

Effect of drying procedures on nutritional composition, bioactive compounds and antioxidant activity of wheatgrass (*Triticum aestivum* L)

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Abstract Fresh wheatgrass was dried using shade, oven and freeze drying methods. The ascorbic acid and β -carotene and content of oven dried wheatgrass was significantly lower (133.13 μ g and 1.98 mg) in comparison to shade dried (193.62 μ g and 2.30 mg) and freeze dried (230.35 μ g and 3.18 mg) wheatgrass. A significantly lower iron and zinc was observed in freeze dried (13.19 and 6.55 mg) wheatgrass in comparison to shade dried (28.94 and 7.40 mg) and oven dried (19.65 and 8.35 mg) wheatgrass. The chlorophyll in freeze dried (3.61 g) wheatgrass was significantly higher than shade dried (2.35 g) and oven dried (2.14 g) wheatgrass. The tannin content in wheatgrass was significantly higher in oven dried wheatgrass (8.18 mg/100 g) as compared to shade dried (6.68 mg/100 g) and freeze dried (6.34 mg/100 g) wheatgrass. The antioxidant activity of shade, oven and freeze dried wheatgrass was 50.13, 48.94 and 53.78%, respectively. The study concluded that freeze drying had preserved maximum amounts of chlorophyll, flavonoids, saponins and antioxidant activity in wheatgrass.

Keywords Wheatgrass · Drying · Proximate analysis · Minerals · Bioactive compounds · Antioxidant activity

Introduction

Wheatgrass is a young plant of common wheat variety, *Triticum aestivum*. It is reported to be a power house of various nutrients like proteins, minerals, vitamins, active enzymes and bioactive compounds like alkaloids, glycosides, saponins, steroids, tannins and flavonoids. The clinical utility of wheatgrass varies from mild illness to life threatening diseases like cancer. Antioxidant property of wheatgrass accounts for the treatment of most of the degenerative diseases like diabetes and cardiovascular diseases. It is proven to be beneficial under various conditions such as anaemia, diabetes, cancer, eczema, constipation, kidney swelling and common cold (Afroz et al. 2012).

The most common forms of wheatgrass available are juice, powder and encapsulated pills. The freshness of wheatgrass can be maintained for 2–3 weeks if stored in refrigerator at 33 to 40 °F (Meyoritz 2010). A loss of nutrients in freezing of wheatgrass was observed by Wigmore (1985). Hence, it is recommended that wheatgrass should be consumed within few days of harvesting. Wheatgrass is as perishable as fruits and vegetables because of its high moisture content which aid its quick deterioration. Therefore, processing may play an important role in the preservation and commercialization of wheatgrass. Drying is one of the major processing technique that can improve the shelf life of wheatgrass. Wheatgrass must be dried at lower possible temperature, so as to prevent loss of the heat sensitive compounds. For this purpose, spray dried and freeze dried powder from wheatgrass juice is the most competent option. The attempts made to commercially preserve the wheatgrass through vacuum drying processes indicated that the elevated temperatures to which the product was exposed during this processing resulted

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into the product that was inconsistent in composition, had a poor flavour and was diminished in nutrient assay, particularly showing a marked decrease in the levels of viable enzymes (Sagliano and Sagliano 1998).

The nutritional quality of the food is greatly influenced by drying methods. The removal of water by heat has been reported to affect the nutrients content of foods in various ways. It can either increase the concentration of some nutrients by making them more available or decrease the concentration of other nutrients (Hassan et al. 2007). In various aromatic and medicinal plants, freeze drying method is the most recommended method in retention of plant components (Abascal et al. 2005).

Comparative studies on suitable methods of drying wheatgrass are limited till date. The present study was undertaken in order to provide comparative data of different drying methods and best method to opt for preparation of wheatgrass powder with maximum health promoting properties.

Materials and methods

Wheat variety PBW-621 was procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Punjab, India. Wheatgrass was cultivated under indoor ($3 \times 1\frac{1}{2}$ feet of soil bed) conditions. 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 so as to moist the soil. The wheatgrass was harvested at 7th day from the date of sowing. The harvested grass was rinsed with distilled water followed by drying.

The harvested wheatgrass was dried with three methods viz. shade drying, oven drying and freeze drying. The wheatgrass was cut into 2 cm long pieces and was divided into three parts. A part of wheatgrass was dried at room temperature under shade till the constant weight was achieved. The second portion was spread over a tray lined with butter paper. The wheatgrass was dried to a constant weight at 50 °C for 8 h. The third part was freeze dried at – 40 °C for 72 h. The wheatgrass samples dried by three methods were ground into fine powder and packed in airtight plastic pouches. The dried samples were stored in deep freezer at – 18 °C until further analysis.

