder of two wheat varieties. The difference in MC and PC of WJP could be related to the differences in harvesting conditions, environmental factors and genotype of the wheat varieties. The highest and lowest PC in WJP was observed in C-306 and HD-2967 variety, respectively. PC was higher in WJP of wheat varieties grown using CNS followed by soil and water. Our results concur with Oyedeji et al. (2014) who reported that the NPK grown *Amaranthus* species had the highest PC than the control samples. Özköse, Arslan, and Aysenur (2016) observed similar results and explained that fertilization led to an increase in the PC of WJP. Another study by Zhang et al. (2017) also reported higher PC in grains of wheat plants grown using NPK fertilizer. The higher significant effect ($P \leq 0.005$) of growing conditions than the wheat varieties on MC, AC and PC of WJP was observed (Table S1). MC exhibited a significant negative correlation with AC ($r = -0.714$, $p \leq 0.05$) as shown in Table 5.

3.3. Total chlorophyll content (TCC)

TCC in WJP of four wheat varieties grown using soil, CNS and water varied from 3.01 to 5.63 mg/g (Table 1). The previous study by Niroula et al. (2019) reported chlorophyll content of 2.69, 4.25, 5.32 and 6.08 mg/g in wheatgrass extracts after 7, 10, 13 and 16 days of germination, respectively. Another study by Ghumman et al. (2017) reported TCC in the range of 0.24–7.05 mg/g in freeze-dried WJP and wheatgrass shoots of two wheat varieties. TCC was observed higher in WJP of wheat varieties grown using soil (4.23–5.63 mg/g) followed by CNS (3.73–5.49 mg/g) and water (3.01–4.88 mg/g). Özköse, Arslan, and Aysenur (2016) also reported that the application of the fertilizer during sprouting decreases TCC in grass shoots of different turfgrass and wheatgrass cultivars. The highest TCC was observed in HD-3086 and the lowest in HS-490 grown using soil. TCC showed higher significant variation among wheat varieties than the growing conditions ($P \leq 0.005$; Table S1). TCC exhibited a significant positive correlation with Mg ($r = 0.846$, $p \leq 0.05$) and a highly significant positive correlation with TPC and TAA ($r = 0.895$ and 0.914, respectively; $P \leq 0.005$) as shown in Table 5. This indicates that the WJP of wheat varieties with higher TCC also had higher TAA. Ghumman et al. (2017) also reported that WJP and pulse juice powder with higher TCC had more Mg and higher antioxidant capacity.

3.4. Mineral composition

The mineral composition of WJP from four wheat varieties grown under different conditions is shown in Table 1. The mineral components quantified in WJP from different wheat varieties were K (3097.00 to 9571.66 mg/kg), P (2608.60 to 6243.66 mg/kg), Ca (441.53 to 2996.40 mg/kg), Mg (710.66 to 1645.66 mg/kg), Na (655.33 to 1150.00 mg/kg) and Fe (450.63 to 1568.33 mg/kg). The high levels of K, Ca and Mg observed in WJP were consistent with those reported previously in wheatgrass shoots (Kulkarni, Acharya et al., 2006). The other mineral components quantified in WJP of wheat varieties were Zn (41.66 to 88.00 mg/kg), Mn (23.28 to 97.33 mg/kg) and Cu (1.02 to 5.16 mg/kg). The previous studies also reported low contents of Zn, Mn and Cu in WJP (Ghumman et al., 2017) and wheatgrass shoots (Kulkarni, Acharya et al., 2006). The levels of K, P, Ca, Mg, Na, Fe, Mn and Cu were higher in WJP obtained from HD-3086 compared to other wheat varieties. Among the growing conditions, the levels of K, Ca, Mg and Mn was higher in WJP obtained from wheat varieties grown using soil than those grown using CNS and water. While, P, Fe, Na, Zn and Cu contents were higher in WJP obtained from wheat varieties grown using CNS compared to those grown using soil and water. The low concentrations of mineral elements in WJP of all wheat varieties grown using water might be due to no external nutrient input other than the nutrients available in tap water. Kulkarni, Acharya et al. (2006) observed similar results that wheatgrass grown using tap water had the lowest mineral content than those grown in nutrient solution and soil. The concentration of minerals in wheatgrass depends on their biological availability in the soil and water. A higher significant variation of growing conditions than the wheat varieties was observed on the level of K, P and Na in WJP ($P \leq 0.005$, Table S1). While, Ca, Mg, Fe, Mn, Zn and Cu contents showed higher significant variation among WJP of different wheat varieties. A significant positive correlation of K with AC ($r = 0.641$, $p \leq 0.05$) and Mg with TCC ($r = 0.846$, $p \leq 0.05$) was observed (Table 5). The correlation of Mg with chlorophyll could be due to the element Mg present in the centre of the porphyrin ring of the chlorophyll molecule (Ghumman et al., 2017). Mg also exhibited a highly significant positive correlation with TPC and TAA ($r = 0.865$ and 0.888, respectively, $p \leq 0.005$) as shown in Table 5. Furthermore, Mn displayed a significant positive correlation with TCC, TPC and TAA ($r = 0.596$, 0.766 and 0.780, respectively, $p \leq 0.05$). Mn is associated with an enzyme superoxide dismutase responsible for radical scavenging activities in wheat seedlings (Lai, Chang, & Chang, 2008).

3.5. Amino acid profile

Table 2 shows the AA profile of WJP obtained from four wheat varieties grown using soil, CNS and water. Results revealed that WJP contained twenty one AAs including nine essential AAs (EAAs) such as histidine, threonine, tryptophan, phenylalanine, valine, leucine, lysine, methionine and isoleucine. The AA composition of WJP is somewhat similar to those reported for wheat sprouts in the previous study (Ohm, Lee, & Cho, 2016). However, their contents varied significantly in WJP of different wheat varieties ($P < 0.005$). The WJP of four wheat varieties were characterized by the high content of EAAs such as threonine (2175.83 to 5101.37 mg/100 g), histidine (985 to 3518.67 mg/100 g) and leucine (1095.02 to 3475.00 mg/100 g). Among non-essential AAs (NEAAs), glutamine (1197.19 to 8085 mg/100 g), arginine (1655.50 to 7514.08 mg/100 g), citrulline (2135.75 to 5735.42 mg/100 g), GABA (1112.04 to 4737.07 mg/100 g) and serine (610.94 to 3065.58 mg/100 g) were prevalent in WJP. The previous study also reported threonine, arginine, citrulline and GABA were the abundant AAs in WJP (Ghumman et al., 2017). The WJP obtained from wheat varieties grown using CNS had the highest content of EAAs and NEAAs than those grown using soil and water. CNS contains nitrogen which play an important role in the synthesis of AAs. A recent study by Zhang et al. (2017) reported nitrogen application during the growth of wheat plants significantly increases the level of essential, non-essential and total AAs in harvested wheat grains. The level of EAAs and NEAAs were highest in WJP of C-306 wheat variety grown using CNS than the

Table 2
Effect of growing conditions on amino acid composition (mg/100 g) of WJP from different wheat varieties.

