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Nutritional composition, bioactive compounds and free radical scavenging activity of wheatgrass (*Triticum aestivum* L.) as influenced by harvesting stages and cultivation method

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Wheatgrass is a rich source of protein (24.08 to 30.40 g/100 g DM) when cultivated under indoor and outdoor conditions and harvested at different stages. The ash ranged being 7.68 to 8.46 g/100 g DM. The crude fibre content was high especially under indoor cultivation, the values were in the range of 19.06 to 27.68 g/100 g. Indoor cultivation was far better than outdoor cultivation in terms of higher protein and ash. Late harvesting stage was better for crude fibre but for proteins, the early stage *i.e* 7th day was superior. The early harvesting (7th day and indoor cultivation) was superior for obtaining maximum ascorbic acid from the wheatgrass. For maximum β-carotene in wheatgrass, the optimum stage of harvesting was the 10th day from the day of sowing during indoor conditions while 7th day was the right stage for harvesting wheatgrass during the outdoor cultivation. It has an abundant amount of chlorophyll and flavonoids, the content was varying between 4.14 to 17.72 g/100 g and 115.67 to 460.18 QE/g, respectively under different harvesting stages and cultivation conditions. The free radical scavenging activity of indoor-grown wheatgrass was significantly ($P \leq 0.05$) higher in comparison to outdoor cultivated wheatgrass on 7th and 10th day of harvesting.

Keywords: Bioactive compounds, Cultivation, Free radical scavenging activity, Harvesting, Minerals, Proximate composition, Wheatgrass.

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Introduction

The horizon of clinical utilities of wheatgrass rises from mild illness to critically ill cancer patients. Wheatgrass has been reported to have hypo-cholesterolemic effect resulting from increased excretion of faecal cholesterol[1]. The antioxidant properties of wheatgrass causing an antimutagenic effect have also been well documented[2]. Wheatgrass is also believed to be an abundant source of various bioactive compounds like alkaloids, glycosides, saponins, steroids and phenolic compounds like tannins and flavonoids. The presence of such compounds further enhanced the therapeutic properties of wheatgrass[3]. A systematic evaluation and enhancement of nutritional and health-promoting components provided by wheatgrass in the suitable health foods hold significance. The wheatgrass enriched foods can be recommended to the population for general good health as well as for cure of disorders like metabolic syndrome, cancer, thalassemia, anaemia etc. Cultivation method and harvesting stage pose a significant influence regarding nutrients and many health-promoting compounds of wheatgrass. For the laboratory purpose, various methods of cultivation techniques were employed and most of the methods opted were indoor methods of cultivation with slight variations in the length of the germination process, however, the outdoor cultivation method was rarely employed[4]. A comparison of different harvesting stages of wheatgrass i.e., 7th, 9th, 10th, and 13th day from the day of sowing has been reported in the literature. Length of grasses was also found to be other criteria for wheatgrass in which it can be harvesting starting 7 inches to 15-16 inches[5]. A study explained that the chlorophyll, minerals (Ca, Fe, Zn and Se) and amino acids in wheatgrass grown outdoor conditions were higher when compared with indoor cultivated wheatgrass[6]. Undoubtedly various data has been established regarding wheatgrass even for medical research, but no comprehensive data is available clearly on exact days that wheatgrass is to

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be harvested when cultivated at either indoor or outdoor location. Comparison of different growth stages and different cultivation methods will give knowledge about the appropriate harvesting stage for the optimum amount of most of the nutrients of interest under a specific location of cultivation.

Materials and Methods

Preparation of samples for analysis

Wheat variety PBW-621 was obtained from the wheat section of Department of Plant Breeding and Genetics, Punjab Agricultural University, Punjab, India. Wheatgrass was cultivated under both indoor (3×1 ½ feet of soil bed) and outdoor (6×2 feet of soil bed) conditions. Soil from the same location was used for both the types of cultivation. Before broadcasting, the soil was tilted and moistened. The germinated seeds were broadcasted densely, covered with a layer of soil and sprinkled with sufficient water to moist the soil. No additional nutrients/fertilizers were added. Tap water was used for watering the grass. The wheatgrass was allowed to grow upto 13th day and was harvested at 7th, 10th, and 13th day from the date of sowing. The wheatgrass was harvested by cutting with a clean sharp knife right above the surface of the soil. The harvested grass was rinsed with distilled water followed by freeze-drying (-40°C for 72 hours) using Labconco, Freezone Benchtop Freeze Dry System). Dried wheatgrass was ground into a fine powder and packed in airtight plastic bags for further analysis.