The proximate composition and ascorbic acid were determined according to the standard AOAC (2005) methods. β -carotene of samples was determined by column chromatography (Ranganna 2002). The minerals were determined in the samples by digesting in diffused microwave system (MLS 1200 Mega; Milestone S.r.L., Sorisole, Italy) using polytetrafluoroethylene digestion vessels. Minerals namely calcium, magnesium, manganese, iron,

copper, zinc, selenium and chromium in the digested samples were estimated by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700 series, Agilent Technology International Pvt. Ltd).

The chlorophyll content was determined by the method of Thimmaiah (1999) where 1 g of sample was homogenized with 10 ml of 80% acetone and centrifuged at 5000 rpm for 5 min to collect the filtrate. The residue was again extracted with 80% acetone until the residue became colourless. The final volume was made to 100 ml with 80% acetone and the absorbance was measured at 663 nm and 645 nm on spectrophotometer. The amount of chlorophyll pigments was calculated using the following formula:

$$\begin{aligned} \text{mg total chlorophyll (per g tissue)} \\ = 20.2(A_{645}) + 8.02(A_{663}) \times V/1000 \times W \end{aligned}$$

where A = absorbance at specific wavelength; V = final volume of the chlorophyll extract in 80% acetone and W = fresh weight of the tissue extracted.

The aluminium chloride colorimetric method was used to determine flavonoids in the samples (Woisky and Salatino 1998). One gram of sample was extracted with 25 ml of 95% ethanol under 200 rpm shaking for 24 h. After filtration, the filtrate was adjusted to 25 ml with 80% ethanol. The extract (0.5 ml) was taken in a test tube and added 0.5 ml of 96% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance of the mixture was read at 415 nm. Quercetin was used to make the standard curve. Ten milligrams of quercetin were dissolved in 80% ethanol and then diluted to 25, 50 and 100 $\mu\text{g/ml}$. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The absorbance was read at 415 nm.

The tannins were determined with Follin–Denis reagent according to the method of Tamilselvi et al. (2012). The phytic acid content was assessed using the method given by Haug and Lantzsch (1983). Double extraction gravimetric method was followed to determine saponins in the samples (Harborne 1973).

The free radical scavenging activity was determined by using the DPPH assay (Dehshahri et al. 2012). Two grams of sample was extracted with 20 ml of methanol by shaking for 2 h. Extraction process was repeated twice. The extract was pulled together and centrifuged at 10,000 rpm for 15 min. The supernatant was stored at – 20 °C till analysis. 100 μl of the aliquot of extract was taken in a test tube and added 2.9 ml of DPPH solution. The mixture was vortexed for 1 min and incubated in dark for 3 min. Discolouration of DPPH was measured against blank at 517 nm. The DPPH scavenging effect was measured using

by formula: Percent inhibition = $A_B - A_A/A_B \times 100$, where A_B = absorbance of blank; A_A = absorbance of sample

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 drying methods using Microsoft Excel (2010) Statistical Analysis Tool Pack. Least Significant Difference (LSD) at 5% was calculated for the comparison among the parameters.

Results and discussion

The nutritional composition of the shade, oven and freeze dried wheatgrass has been given in Table 1. The moisture content of wheatgrass for shade, oven and freeze drying was 3.85, 2.33 and 1.55%, respectively. The statistical analysis revealed that the moisture content of freeze dried wheatgrass was significantly ($p \leq 0.05$) lower when compared to shade and oven dried wheatgrass. Desai (2005) and Chouhan and Mogra (2014) reported a higher moisture content of 4.4 and 11.4 g/per 100 g, respectively for shade dried wheatgrass powder. The moisture content of wheatgrass observed in the present study is lower than the values

reported in the literature. A decrease in moisture content of oven dried wheatgrass may be due to application of heat that leads to subsequent reduction in moisture content. On the other hand, freeze drying is based on sublimation process of frozen products and hence leads to complete removal of water.

A significant ($p \leq 0.05$) reduction in protein content was observed in oven dried (27.83%) in comparison to shade (31.37%) and freeze dried (30.04%) wheatgrass. Desai (2005) found closer values of 25.5 and 28.38% for protein in wheatgrass. The decrease in protein could be attributed to the ability of the drier to concentrate energy which could in turn cause some denaturation of protein in dried samples (Hassan et al. 2007). No significant difference in ash content of wheatgrass was observed in all the three methods of drying as the values were ranged between 8.27 and 8.54%. Desai (2005) reported the lower values of ash in wheatgrass powder (4.15–4.80%). The variation in ash content of wheatgrass may be attributed to genetic changes during growth and different methods of cultivation. The soil mineral content may also greatly influence the ash content in wheatgrass, hence, may be a cause of variation in the mineral content of wheatgrass reported by different researchers. The crude fat content of the oven dried wheatgrass powder was significantly ($p \leq 0.05$)