		Wheat varieties				
		C306			HD3086	
Amino acids		Soil	CNS	Water	Soil	CNS
EAAs	Val	1491.59 ± 21.24^{d}	2554.33 ± 26.95^{e}	1554.57 ± 24.94^{d}	1452.91 ± 22.49^{d}	1541.12 ± 12.58^{d}
	Met	1142.65 ± 12.50^{e}	1321.12 ± 21.00^{f}	1041.67 ± 15.28^{d}	1134.37 ± 5.24^{e}	1178.27 ± 13.53^{e}
	Leu	2214.85 ± 14.54^{c}	3475.00 ± 25.00^{d}	2052.15 ± 14.49^{c}	2067.60 ± 20.33^{c}	3328.09 ± 17.53^{d}
	Ile	1310.75 ± 12.86^{d}	1714.00 ± 20.07^{f}	1110.00 ± 13.00^{b}	1200.85 ± 8.11^{c}	2539.52 ± 20.02^{h}
	Phe	1264.52 ± 10.03^{c}	2068.67 ± 21.22^{e}	1235.67 ± 22.50^{c}	1179.42 ± 15.03^{b}	1362.17 ± 15.69^{c}
	Trp	1368.51 ± 20.51^{d}	2047.72 ± 27.40^{g}	1268.26 ± 23.61^{c}	1057.59 ± 17.01^{b}	1504.92 ± 5.04^{e}
	His	2819.22 ± 20.24^{a}	3518.67 ± 19.01^{e}	2712.49 ± 15.72^{d}	2733.54 ± 19.97^{d}	3168.81 ± 23.50^{d}
	Lys	287.24 ± 12.26^{a}	820.55 ± 4.90^{f}	50.78 ± 2.35^{a}	164.59 ± 18.01^{b}	434.94 ± 25.01^{e}
	Thr	4889.59 ± 20.50^{f}	5101.37 ± 15.19^{f}	4625.58 ± 20.82^{e}	4614.25 ± 15.10^{e}	5005.48 ± 13.40^{f}
	Total	16788.92	22621.43	15651.17	15605.12	20063.32
NEAAs	Gly	283.79 ± 6.87^{b}	380.85 ± 16.54^{d}	264.06 ± 4.00^{b}	212.12 ± 2.58^{a}	362.25 ± 17.52^{d}
	Ala	81.26 ± 1.28^{f}	67.81 ± 3.17^{e}	54.13 ± 3.90^{d}	81.99 ± 3.48^{f}	55.24 ± 5.00^{d}
	Pro	98.75 ± 3.31^{a}	122.18 ± 7.73^{c}	98.67 ± 3.06^{a}	124.89 ± 6.39^{c}	225.03 ± 5.01^{g}
	Asp	1088.86 ± 20.74^{c}	2528.23 ± 25.60^{h}	1743.63 ± 10.45^{e}	851.42 ± 19.00^{b}	1862.49 ± 27.5^{g}
	Glu	122.60 ± 12.24^{c}	851.34 ± 15.02^{h}	209.47 ± 10.27^{d}	337.34 ± 17^{e}	523.32 ± 12.54^{g}
	Arg	5832.59 ± 23.03^{e}	7514.08 ± 23.31^{f}	5630.29 ± 24.95^{e}	4766.50 ± 17.48^{d}	5489.25 ± 14.93^{e}
	Ser	2332.88 ± 23.07^{d}	3065.58 ± 20.01^{e}	2309.08 ± 10.72^{d}	1518.25 ± 14.00^{c}	2038.20 ± 27.51^{d}
	Asn	255.28 ± 14.12^{d}	845.26 ± 20.00^{h}	189.40 ± 21.14^{b}	300.50 ± 12.03^{e}	625.24 ± 17.58^{g}
	Gln	6431.73 ± 25.77^{d}	8085.00 ± 25.98^{e}	6846.83 ± 22.67^{d}	6928.17 ± 27.18^{d}	7127.92 ± 19.14^{d}
	Cys	328.27 ± 8.88^{c}	521.30 ± 12.10^{g}	224.92 ± 10.50^{a}	376.25 ± 3.60^{d}	422.76 ± 12.55^{e}
	Tyr	185.07 ± 14.75^{b}	255.78 ± 15.51^{c}	194.82 ± 4.50^{b}	309.50 ± 11.01^{d}	295.40 ± 15.00^{c}
	Cit	4638.29 ± 17.52^{e}	5735.42 ± 20.53^{g}	4318.59 ± 17.01^{e}	4221.92 ± 12.58^{e}	4985.75 ± 12.94^{f}
	GABA	3966.74 ± 22.54^{e}	3154.74 ± 20.40^{d}	1546.67 ± 23.63^{b}	4737.07 ± 26.62^{f}	4125.74 ± 27.67^{e}
	Total	25646.11	33127.57	23630.56	24765.92	28138.59

	Wheat varieties						
	HD3086	HD2967			HS490		
Amino acids	Water	Soil	CNS	Water	Soil	CNS	Water
EAAs	1260.34 ± 25.2^{c}	513.75 ± 11.92^{a}	926.48 ± 23.02^{b}	645.27 ± 24.96^{a}	1348.67 ± 26.63^{c}	1574.08 ± 10.13^{d}	1183.42 ± 17.43^{c}
	1160.38 ± 15.00^{e}	759.87 ± 14.88^{c}	1066.44 ± 31.50^{e}	537.25 ± 10.58^{b}	568.17 ± 22.37^{b}	905.73 ± 17.70^{a}	417.33 ± 2.60^{a}
	2233.42 ± 30.02^{c}	1195.03 ± 14.00^{a}	1338.88 ± 21.10^{b}	1119.75 ± 19.76^{a}	1095.02 ± 14.90^{a}	1272.33 ± 22.50^{b}	1110.84 ± 11.00^{a}
	1182.50 ± 17.38^{b}	1579.50 ± 15.06^{e}	2012.33 ± 12.50^{g}	1254.42 ± 21.42^{c}	1285.78 ± 24.76^{c}	1512.33 ± 12.50^{e}	1038.37 ± 15.26^{a}
	1007.49 ± 15.22^{b}	1023.08 ± 10.26^{b}	1731.36 ± 23.50^{d}	1026.42 ± 12.55^{b}	888.68 ± 11.86^{a}	1014.67 ± 16.74^{b}	926.65 ± 16.90^{a}
	983.68 ± 10.30^{a}	1016.50 ± 14.30^{a}	1122.67 ± 17.01^{b}	1012.00 ± 12.00^{a}	1229.37 ± 26.65^{c}	1651.75 ± 11.48^{f}	1086.94 ± 19.11^{b}
	2812.27 ± 28.24^{d}	986.79 ± 18.93^{a}	1164.86 ± 25.00^{b}	985.00 ± 17.32^{a}	1442.86 ± 7.39^{b}	2009.07 ± 13.84^{c}	1250.08 ± 15^{b}
	24.89 ± 5.04^{a}	156.42 ± 5.87^{b}	419.04 ± 18.57^{d}	51.53 ± 1.60^{a}	170.24 ± 20.26^{b}	387.18 ± 12.51^{d}	54.36 ± 2.51^{a}
	4582.92 ± 17.50^{e}	3645.58 ± 27.75^{d}	3929.38 ± 30.02^{d}	3539.58 ± 22.68^{c}	2545.92 ± 21.83^{b}	3213.73 ± 19.62^{c}	2175.83 ± 8.89^{a}
	15247.89	10876.52	13711.44	10171.22	10574.74	13540.87	9243.82
NEAAs	262.84 ± 9.00^{b}	288.51 ± 8.50^{b}	803.18 ± 20.28^{e}	318.50 ± 1.39^{c}	255.07 ± 3.54^{b}	327.43 ± 14.03^{c}	219.42 ± 2.45^{a}
	46.14 ± 1.72^{d}	29.99 ± 5.00^{b}	66.82 ± 7.52^{e}	44.36 ± 4.17^{c}	12.17 ± 1.89^{a}	39.96 ± 5.00^{c}	5.90 ± 1.01^{a}
	111.45 ± 1.31^{b}	117.76 ± 8.11^{b}	197.65 ± 6.66^{f}	103.00 ± 7.55^{a}	129.52 ± 5.50^{c}	143.40 ± 12.60^{d}	156.52 ± 5.65^{e}
	982.58 ± 7.51^{c}	572.20 ± 22.51^{a}	812.80 ± 12.52^{b}	755.52 ± 15.50^{b}	713.54 ± 13.78^{b}	1234.00 ± 15.10^{d}	580.05 ± 22.65^{a}
	106.52 ± 8.10^{c}	58.47 ± 6.50^{a}	106.93 ± 7.11^{c}	88.65 ± 3.56^{b}	51.57 ± 4.39^{a}	212.52 ± 22.02^{d}	13.55 ± 1.50^{a}
	4794.62 ± 20.03^{d}	1655.50 ± 25.00^{a}	2451.00 ± 24.67^{b}	1725.67 ± 26.01^{a}	3894.17 ± 20.00^{c}	4515.00 ± 15.52^{d}	3212.17 ± 22.39^{c}
	1269.08 ± 21.42^{b}	610.94 ± 9.09^{a}	1076.36 ± 21.50^{b}	663.30 ± 22.01^{a}	1364.17 ± 10.01^{b}	1756.08 ± 17.67^{c}	1059.25 ± 51.21^{b}
	131.92 ± 8.02^{a}	163.49 ± 13.50^{b}	625.59 ± 17.90^{g}	205.00 ± 8.72^{c}	246.94 ± 27.52^{c}	566.25 ± 14.53^{f}	178.42 ± 6.00^{b}