Biochemical analysis

The freeze-dried samples of WG harvested at three harvesting stages and grown at indoor and outdoor locations were subjected to biochemical analysis using standard analytical procedures. Proximate analysis was carried out by AOAC method[7]. The methods of Arnon[8] and Woisky and Salatino[9] were followed for the estimation of chlorophyll and flavonoids, respectively. The tannins were determined with Follin-Denis reagents[10]. Phytin phosphorous was determined by the method of Haugand Lantzsch[11]. Saponins were determined gravimetrically[12]. The fresh samples were analyzed for β-carotene by column chromatography[13] and ascorbic acid by AOAC method[7]. The free radical scavenging activity was determined by using the DPPH assay[14].

Statistical analysis

All the experiments were conducted thrice. Mean and standard deviations for the various parameters were computed. Analysis of variance (ANOVA) was employed to assess the difference in parameters as influenced by harvesting while t-test was used to study differences due to cultivation location i.e. indoor and outdoor using Microsoft Excel (2003) statistical analysis tool pack. Least significant difference (LSD) at 5% was calculated for the comparison among the parameters.

Results and Discussion

Moisture

The moisture content of wheatgrass under indoor cultivation was 87.3, 88.9 and 87.3% on 7th, 10th, and 13th day, respectively. The corresponding values of outdoor cultivation were 87.7, 88.6 and 87.3%. The moisture content of fresh wheatgrass on 10th day was significantly ($P \leq 0.05$) higher in comparison to 7th and 13th day under both indoor and outdoor cultivation. Further, the moisture content of outdoor cultivated wheatgrass was significantly ($P \leq 0.05$) higher than the indoor cultivated grass when harvested on 7th and 10th day. Under both indoor and outdoor cultivation, the wheatgrass harvested on 10th day exhibited the highest moisture content. The moisture in wheatgrass at par with moisture in common green leafy vegetables such as mustard leaves, radish leaves, celery leaves and bottle gourd leaves (87.9-89.8%) have been reported[15].

Proximate composition

The proximate composition of wheatgrass harvested on 7th, 10th, and 13th day after sowing under both indoor and outdoor locations on dry weight basis has been discussed in Table 1. The protein content of WG in outdoor cultivation decreased significantly ($P \leq 0.05$) when harvested at 13th day from the day of sowing in comparison to 7th and 10th day of harvesting stage. A significantly ($P \leq 0.05$) higher protein was observed in outdoor cultivation of wheatgrass during all the three stages of harvesting. The protein content of 25.5 to 28.38% in wheatgrass has been reported[16,17]. On the other hand, the higher protein content of 35.12 to 38.67% was also reported[18]. A significantly ($P \leq 0.05$) lower ash content was found in outdoor cultivated wheatgrass when harvested on 7th and 10th day. Literature showed plenty of variation in the ash content of wheatgrass as a higher ash content in the range of 10.63 to 12.59% was reported[17] while a lower value of 4.15 and 4.80% was also observed[16,17]. The wide difference in ash content of the grasses may

be attributed to the mineral content of the soil in which wheatgrass was cultivated. The statistical analysis showed that crude fat was significantly ($P \leq 0.05$) lower in wheatgrass harvested on 7th and 10th day. Fat values in the range of 0.6 to 1.9 have been reported in the literature[16,17,19] which were higher than the values obtained in the present study. The findings of the present study indicated that wheatgrass contained a negligible amount of fat. A significant ($P \leq 0.05$) increase in fibre content was found in the wheatgrass on 13th day of harvesting in comparison to 7th and 10th during both indoor and outdoor cultivation. Further, significantly ($P \leq 0.05$) higher values of fibre were observed during indoor cultivation in comparison to outdoor cultivation when wheatgrass was harvested on 7th and 10th day of sowing.

The results highlighted that indoor cultivation was found to be preferable than outdoor cultivation in terms of higher protein, ash and crude fat. Late harvesting stage was better for crude fibre but for proteins, the early stage i.e., 7th day was superior.

Ascorbic acid and β-carotene

As shown in Table 2, a significant ($P \leq 0.05$) reduction in ascorbic acid was found with an increase

Table 1 — Proximate composition (g/100 g dry matter) of wheatgrass harvested at different stages during indoor and outdoor cultivation

Parameter	Stage of harvesting			LSD at 5%
	7th day	10th day	13th day	
		Moisture		
Indoor	2.80±0.37	2.07±0.32	2.88±0.36	NS
Outdoor	2.58±0.30	2.36±0.26	2.62±0.44	NS
t-value	NS	NS	NS	
		Protein		
Indoor	30.40±0.84	29.89±0.40	28.97±0.57	NS
Outdoor	27.41±0.96	25.10±0.80	24.08±0.53	2.68
t-value	2.33**	5.31**	6.29**	
		Ash		
Indoor	8.30±0.08	8.46±0.09	8.24±0.07	NS
Outdoor	7.68±0.15	8.07±0.07	8.02±0.09	NS
t-value	3.62**	3.34**	NS	
		Crude fat		
Indoor	0.55±0.05	0.52±0.02	0.38±0.05	0.15
Outdoor	0.59±0.05	0.53±0.05	0.36±0.04	0.16
t-value	NS	NS	NS	
		Crude fiber		
Indoor	22.91±0.64	24.45±0.99	27.68±1.47	3.70
Outdoor	19.06±0.34	20.18±0.44	24.70±0.67	1.71
t-value	5.31**	3.94**	NS	