Table 1 Nutritional composition of shade, oven and freeze dried wheatgrass (per 100 g)

Parameter	Method of drying			LSD at 5%
	Shade	Oven	Freeze	
<i>Proximate composition</i>				
Moisture, g	3.85 ± 0.17	2.33 ± 0.19	1.55 ± 0.11	0.56
Protein, g	31.37 ± 0.63	27.83 ± 0.52	30.04 ± 0.43	1.98
Ash, g	8.54 ± 0.09	8.33 ± 0.08	8.27 ± 0.07	NS
Crude fat, g	0.59 ± 0.04	0.34 ± 0.05	0.54 ± 0.03	0.15
Crude fiber, g	25.61 ± 1.01	28.13 ± 1.49	21.29 ± 0.46	3.96
<i>Vitamins</i>				
Ascorbic acid, mg	2.30 ± 0.06	1.98 ± 0.15	3.18 ± 0.42	0.88
β carotene, μg	193.62 ± 36.62	133.13 ± 22.75	230.35 ± 36.57	72.07
<i>Minerals</i>				
Calcium, mg	72.72 ± 2.80	38.67 ± 4.01	38.16 ± 7.17	18.47
Magnesium, mg	287.97 ± 9.48	259.99 ± 18.60	260.40 ± 20.56	NS
Manganese, mg	4.09 ± 0.15	3.44 ± 0.15	3.19 ± 0.37	NS
Iron, mg	28.94 ± 1.90	19.65 ± 4.15	13.34 ± 1.71	10.36
Copper, mg	1.45 ± 0.07	1.24 ± 0.04	1.32 ± 0.25	NS
Zinc, mg	7.40 ± 0.27	8.35 ± 0.36	6.55 ± 0.29	1.14
Selenium, μg	45.10 ± 2.92	51.48 ± 2.49	49.29 ± 2.33	NS
Chromium, mg	0.27 ± 0.01	0.16 ± 0.05	0.12 ± 0.03	0.13
Cobalt, μg	9.57 ± 0.69	5.53 ± 1.49	4.83 ± 1.06	4.15

Values are mean ± SD

NS non-significant

lower (0.34%) in comparison to the shade dried (0.59%) and freeze dried (0.54%) wheatgrass. A significantly ($p \leq 0.05$) lower crude fiber in wheatgrass was observed in freeze dried (21.29%) as compared to shade dried (25.61%) and oven dried (28.13%) wheatgrass powder. Chouhan and Mogra (2014) reported 16.6% of crude fibre in wheatgrass. The results of the proximate analysis indicated that the drying methods influenced the proximate composition of wheatgrass except total ash content. Better nutrient retention was found in shade and freeze dried wheatgrass. Hence, either shade drying or freeze drying can be opted as processing methods when proximate composition is under consideration.

The ascorbic acid content of oven dried wheatgrass was significantly ($p \leq 0.05$) lower in comparison to shade dried and freeze dried wheatgrass. The retention of ascorbic acid was found to be the highest in freeze dried samples. Puranik et al. (2012) found a reduction in ascorbic acid when medicinal herbs were subjected to different drying methods. The β -carotene content in wheatgrass was significantly ($p \leq 0.05$) lower in oven dried wheatgrass in comparison to shade dried and freeze dried wheatgrass. Desai (2005) reported the β carotene concentration of 10.91 IU/100 g for shade dried wheatgrass, which is lower than the values obtained even from shade dried wheatgrass.

The calcium content of shade dried wheatgrass was significantly ($p \leq 0.05$) higher in comparison to oven dried and freeze dried wheatgrass. Desai (2005) reported the calcium content of 32 mg/100 g for shade dry wheatgrass. The magnesium was maximum in shade dried followed by freeze and oven dried wheatgrass though no significant difference was observed among the three drying methods. The results of the present study were in line with those reported by Kulkarni et al. (2006). Further, no significant difference in manganese was observed in all three drying methods, the range of manganese content being 3.19–4.09 mg/100 g. Kulkarni et al. (2006) reported manganese content of 1.42–4.01 mg/100 g while Premkumari and Haripriya (2010) reported a higher value of 10.2 mg/100 g in wheatgrass powder. A significantly ($p \leq 0.05$) lower iron content was observed in freeze dried in comparison to shade dried and oven dried wheatgrass. The values obtained in the present study were within a range reported by Kulkarni et al. (2007) and Desai (2005). No significant difference was found in the copper content of shade, oven and freeze dried wheatgrass. The values observed in the present study were within the range reported by Premkumari and Haripriya (2010) and Girmes Wheatgrass Organization (2016) i.e. 0.2 mg/100 g and 49.67 μ g/100 g, respectively. A significantly ($p \leq 0.05$) lower zinc content in freeze dried wheatgrass in comparison to shade and oven dried wheatgrass was found. A higher value of zinc with concentration of 97.8 mg/100 g