(*continued on next page*)

Table 2 (*continued*)

Amino acids	Wheat varieties						
	HD3086	HD2967			HS490		
	Water	Soil	CNS	Water	Soil	CNS	Water
	6960.58 ± 20.50[d]	1197.19 ± 22.00[a]	1531.68 ± 26.70[b]	1265.87 ± 25.89[a]	2009.27 ± 34.50[b]	3131.75 ± 16.02[c]	1928.42 ± 25.76[b]
	336.92 ± 21.39[c]	309.86 ± 10.01[c]	719.92 ± 20.13[h]	303.49 ± 5.86[c]	277.43 ± 7.38[b]	347.47 ± 27.75[c]	209.58 ± 6.43[a]
	324.17 ± 13.13[d]	145.13 ± 10.00[a]	261.29 ± 11.95[c]	150.69 ± 4.12[b]	151.52 ± 9.63[b]	189.96 ± 9.75[b]	123.51 ± 2.41[a]
	4116.25 ± 13.53[e]	2711.48 ± 16.50[b]	3255.28 ± 20.06[c]	2135.75 ± 17.68[a]	3742.17 ± 22.39[d]	4056.74 ± 16.03[d]	3468.75 ± 15.96[d]
	2353.67 ± 18.58[c]	2332.18 ± 32.50[c]	2402.74 ± 12.51[c]	1810.07 ± 25.00[b]	1218.53 ± 16.50[a]	1621.32 ± 21.01[b]	1112.04 ± 12.53[a]
	21796.74	10192.7	14311.24	9569.87	14066.07	18141.88	12267.58

Values with similar superscripts in a row do not differ significantly ($p < 0.05$). CNS, Cocopeat with nutrient solution; EAAs, Essential amino acids; NEAAs, Non-essential amino acids; Gly, Glycine; Ala, Alanine; Val, Valine; Met, Methionine; Leu, Leucine; Ile, Isoleucine; Pro, Proline; Phe, Phenylalanine; Trp, tryptophan; Asp, Aspartic acid; Glu, Glutamic acid; His, Histidine; Lys, Lysine; Arg, Arginine; Ser, Serine; Thr, Threonine; Asn, Asparagine; Gln, Glutamine; Cys, Cysteine; Tyr, Tyrosine; Cit, Citrulline; GABA, γ-aminobutyric acid.

other wheat varieties.

Glutamine was abundant in WJP of four wheat varieties with the highest content in C-306 grown using CNS (8085 mg/100 g). The level of aspartic and glutamic acid varied from 572.20 to 2528.23 mg/100 g and 13.55 to 851.34 mg/100 g, respectively, with the highest in WJP of C-306 grown using CNS. The highest content of leucine (3475.00 mg/100 g) and isoleucine (2539.52 mg/100 g) were observed in WJP of CNS grown C-306 and HD-3086, respectively. The level of cysteine and methionine (sulfur AAs) varied from 209.58 to 719.92 mg/100 g and 417.33 to 1321.12 mg/100 g, respectively, with the highest in WJP of HD-2967 and C-306 grown using CNS, respectively. The level of GABA was highest in WJP of HD-3086 grown using soil (4737.07 mg/100 g). GABA is synthesized from glutamate during germination and sprouting of wheat grains and has the potential to relieve neurological disorders, inflammation, diabetics and other related disorders (Ohm et al., 2016). Alanine, valine, methionine, leucine, aspartic acid, histidine, arginine, serine, threonine, glutamine, tyrosine, citrulline and GABA contents in WJP showed higher significant variation among wheat varieties (($P \leq 0.005$, Table S1). While, the level of glycine, isoleucine, proline, phenylalanine, tryptophan, glutamic acid, lysine, cysteine and asparagine in WJP were greatly and significantly influenced by the growing conditions. EAAs and NEAAs exhibited a highly significant positive correlation with PC ($r = 0.860$ and 0.872, respectively; $p \leq 0.05$) as shown in Table 5.

3.6. Total phenolic content (TPC) and flavonoid content (TFC)

TPC and TFC in WJP of different wheat varieties grown using soil, CNS and water are shown in Table 3. The TPC of WJP ranged from 12.02 to 17.44 mg GAE/g. The study conducted by Akbas et al. (2017) reported lower TPC (6.73 mg GAE/g) in freeze-dried WJP. The differences observed in the level of phenolic compounds could be related to differences in wheat genotypes, growing environment and harvesting conditions (Mpofu, Sapirstein, & Beta, 2006). TPC was highest in WJP of wheat varieties grown in soil (14.43–17.44 mg GAE/g) followed by those grown in CNS (13.07–15.76 mg GAE/g) and water (12.02–14.62 mg GAE/g). The highest and the lowest TPC was observed for HD-3086 and HS-490 variety, respectively. TFC of WJP varied from 4.38 to 10.10 mg QE/g, where HD-2967 and HS-490 had the highest and the lowest contents, respectively. Among growing conditions, the highest TFC was observed for wheat varieties grown using soil (6.18–10.10 mg QE/g) followed by those grown using CNS (4.63–8.39 mg QE/g) and water (4.38–7.56 mg QE/g). Our results concur with the previous study reporting the influence of growth under different conditions on TPC and TFC of wheat plants (Kulkarni, Tilak et al., 2006). The higher significant effect of growing conditions on TPC and wheat varieties on TFC of WJP was observed ($P \leq 0.005$; Table S1).

3.7. Phenolic composition

The phenolic compounds in free and bound form were identified and quantified from WJP of wheat varieties grown using soil, CNS and water (Table 4). The phenolic acids identified in WJP include hydroxybenzoic (GAC, PCA and SYA) and hydroxycinnamic (FRA, CFA, CGA, SPA, *p*-CA, and CMA) acids. The previous study identified benzoic, p-hydroxybenzoic and ellagic acids in addition to FRA, GAC, SYA, *p*-CA and CFA in dried wheatgrass of amber durum wheat (Kardas & Durucasu, 2014). Among the identified phenolic acids, FRA, SYA and SPA were predominant in WJP and our results are in agreement with previous finding on wheatgrass seedlings (Złotek et al., 2019). The hydroxybenzoic acids were more in free than the bound form in wheat varieties. GAC and PCA were higher in WJP of wheat varieties grown using soil while SYA was more in WJP of wheat varieties grown using CNS. The highest content of free GAC (342.66 μg/g) and PCA (252.88 μg/g) was observed in WJP of HD-2967 and HS-490 wheat varieties grown using soil, respectively while SYA in free form was higher in HD-2967 (236.64 µg/g) grown using CNS. The level of free PCA showed higher significant variation among wheat varieties while free GAC and SYA contents were significantly influenced by the growing conditions ($P \leq 0.005$, Table S1). Among bound hydroxybenzoic acids, the level of bound PCA was highest in HD-3086 variety grown using soil (129.40 µg/g) and lowest in HS-490 grown using water (1.35 µg/g). The variation in bound PCA content was greatly and significantly ($P \leq 0.005$) influenced by growing conditions (Table S1). The bound PCA also showed a significant positive correlation ($p \leq 0.05$) with TPC ($r = 0.687$) of WJP (Table 5).