Values are Mean±SD; NS non-significant; **Significant at 5%

Table 2 — Ascorbic acid and β-carotene content (on fresh weight basis) of wheatgrass powder harvested at different stages during indoor and outdoor cultivation

Parameter	Stage of harvesting			LSD at 5%
	7th day	10th day	13th day	
		Ascorbic acid (mg/100 g)		
Indoor	2.55±0.37	2.85±0.31	2.07±0.15	NS
Outdoor	2.59±0.22	2.11±0.23	1.48±0.15	0.69
t-value	NS	NS	2.79*	
		β-carotene (µg/100 g)		
Indoor	161.15±24.03	216.77±18.25	178.71±13.07	NS
Outdoor	296.86±22.45	319.65±25.07	227.30±30.09	88.69
t-value	NS	NS	NS	

Values are Mean±SD; NS non-significant; *Significant at 5%

in days of harvesting during outdoor cultivation as the wheatgrass harvested at 7th day (2.59 mg) had 75% higher ascorbic acid in comparison to the one harvested on 13th day (1.48 mg). Further, the outdoor grown wheatgrass harvested on 13th day had 39.86% lower ascorbic acid content than that of indoor-grown wheatgrass. A study revealed that the ascorbic acid content of fresh wheatgrass decreased to 0.83 mg/g when harvested on 12th day when compared with a value of 1.17 mg/g on harvested at 4th day from the day of sowing has been reported[20]. The ascorbic acid content in green vegetables decreases when harvested on advanced maturity stage which is related to physiological and climatic conditions[21,22]. The higher the intensity of light during the growing season, the greater is vitamin C content in plant tissues. The results indicated that early harvesting i.e., 7th day and indoor cultivation is superior for obtaining maximum ascorbic acid from the wheatgrass. A reasonably inconsistent result of β-carotene was observed under both indoor and outdoor cultivation location. The β carotene was significantly ($P \leq 0.05$) decreased on 13th day in comparison to 7th and 10th day during outdoor cultivation.

Bioactive compounds

The chlorophyll content of wheatgrass was 2.34, 3.28 and 2.51 g per 100 g dry matter, respectively, under indoor conditions. On the other hand, the chlorophyll content of outdoor cultivated dried wheatgrass was 7.59, 7.68, and 8.11 g per 100 g on 7th, 10th, and 13th day, respectively as shown in Table 3. In a study, Desai[15] reported 5.12% of chlorophyll in 9th day harvested wheatgrass. The variations in chlorophyll concentrations may be attributed to the agronomic factors *viz* time of cultivation, temperature and rate of exposure to sunlight. Chlorophyll concentration of outdoor cultivated grass was significantly higher ($P \leq 0.01$) than indoor cultivated grass. The difference in the production methods appeared to influence the

Table 3 — Bioactive components and free radical scavenging activity (on dry matter basis) of wheatgrass harvested at different stages during indoor and outdoor cultivation

Bioactive Compound	Stage of harvesting			LSD at 5%
	7th day	10th day	13th day	
		Chlorophyll (g/100 g)		
Indoor	2.34±0.27	3.28±0.18	2.51±0.29	0.87
Outdoor	7.59±0.56	7.68±0.70	8.11±0.45	NS
t-value	8.41***	6.07***	10.43***	
		Tannins (mg/100 g)		
Indoor	6.21±0.29	7.40±0.34	7.60±0.34	1.13
Outdoor	6.33±0.22	7.15±0.17	7.89±0.23	0.71
t-value	NS	NS	NS	
		Phytic acid (mg/100 g)		
Indoor	3.03±0.17	3.50±0.09	4.00±0.17	0.57
Outdoor	3.02±015	3.47±0.21	3.83±0.11	0.55
t-value	NS	NS	NS	
		Saponins (g/100 g)		
Indoor	0.74±0.42	1.14±0.11	1.48±0.08	0.29
Outdoor	0.87±0.05	1.64±0.18	2.36±0.11	0.43
t-value	NS	2.36**	6.36**	
		Flavonoids (QE/g)		
Indoor	388.31±31.40	460.18±11.42	115.67±13.29	70.65
Outdoor	452.75±28.17	308.99±29.69	159.22±14.74	86.53
t-value	NS	4.75**	2.19**	
		Free radical scavenging activity (%)		
Indoor	49.75±1.61	49.55±1.86	44.68±2.00	NS
Outdoor	36.39±3.38	43.50±1.79	44.69±2.01	NS
t-value	3.57**	2.33**	NS**	