The level of phenolic acids was more in WJP of wheat varieties grown using soil than those grown using CNS and water. The previous study by Nguyen, Kwee, and Niemeyer (2010) reported that the availability of macronutrients and environmental factors during plant growth significantly influences the level of phenolic acids in basil leaves. Among free hydroxycinnamic acids, FRA (109.97–710.00 µg/g), CFA (35.81–557.71 µg/g), CGA (37.73–294.03 µg/g) and SPA (15.06–157.90 µg/g) were quantified in higher amounts than the *p*-CA (5.65–24.08 µg/g) and CMA (0.23–15.47 µg/g). A study by Skoczylas, Korus, Tabaszewska, Gedos, and Szczepanska (2017) reported higher content of FRA (92.8 to 138.4 mg/l) than CGA (29.6 to 78.4 mg/l) and *p*-CA (15.8–27.8 mg/l) in frozen wheatgrass juice after 3 months of storage. Akbas et al. (2017) reported FRA as major phenolic acid followed by CGA, CFA and GAC (1020, 270, 11.5, and 2.7 µg/g, respectively) in freeze-dried wheatgrass juice. Our results concur with previous studies that FRA is the major phenolic compounds in wheatgrass (Kardas & Durucasu, 2014; Benincasa et al., 2015; Akbas et al., 2017). HD-3086 had higher contents of free FRA, CFA, CGA and *p*-CA while HD-2967 had higher contents of free CMA and SPA. The concentration of phenolic compounds in wheat plants varies with genotype, environmental factors and growing conditions (Mpofu et al., 2006). The level of free CMA was greatly and significantly influenced by the growing conditions while free FRA, CFA, CGA, SPA and *p*-CA contents showed higher significant variation among wheat varieties ($P \leq 0.005$, Table S1). The free FRA content showed a highly significant positive relation with TAA ($r = 0.800$, $p \leq 0.005$) and a significant positive correlation with TPC ($r = 0.703$, $p \leq 0.05$) of WJP (Table 5). The level of bound CGA varied from 3.79 to 35.11 µg/g with the highest in HD-3086 grown using soil and the lowest in HS-490 grown using soil. The level of *p*-CA in bound form varied from 9.92 to 29.42 µg/g with the highest in WJP of HS-490 variety grown using soil. Benincasa et al. (2015) reported similar results for bound *p*-CA in wheatgrass of different *Triticum* species. The bound CGA and *p*-CA showed higher significant variation among wheat varieties than the growing conditions ($P \leq 0.005$, Table S1). As shown in Table 5, the bound CGA exhibited a significant positive correlation ($p \leq 0.05$) with TPC and TAA ($r = 0.690$ and 0.695, respectively).

The flavonoids detected in WJP include flavan-3-ols (catechin and epicatechin), flavonols (quercetin, kaempferol and rutin) and flavones (vitexin and isovitexin). The non-flavonoid polyphenol (resveratrol) was also detected in WJP (Table 4). The level of free and bound flavan-3-ols was more in WJP of wheat varieties grown using soil than those grown using CNS and water. Catechin in free and bound form varied from 12.17 to 986.00 µg/g and 1.42 to 53.88 µg/g, respectively, with the highest content in C-306 wheat variety grown using soil. Skoczylas et al. (2017) reported catechin content in the range of 341.1 to 573.0 mg/l in frozen and stored wheatgrass juice. The free and bound epicatechin content varied from 15.63 to 183.81 µg/g and 86.75 to 192.66 µg/g, respectively. The WJP of HD-2967 and HD-3086 wheat varieties grown using soil exhibited the highest free and bound epicatechin content, respectively. The level of free catechin and epicatechin showed higher significant variation ($P \leq 0.005$) among wheat varieties than the growing conditions (Table S1). While the bound catechin and epicatechin contents showed higher significant variation ($P \leq 0.005$) with growing conditions than the wheat varieties. The bound epicatechin exhibited a significant positive correlation with TPC and TAA

Table 3
Antioxidant properties of WJP from different wheat varieties.

Wheat Varieties	Growing Conditions	Total Phenolic Content (mg GAE/g DW)			Total Flavonoid Content (mg QE/g DW)			Total Antioxidant Activity (µmol TE/g DW)		
		Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
C306	Soil	11.90 ± 0.05^{ef}	4.20 ± 0.07^{de}	16.10 ± 0.08^{f}	3.89 ± 0.04^{d}	3.77 ± 0.06^{e}	7.67 ± 0.04^{f}	9.55 ± 0.08^{f}	6.79 ± 0.24^{e}	16.34 ± 0.26^{h}
	CNS	11.64 ± 0.10^{e}	3.74 ± 0.11^{c}	15.38 ± 0.14^{e}	3.58 ± 0.03^{d}	3.40 ± 0.17^{d}	6.98 ± 0.22^{e}	9.24 ± 0.005^{d}	6.43 ± 0.03^{d}	15.67 ± 0.03^{g}
	Water	10.29 ± 0.20^{c}	3.41 ± 0.07^{b}	13.70 ± 0.23^{c}	3.56 ± 0.03^{d}	3.38 ± 0.06^{d}	6.94 ± 0.03^{e}	9.00 ± 0.02^{c}	6.18 ± 0.06^{c}	15.18 ± 0.06^{e}
HD3086	Soil	12.75 ± 0.10^{g}	4.69 ± 0.06^{f}	17.44 ± 0.12^{g}	3.18 ± 0.01^{c}	3.78 ± 0.09^{e}	6.97 ± 0.11^{e}	9.90 ± 0.04^{f}	7.43 ± 0.03^{g}	17.33 ± 0.02^{j}
	CNS	11.93 ± 0.03^{f}	3.83 ± 0.07^{c}	15.76 ± 0.10^{f}	3.06 ± 0.05^{c}	3.00 ± 0.12^{c}	6.06 ± 0.12^{d}	9.39 ± 0.005^{e}	7.27 ± 0.04^{f}	16.66 ± 0.05^{i}
	Water	10.95 ± 0.09^{d}	3.67 ± 0.05^{c}	14.62 ± 0.14^{d}	2.85 ± 0.22^{b}	2.57 ± 0.38^{b}	5.42 ± 0.52^{c}	8.98 ± 0.008^{c}	7.11 ± 0.10^{f}	16.10 ± 0.10^{h}
HD2967	Soil	11.60 ± 0.04^{e}	4.14 ± 0.12^{d}	15.75 ± 0.07	4.97 ± 0.12^{f}	5.13 ± 0.07^{g}	10.10 ± 0.11^{h}	9.36 ± 0.01^{e}	6.42 ± 0.03^{d}	15.78 ± 0.03^{g}
	CNS	10.77 ± 0.19^{d}	3.56 ± 0.07^{b}	14.33 ± 0.21^{d}	4.09 ± 0.03^{e}	4.30 ± 0.13^{f}	8.39 ± 0.16^{g}	9.18 ± 0.07^{d}	6.17 ± 0.05^{c}	15.35 ± 0.08^{f}
	Water	10.26 ± 0.27^{c}	3.33 ± 0.02^{b}	13.59 ± 0.27^{c}	3.77 ± 0.17^{d}	3.79 ± 0.07^{e}	7.56 ± 0.25^{f}	9.09 ± 0.10^{d}	5.85 ± 0.04^{b}	14.94 ± 0.12^{d}
HS490	Soil	10.36 ± 0.15^{c}	4.07 ± 0.06^{d}	14.43 ± 0.10^{d}	3.03 ± 0.04^{c}	3.15 ± 0.15^{c}	6.18 ± 0.25^{d}	8.77 ± 0.09^{c}	5.80 ± 0.07^{b}	14.56 ± 0.07^{c}
	CNS	9.66 ± 0.10^{b}	3.41 ± 0.05^{b}	13.07 ± 0.06^{b}	2.43 ± 0.26^{a}	2.20 ± 0.13^{a}	4.63 ± 0.33^{b}	8.68 ± 0.05^{b}	5.65 ± 0.04^{b}	14.33 ± 0.07^{b}
	Water	8.94 ± 0.09^{a}	3.08 ± 0.04^{a}	12.02 ± 0.07^{a}	2.29 ± 0.02^{a}	2.09 ± 0.07^{a}	4.38 ± 0.10^{a}	8.25 ± 0.01^{a}	5.28 ± 0.05^{a}	13.54 ± 0.04^{a}

Values with similar superscripts in a column do not differ significantly ($p < 0.05$). CNS, Cocopeat with nutrient solution; GAE, Gallic acid equivalents; QE, Quercetin equivalents; TE, Trolox equivalents; DW, Dry weight.

Table 4
Effect of growing conditions on free and bound phenolic compounds (μg/g) of WJP from different wheat varieties.

Phenolic compounds		C306			HD3086	
		Soil	CNS	Water	Soil	CNS
Phenolic acids (Hydroxybenzoic acids)						
GAC	Free	65.89 ± 2.11^{a}	65.52 ± 5.46^{a}	60.08 ± 1.42^{a}	299.51 ± 10.46^{d}	297.41 ± 12.51^{d}
	Bound	2.59 ± 0.15^{b}	2.30 ± 0.29^{b}	0.97 ± 0.04^{a}	ND	ND
PCA	Free	119.29 ± 11.02^{d}	44.80 ± 3.14^{b}	3.44 ± 0.42^{a}	110.69 ± 12.50^{d}	79.63 ± 5.68^{c}
	Bound	3.16 ± 0.16^{a}	2.96 ± 0.07^{a}	1.47 ± 0.15^{a}	129.40 ± 6.89^{e}	30.45 ± 0.56^{b}
SYA	Free	157.09 ± 11.67^{c}	158.24 ± 7.00^{c}	25.14 ± 0.65^{a}	140.62 ± 2.30^{c}	149.94 ± 12.01^{c}
	Bound	5.13 ± 0.32^{a}	7.48 ± 0.50^{a}	2.98 ± 0.25^{a}	3.54 ± 0.32^{a}	3.92 ± 0.08^{a}
Phenolic acids (Hydroxycinnamic acids)						
FRA	Free	251.19 ± 14.06^{c}	165.02 ± 15.00^{b}	109.97 ± 10.22^{a}	710.00 ± 13.23^{g}	656.11 ± 15.90^{f}
	Bound	105.78 ± 11.90^{a}	167.29 ± 5.16^{b}	162.23 ± 13.13^{b}	267.23 ± 11.69^{c}	177.21 ± 10.34^{b}
CFA	Free	80.5 ± 3.52^{b}	50.66 ± 1.51^{a}	67.7 ± 2.57^{a}	557.71 ± 12.50^{d}	105.54 ± 9.25^{b}
	Bound	43.18 ± 2.33^{f}	13.54 ± 0.41^{e}	6.93 ± 0.36^{c}	2.23 ± 0.28^{a}	3.68 ± 0.33^{b}
CGA	Free	89.70 ± 1.40^{a}	72.11 ± 3.10^{a}	67.59 ± 2.10^{a}	294.03 ± 8.38^{b}	103.58 ± 4.88^{b}
	Bound	8.36 ± 0.38^{a}	7.14 ± 0.35^{a}	6.96 ± 0.30^{a}	35.11 ± 1.80^{c}	14.81 ± 0.39^{b}
SPA	Free	136.69 ± 8.50^{c}	136.78 ± 11.60^{c}	109.42 ± 6.01^{b}	42.23 ± 1.22^{a}	38.18 ± 2.97^{a}
	Bound	16.88 ± 0.66^{c}	0.94 ± 0.08^{a}	4.39 ± 0.37^{a}	7.44 ± 0.11^{b}	4.54 ± 0.33^{a}
p-CA	Free	20.52 ± 0.57^{d}	15.51 ± 0.45^{c}	13.81 ± 0.23^{b}	24.08 ± 2.00^{e}	17.60 ± 0.46^{c}
	Bound	13.37 ± 0.44^{b}	11.91 ± 0.66^{b}	9.92 ± 0.34^{a}	21.13 ± 1.00^{e}	19.48 ± 0.12^{d}
CMA	Free	0.23 ± 0.03^{a}	0.83 ± 0.08^{a}	0.24 ± 0.05^{a}	1.77 ± 0.23^{a}	2.93 ± 0.08^{a}
	Bound	5.77 ± 0.24^{c}	0.91 ± 0.10^{a}	1.85 ± 0.29^{a}	1.76 ± 0.44^{a}	0.45 ± 0.05^{a}
Flavonoids (Flavan-3-ols)						
CCN	Free	986.00 ± 8.54^{c}	937.72 ± 20.51^{c}	962.13 ± 19.21^{c}	229.19 ± 19.00^{a}	12.17 ± 0.21^{a}
	Bound	53.88 ± 2.87^{h}	13.58 ± 0.40^{c}	24.20 ± 2.15^{e}	33.12 ± 1.90^{f}	11.18 ± 0.22^{b}
ECN	Free	40.10 ± 3.10^{a}	57.28 ± 3.02^{a}	63.70 ± 2.43^{a}	39.84 ± 2.24^{a}	15.63 ± 0.20^{a}
	Bound	187.58 ± 3.39^{e}	105.45 ± 9.93^{b}	86.75 ± 3.50^{a}	192.66 ± 2.51^{e}	178.18 ± 11.50^{e}
Flavonoids (Flavonols)						
RUT	Free	76.94 ± 3.06^{a}	88.30 ± 2.62^{a}	15.09 ± 0.68^{a}	263.70 ± 11.50^{b}	332.68 ± 15.58^{c}
	Bound	17.37 ± 0.55^{a}	20.60 ± 1.35^{b}	10.49 ± 0.39^{a}	5.80 ± 0.36^{a}	30.48 ± 0.42^{b}
QUE	Free	11.18 ± 0.24^{f}	10.57 ± 0.40^{e}	9.58 ± 0.40^{d}	10.58 ± 0.43^{e}	9.61 ± 0.27^{d}
	Bound	ND	ND	ND	0.25 ± 0.04^{a}	ND
KML	Free	1.21 ± 0.20^{a}	0.33 ± 0.05^{a}	0.16 ± 0.02^{a}	31.19 ± 0.97^{g}	22.28 ± 0.49^{e}
	Bound	ND	0.11 ± 0.01^{b}	0.08 ± 0.01^{a}	0.28 ± 0.04^{c}	0.29 ± 0.03^{c}
Flavonoids (Flavones)						
VIT	Free	72.71 ± 2.45^{c}	38.14 ± 0.88^{b}	36.89 ± 1.11^{b}	31.69 ± 1.70^{b}	17.84 ± 0.17^{a}
	Bound	16.69 ± 0.56^{d}	10.51 ± 0.62^{c}	0.58 ± 0.04^{a}	4.66 ± 0.34^{b}	1.28 ± 0.18^{a}
ISV	Free	19.39 ± 0.55^{b}	19.88 ± 0.86^{b}	12.64 ± 0.46^{b}	46.85 ± 1.66^{e}	25.66 ± 0.34^{c}
	Bound	115.17 ± 8.52^{c}	112.42 ± 6.64^{c}	100.33 ± 5.03^{c}	181.35 ± 5.86^{f}	175.52 ± 11.77^{e}
Non-flavonoid polyphenol						
RVL	Free	18.45 ± 1.51^{c}	4.14 ± 0.01^{a}	2.51 ± 0.33^{a}	19.35 ± 0.54^{c}	6.79 ± 0.33^{a}
	Bound	1.24 ± 0.22^{f}	0.52 ± 0.09^{d}	ND	0.35 ± 0.05^{b}	0.73 ± 0.02^{e}