Values are Mean±SD; NS Non-significant; **Significant at 5%; *** Significant at 1%; QE- Quercetin equivalent

chlorophyll concentration of wheatgrass. Outdoor grown wheatgrass, in the present study, contained approximately more than 70% of chlorophyll than indoor grass. This could be because outdoor grown wheatgrass was subjected to more direct sunlight. A significantly ($P \leq 0.05$) lower tannins were found on 7th day in comparison to 10th and 13th day during indoor as well as outdoor cultivation. However, no significant difference was found in the tannins content between indoor and outdoor cultivated wheatgrass. A significantly ($P \leq 0.05$) lower phytic acid was found on 7th day in comparison to 10th and 13th day under both indoor and outdoor cultivation. Saponins increased significantly ($P \leq 0.05$) with the growth of wheatgrass during indoor and outdoor cultivation. The saponin content of outdoor cultivated wheatgrass was significantly ($P \leq 0.05$) higher on 7th and 10th day. There are limited quantitative studies of saponins, nevertheless, various research on qualitative analysis showed that wheatgrass has an abundant amount of saponins. Haemolytic and foam test of wheatgrass juice indicates the presence of saponins[24]. Flavonoids contribute to antioxidant properties of green vegetables, fruits and various foods. There was a significant ($P \leq 0.05$) difference in flavonoid content of wheatgrass at three different stages under indoor cultivation, the maximum flavonoids were on 10th day followed by 7th day and 13th day. On the other hand, flavonoids content in outdoor cultivated wheatgrass was significantly ($P \leq 0.05$) decreased with increasing stage of growth. The flavonoid content in indoor cultivated wheatgrass was significantly ($P < 0.05$) higher than outdoor cultivated grass on 10th day. On the contrary, outdoor cultivated grass had significantly ($P < 0.05$) higher flavonoid content on 13th day. Sprouting of seeds of wheat at indoor and outdoor locations improve the nutritional value of the grass with preferably to indoor cultivation has been reported[6].

Free radical scavenging activity

Under indoor cultivation, the DPPH Free radical scavenging activity of wheatgrass was 49.75, 49.55 and 44.68% on 7th, 10th, and 13th day, respectively. The corresponding values under outdoor cultivation were 36.39, 43.50, and 44.69%. The statistical analysis revealed that the free radical scavenging activity of indoor-grown wheatgrass was significantly ($P \leq 0.05$) higher in comparison to outdoor cultivated wheatgrass powder on 7th and 10th day of harvesting. Various bioactive components like phenols, flavonoids, ascorbic acid, vitamin E, β-carotene and antioxidant enzymes namely superoxide dismutase, cytochrome oxidase were all responsible for antioxidant activity of wheatgrass[25]. These antioxidant compounds were likely to changes with certain exposure to air. A decrease in antioxidant content may be attributed to the loss of such antioxidants compound in post-harvesting. The percentage capacity to scavenge free radicals by wheatgrass depends upon the synergistic effect of bioactive compounds present in it[24]. An abundant amount of chlorophyll, tannins, flavonoids, ascorbic acid, and β-carotene observed in the present study might have contributed to the antioxidant activity of wheatgrass. Certain antioxidative enzymesare to be considered which were not analyzed in the present study.

Conclusion

The results concluded that wheatgrass is a rich source of protein. The ash content was also high, but the fat content was very low. The crude fibre content was high, especially under indoor cultivation. The indoor cultivation was better than outdoor cultivation in terms of higher protein, ash and crude fat. Late harvesting stage was better for crude fibre but for proteins, the early stage i.e., 7th day was superior. In comparison to wheat grain, wheatgrass had much higher protein, minerals and crude fibre but lesser fat; therefore, wheatgrass can be a suitable component for the enrichment in various food products. Wheatgrass has plenty of minerals. A considerable amount of calcium was observed in wheatgrass Core mineral for chlorophyll synthesis, magnesium was also found in high amounts. High amounts of manganese, iron, copper and chromium were also observed. Minerals responsible for antioxidant activity of wheatgrass namely zinc and selenium were also found in appreciable amounts. The outdoor cultivation was a better location for wheatgrass cultivation when certain minerals of interest are to be considered except zinc and selenium. Analysis of bioactive compounds revealed that plenty of bioactive compounds namely chlorophyll, flavonoids, saponins, phytic acid and tannins were present in wheatgrass. High free radical scavenging activity of wheatgrass makes it a highly potent functional food component when harvested between 7 to 13 days from the day of sowing during indoor cultivation and 13th day from the day of sowing during outdoor cultivation.

Conflicts of interest

The authors declare no conflict of interest.

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