Phenolic compounds	HD3086	HD2967			HS490		
	Water	Soil	CNS	Water	Soil	CNS	Water
Phenolic acids (Hydroxybenzoic acids)							
GAC	205.67 ± 10.54^{d}	342.66 ± 9.13^{e}	321.53 ± 14.07^{e}	300.17 ± 19.87^{e}	154.38 ± 15.87^{c}	107.63 ± 7.50^{b}	104.15 ± 7.10^{b}
	ND	3.88 ± 0.34^{c}	6.20 ± 0.16^{d}	2.34 ± 0.32^{b}	0.95 ± 0.13^{a}	3.64 ± 0.35^{c}	2.37 ± 0.37^{b}
PCA	81.20 ± 2.00^{c}	110.92 ± 9.02^{d}	104.49 ± 5.73^{d}	5.31 ± 0.32^{a}	252.88 ± 27.01^{e}	44.30 ± 4.08^{b}	6.17 ± 0.22^{a}

(*continued on next page*)

Table 4 (*continued*)

Phenolic compounds	HD3086	HD2967			HS490		
	Water	Soil	CNS	Water	Soil	CNS	Water
	2.77 ± 0.23^{a}	85.01 ± 3.85^{c}	4.51 ± 0.33^{a}	2.36 ± 0.37^{a}	30.76 ± 1.22^{b}	2.57 ± 0.48^{a}	1.35 ± 0.20^{a}
SYA	110.94 ± 13.05^{b}	230.76 ± 5.61^{d}	236.64 ± 13.47^{d}	202.26 ± 7.00^{d}	104.42 ± 14.14^{b}	142.58 ± 8.74^{c}	100.22 ± 1.79^{b}
	28.77 ± 1.35^{b}	3.41 ± 0.40^{a}	0.85 ± 0.05^{a}	50.58 ± 2.51^{d}	46.79 ± 1.76^{c}	45.89 ± 0.89^{c}	55.86 ± 1.21^{d}
Phenolic acids (Hydroxycinnamic acids)							
FRA	561.01 ± 19.50^{e}	495.76 ± 17.88^{d}	388.45 ± 12.54^{d}	165.28 ± 10.00^{b}	149.14 ± 9.22^{b}	140.39 ± 8.06^{b}	135.61 ± 8.92^{a}
	115.20 ± 10.05^{a}	532.33 ± 8.50^{e}	105.50 ± 6.63^{a}	170.61 ± 10.47^{b}	327.07 ± 22.14^{d}	171.34 ± 19.01^{b}	176.77 ± 11.65^{b}
CFA	263.56 ± 12.17^{c}	85.84 ± 4.83^{b}	35.81 ± 4.81^{a}	54.75 ± 3.03^{a}	84.76 ± 2.71^{b}	36.83 ± 1.24^{a}	59.90 ± 2.02^{a}
	10.88 ± 0.34^{d}	9.86 ± 0.13^{d}	12.43 ± 0.50^{e}	4.41 ± 0.51^{b}	8.94 ± 0.17^{c}	4.94 ± 0.20^{b}	4.48 ± 0.50^{b}
CGA	37.73 ± 0.24^{a}	66.03 ± 1.54^{a}	62.31 ± 0.67^{a}	60.61 ± 4.51^{a}	114.69 ± 4.19^{b}	74.63 ± 3.76^{a}	67.75 ± 2.13^{a}
	4.72 ± 0.38^{a}	5.53 ± 0.50^{a}	5.44 ± 0.04^{a}	5.30 ± 0.30^{a}	3.99 ± 0.13^{a}	3.79 ± 0.23^{a}	5.43 ± 0.42^{a}
SPA	20.93 ± 0.53^{a}	157.90 ± 4.01^{d}	152.48 ± 2.53^{d}	150.27 ± 14.85^{d}	15.06 ± 1.46^{a}	15.81 ± 0.40^{a}	16.95 ± 0.18^{a}
	4.17 ± 0.16^{a}	75.13 ± 4.84^{e}	20.82 ± 0.70^{d}	9.27 ± 0.27^{b}	21.41 ± 1.38^{d}	13.61 ± 0.40^{b}	10.04 ± 0.02^{b}
p-CA	8.48 ± 0.27^{a}	ND	ND	ND	18.83 ± 1.29^{c}	14.34 ± 0.43^{c}	5.65 ± 0.21^{a}
	13.15 ± 0.16^{b}	18.77 ± 1.27^{d}	15.48 ± 0.44^{c}	18.17 ± 2.00^{d}	29.42 ± 1.58^{f}	22.57 ± 1.51^{e}	19.28 ± 0.54^{d}
CMA	3.28 ± 0.28^{a}	15.47 ± 0.50^{b}	10.51 ± 1.50^{b}	3.01 ± 0.45^{a}	13.61 ± 0.38^{b}	2.01 ± 0.13^{a}	3.31 ± 0.29^{a}
	1.48 ± 0.47^{a}	15.26 ± 4.89^{d}	0.53 ± 0.03^{a}	3.04 ± 0.17^{b}	5.81 ± 0.40^{c}	0.50 ± 0.03^{a}	3.67 ± 0.46^{b}
Flavonoids (Flavan-3-ols)							
CCN	12.75 ± 0.23^{a}	253.54 ± 10.36^{b}	227.35 ± 12.37^{b}	75.50 ± 4.38^{a}	152.12 ± 22.53^{a}	97.74 ± 2.36^{a}	84.70 ± 3.51^{a}
	18.80 ± 0.87^{d}	9.22 ± 0.72^{b}	14.17 ± 1.87^{c}	18.38 ± 0.43^{d}	17.45 ± 0.50^{c}	12.08 ± 1.06^{c}	1.42 ± 0.79^{a}
ECN	41.87 ± 3.03^{a}	183.81 ± 8.30^{b}	143.63 ± 8.52^{b}	50.34 ± 1.87^{a}	131.83 ± 3.29^{b}	80.59 ± 1.38^{a}	54.85 ± 2.99^{a}
	113.68 ± 13.62^{b}	161.85 ± 13.34^{d}	159.72 ± 14.50^{b}	125.25 ± 10.00^{c}	179.6 ± 5.61^{e}	100.22 ± 2.28^{b}	100.82 ± 2.64^{b}
Flavonoids (Flavonols)							
RUT	62.58 ± 0.38^{a}	203.56 ± 10.67^{b}	279.39 ± 0.68^{a}	70.50 ± 4.38^{a}	76.41 ± 3.84^{a}	86.96 ± 4.26^{b}	77.26 ± 1.97^{a}
	20.74 ± 0.28^{b}	18.18 ± 0.42^{a}	27.18 ± 2.57^{b}	20.29 ± 1.72^{b}	36.48 ± 1.49^{b}	64.15 ± 3.88^{c}	27.20 ± 1.28^{b}
QUE	8.68 ± 0.13^{c}	22.46 ± 1.20^{h}	21.08 ± 0.89^{g}	9.22 ± 0.38^{d}	2.96 ± 0.14^{b}	1.22 ± 0.22^{a}	1.13 ± 0.08^{a}
	ND	5.87 ± 0.24^{c}	ND	ND	2.50 ± 0.35^{b}	ND	ND
KML	21.61 ± 1.45^{f}	21.07 ± 0.06^{e}	6.09 ± 0.03^{b}	3.04 ± 0.05^{a}	17.62 ± 1.04^{d}	13.54 ± 0.43^{c}	4.12 ± 0.11^{a}
	ND	0.42 ± 0.13^{d}	0.54 ± 0.06^{e}	0.23 ± 0.03^{c}	ND	0.45 ± 0.06^{d}	0.11 ± 0.01^{b}
Flavonoids (Flavones)							
VIT	16.76 ± 0.10^{a}	105.82 ± 12.76^{e}	81.87 ± 6.17^{d}	40.84 ± 2.91^{b}	83.65 ± 0.32^{d}	81.45 ± 3.39^{d}	43.44 ± 2.41^{b}
	0.79 ± 0.02^{a}	25.60 ± 3.98^{e}	11.25 ± 0.40^{c}	0.34 ± 0.06^{a}	5.82 ± 0.28^{b}	2.61 ± 0.40^{a}	0.29 ± 0.01^{a}
ISV	25.19 ± 0.20^{c}	48.68 ± 0.76^{e}	46.35 ± 3.93^{e}	28.97 ± 1.80^{c}	5.41 ± 0.53^{a}	2.20 ± 0.21^{a}	ND
	126.83 ± 7.50^{d}	95.42 ± 1.51^{e}	82.59 ± 4.53^{b}	75.22 ± 2.90^{b}	76.86 ± 6.14^{b}	40.28 ± 2.26^{a}	42.7 ± 2.72^{a}
Non-flavonoid polyphenol							
RVL	4.50 ± 0.12^{a}	41.06 ± 0.90^{d}	18.06 ± 0.82^{c}	0.45 ± 0.06^{a}	97.55 ± 2.51^{e}	4.08 ± 0.93^{a}	0.57 ± 0.06^{a}
	ND	0.44 ± 0.06^{c}	0.14 ± 0.01^{a}	0.51 ± 0.09^{d}	0.54 ± 0.09^{d}	0.52 ± 0.12^{d}	0.48 ± 0.07^{c}

Values with similar superscripts in a row do not differ significantly ($p < 0.05$). CNS, Cocopeat with nutrient solution; RUT, Rutin; QUE, Quercetin; KML, Kaempferol;CCN, Catechin; ECN, Epicatechin; FRA, Ferulic acid; VIT, Vitexin; PCA, protocatechuic acid; GAC, Gallic acid; SYA, Syringic acid; CFA, caffeic acid; CGA, Chlorogenic acid; ISV, Isovitexin; SPA, Sinapic acid; p-CA, p-Coumaric acid; CMA, Cinnamic acid; RVL, Resveratol; ND, Not detected.

Table 5
Pearson correlation coefficients between the various parameters of freeze-dried WJP.

	TCC	TPC	TAA	MC	AC	Yield %	PC
TPC	0.895**						
TAA	0.914**	0.930**					
AC				−0.714*			
Yield (%)					0.839**		
K					0.641*		
Mg	0.846*	0.865**	0.888**				
Mn	0.596*	0.766*	0.780*				
P			0.697*			0.596*	
Free FRA		0.703*	0.800**				
Free ISV		0.653*	0.770**				
Bound PCA		0.687*					
Bound ECN		0.737*	0.597*				
Bound CGA		0.690*	0.695*				
EAAs							0.860**
NEAAs							0.872**

TCC, Total chlorophyll content; TPC, Total phenolic content; TAA, Antioxidant activity; AC, Ash content; MC, Moisture content; PC, Protein content; K, Potassium; P, Phosphorus; Mg, Magnesium; Mn, Manganese; EAAs, Essential amino acids; NEAAs, Non-essential amino acids; ECN, Epicatechin, ISV, Isovitexin; FRA, Ferulic acid; PCA, Protocatechuic acid; CGA, Chlorogenic acid; $*p \leq 0.05$; $**p \leq 0.005$.

(r = 0.737 and 0.597, p ≤ 0.05) as shown in Table 5.

Among flavonols, the contents of free rutin, quercetin and kaempferol varied from 15.09 to 332.68, 1.13 to 22.46 and 0.16–31.19 µg/g, respectively in WJP of wheat varieties grown under different conditions. The level of rutin was higher than quercetin and kaempferol in WJP of all wheat varieties. Skoczylas et al. (2017) also reported higher content of rutin (27.9 to 43.7 mg/l) than quercetin (0.7 to 1.1 mg/l) in frozen and stored wheatgrass juice. Among the four wheat varieties, HD-3086 exhibited the highest free rutin (332.68 µg/g) and kaempferol content (31.19 µg/g) while the level of free quercetin (22.46 µg/g) was more in HD-2967. The previous study by Kardas and Durucasu (2014) reported a higher content of quercetin (115 µg/g) in comparison to our results. The bound rutin varied from 5.80 to 64.15 µg/g with the highest level in HS-490 grown using CNS. The level of free and bound rutin was more in WJP of wheat varieties grown using CNS than those grown using soil and water. While, free quercetin and kaempferol contents were highest in WJP of wheat varieties grown using soil. The level of free rutin, quercetin and kaempferol showed higher significant variation (P ≤ 0.005) among wheat varieties than the growing conditions (Table S1). While, bound rutin, quercetin and kaempferol showed higher significant variation (P ≤ 0.005) with growing conditions than the wheat varieties.

Among flavones, the level of bound isovitexin (40.28 to 181.35 µg/g) was higher than the free isovitexin (2.20 to 48.68 µg/g) in WJP of all wheat varieties. The level of free and bound isovitexin was more in WJP of wheat varieties grown using soil than those grown using CNS and water. HD-3086 exhibited the highest level of bound isovitexin while free isovitexin content was higher in HD-2967. The level of free and bound vitexin varied from 16.76 to 105.82 µg/g and 0.29 to 25.60 µg/g, respectively. The levels of free and bound isovitexin and free vitexin showed higher significant variation (P ≤ 0.005) with the wheat varieties than the growth conditions (Table S1). However, the bound vitexin showed higher significant variation (P ≤ 0.005) with growing conditions than the wheat varieties. The free resveratrol content ranged from 0.45 to 41.06 µg/g in WJP of four wheat varieties. The highest free vitexin, bound vitexin and free resveratrol contents were reported in WJP of HD-2967 wheat variety grown using soil. The free and bound resveratrol showed higher significant variation (P ≤ 0.005) with growing conditions than the wheat varieties (Table S1). The free isovitexin exhibited significant positive correlation with TPC (r = 0.653, P ≤ 0.05) and highly significant positive correlation with TAA (r = 0.770, P ≤ 0.005) of WJP (Table 5).

3.8. Total antioxidant activity (TAA)

The TAA of the WJP ranged from 13.54 to 17.33 µmol TE/g, where the highest and the lowest TAA was exhibited by HD-3086 and HS-490 variety, respectively (Table 3). The previous study reported that WJP had a TAA of 0.67 mg DPPH/g (Akbas et al., 2017). WJP had high TAA as it is a good source of various phenolics and flavonoids known for antioxidant properties (Benincasa et al., 2015; Akbas et al., 2017). Among growing conditions, the highest TAA was observed for wheat varieties grown using soil (14.56–17.33 µmol TE/g) followed by CNS (14.33–16.66 µmol TE/g) and water (13.54–16.10 µmol TE/g). Similar changes in the antioxidant potential of wheat plants grown under different conditions was reported by Kulkarni, Tilak et al. (2006). TAA of WJP showed a higher significant variation among wheat varieties than the growing conditions (P ≤ 0.005, Table S1). The level of various phenolic acids and flavonoids determines the antioxidant potential of wheat plants and their contents mainly vary with genotype, growing location and harvesting time (Mpofu et al., 2006; Kulkarni, Tilak et al., 2006). TAA exhibited a highly significant positive correlation with TPC and TCC (r = 0.930 and 0.914, respectively; p ≤ 0.005) as shown in Table 5. Several studies suggested that the antioxidant activity has a strong correlation with phenolic compounds present in the wheatgrass samples (Akbas et al., 2017; Złotek, Szymanowska, Jakubczyk, Sikora, & Świeca, 2019).

3.9. Principle component analysis (PCA)

The PCA was carried out to correlate the results of various parameters such as proximate composition, phenolic profile, amino acid profile and antioxidant properties (Fig. 1 and Table S2). Multivariate analysis was used to characterize the effect of growing conditions on WJP obtained from different wheat varieties. Eigenvalues greater than 1 were considered for determining the relative contribution of components in total data variability. The first to eight principal components (PC1 to PC8) explained 96.5% variability within the observations. PC1, PC2, PC3, PC4, PC5, PC6, PC7 and PC8 accounted for 28.8%, 25.1%, 15.2%, 11.3%, 5.5%, 4.5%, 3.2% and 2.9% of the total variability, respectively (Table S2). Different variables such as TCC (0.200), TPC (0.255), TAA (0.227), Mg (0.218), free rutin (0.206), free FRA (0.225), free CFA (0.160), bound PCA (0.239), bound epicatechin (0.233) and bound CGA (0.178) were correlated the most with the PC1. While, PC (0.174), EAAs (0.226), NEAAs (0.258), free epicatechin (−0.271), free CMA (−0.259), free vitexin (−0.253), bound quercetin (−0.222), bound CMA (−0.206), bound isovitexin (0.207) and bound SPA

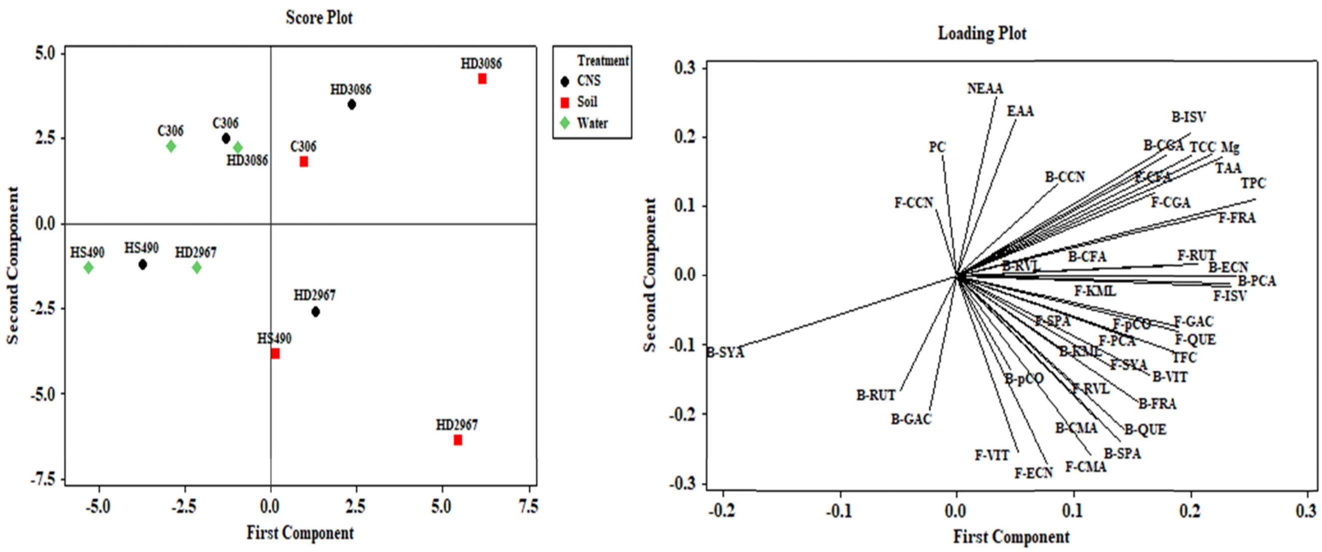

Fig. 1. Principal component analysis score plot (A) and loading plot (B) describing relationship among different properties of WJP obtained from wheat varieties grown under different growing conditions (MC, Moisture content; PC, Protein content; AC, Ash content; TCC, Total chlorophyll content; TPC, Total phenolic content; TFC, Total flavonoid content; TAA, Total Antioxidant activity; EAA, Essential amino acids; NEAA, Non-essential amino acids; Mg, Magnesium; F, Free; B, Bound; RUT, Rutin; QUE, Quercetin; KML, Kaempferol*;* CCN, Catechin, ECN, Epicatechin, FRA, Ferulic acid; VIT, Vitexin; PCA, Protocatechuic acid; GAC, Gallic acid; CFA, Caffeic acid; CGA, Chlorogenic acid; ISV, Isovitexin.; SYA, Syringic acid; CFA, Caffeic acid; SPA, Sinapic acid; *p*-CA, *p*-Coumaric acid; CMA, Cinnamic acid; RVL, Resveratol).

(−0.240) mainly contributed to the PC2. As shown in Fig. 1 A, WJP of HD-3086 wheat variety grown using soil placed on the extreme right-hand side near to the y-axis of the plot was characterized by high TCC, TPC, TAA, Mg content, level of free (FRA and CFA) and bound (CGA and PCA) phenolic acids and free (kaempferol) and bound (isovitexin and epicatechin) flavonoids. It was followed by HD-3086 grown using CNS and C-306 grown using soil and CNS, respectively. The WJP of wheat varieties grown using water were placed on the left-hand side of score plot as they had low PC, TCC, TPC, TAA, Mg content, EAAs, NEAAs, level of free phenolic acids (FRA and *p*-CA) and flavonoids in free (kaempferol) and bound (isovitexin and epicatechin) forms. As shown in Fig. 1B, TCC, TPC, Mg content, phenolic acids and flavonoids were positively correlated with TAA indicating that the increase in their contents increases the antioxidant activity of WJP. The free FRA was closely related to TPC and was the predominant phenolic acid in WJP.

4. Conclusion

The present work showed that WJP of HD-3086 and C-306 exhibited higher antioxidant properties, TCC, Mg content, EAAs and NEAAs than the other two wheat varieties. Growing conditions significantly influenced the nutritional composition and antioxidant properties of WJP. The soil grown WJP exhibited higher AC, TCC, TPC, TFC, TAA, minerals (K, Ca, Mg and Mn contents), phenolic acids (GAC, PCA, FRA, CFA, CGA and *p*-CA) and flavonoids (catechin, quercetin, kaempferol, vitexin). While, CNS grown WJP showed higher yield, PC, minerals (P and Fe contents), level of essential AAs and rutin content. The wheatgrass grown using water exhibited the lowest mineral content, AAs, TPC and antioxidant activities. Thus, wheatgrass grown using soil and CNS had better nutritional composition and antioxidant properties. Principal component analysis also confirmed the observed differences between WJP of four wheat varieties and three growing conditions.

CRediT authorship contribution statement

Nancydeep Kaur: Investigation, Data curation, Writing - original draft. **Balwinder Singh:** Conceptualization, Data curation, Formal analysis, Resources, Software. **Amritpal Kaur:** Conceptualization, Methodology, Funding acquisition, Project administration, Resources, Formal analysis. **Madhav P. Yadav:** Conceptualization, Resources, Supervision, Validation. **Narpinder Singh:** Resources, Data curation, Validation, Formal analysis. **Arvind Kumar Ahlawat:** Resources, Validation, Formal analysis. **Anju Mahendru Singh:** Resources, Validation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128201.

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