was reported by Desai (2005). On the other hand, Premkumari and Haripriya (2010) reported 0.33 mg/100 g zinc in wheatgrass powder. A significantly ($p \leq 0.05$) higher selenium was found in regard to shade drying method in comparison to other methods. Selenium concentration of 52.3 μ g/100 g in wheatgrass was reported by Premkumari and Haripriya (2010), which is comparable with the values obtained in the present study. In another study, 63.89 μ g of selenium in 100 g of wheatgrass powder was reported by Girmes wheatgrass (2016). The statistical analysis showed that chromium and cobalt was significantly ($p \leq 0.05$) lower in freeze dried in comparison to shade and oven dried wheatgrass. Elemental concentration study of wheatgrass shoots by Kulkarni et al. (2006) reported the chromium and cobalt concentration of 0.7–10.5 mg and 3.92–4.93 mg/100 g respectively.

The bioactive compounds namely chlorophyll, tannins, phytic acid, flavonoids and saponins in wheatgrass dried by the three methods have been presented in Table 2. The chlorophyll in freeze dried wheatgrass was significantly ($p \leq 0.01$) higher (3.61 g) than shade dried (2.35 g) and oven dried (2.14 g) wheatgrass per 100 g. Desai (2005) reported 5.12% of chlorophyll for wheatgrass powder however, according to Grime's wheatgrass (2016), 6.15 g of chlorophyll is present in dehydrated wheatgrass. Wheat grass shoot powder showed higher chlorophyll as well as ash as compared to pulse shoot powders as observed by Ghumman et al. (2017). The growing conditions such as indoor and outdoor cultivation influenced the chlorophyll content of wheatgrass as indoor grown wheatgrass has lesser chlorophyll (48%) less as compared to outdoor grown wheatgrass (Wakehem 2013).

The tannin content in wheatgrass was significantly ($p \leq 0.05$) higher in oven dried wheatgrass (8.18 mg/100 g) as compared to shade dried (6.68 mg/100 g) and freeze dried (6.34 mg/100 g) wheatgrass. Contrary to tannins, a significantly ($p \leq 0.05$) lower phytic acid content was observed in oven dried wheatgrass (3.03 mg/100 g) in comparison to shade dried and freeze dried wheatgrass (3.76 mg/100 g). The phytic acid content of wheatgrass was found to be higher than the reported values of phytates for various leafy vegetables (Gopalan et al. 2009). Sakac et al. (2010) observed that phytic acid influenced the catalytic oxidation by chelating Fe^{2+} ions, and thus inhibits generation of lipid oxy radicals. By the mechanism, dietary phytic acid could lower the incidence of cancer or inhibit oxidation during processing, preservation and storage of foods (Shamsuddin 2002; Akbas et al. 2017) suggested that wheatgrass powder could be used as functional food ingredient due to its high phenolic content and antioxidant activity for different food applications.

No significant difference in saponins (1.04–1.20%) was found in wheatgrass dried by the three drying methods.

Table 2 Bioactive components and antioxidant activity of shade, oven and freeze dried wheatgrass (per 100 g)

Parameter	Method of drying			LSD at 5%
	Shade	Oven	Freeze	
Chlorophyll, g	2.35 ± 22.49	2.14 ± 95.72	3.61 ± 97.39	0.61
Tannins, mg	6.68 ± 0.36	8.18 ± 0.30	6.34 ± 0.10	0.94
Phytic acid, mg	3.76 ± 0.17	3.03 ± 0.17	3.76 ± 0.16	0.56
Saponins, %	1.04 ± 0.12	1.12 ± 0.21	1.20 ± 0.18	NS
Flavonoids, QE/g	333.78 ± 50.72	266.86 ± 53.80	363.53 ± 58.91	NS
Antioxidant activity, %	50.13 ± 1.89	48.94 ± 1.13	53.78 ± 1.79	NS

Values are mean ± SD

NS non-significant

Saponins could be used to prevent the damage produced by free radicals (Akinpelu et al. 2014). Saponins have antioxidant and free radical scavenging ability hence, could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases (Alli Smith and Adanlawo 2014).

The flavonoids were lower in oven dried wheatgrass (266.86 QE/g) in comparison to the wheatgrass dried by shade drying (333.78 QE/g) and freeze drying (363.53 QE/g). Durairaj et al. (2014) observed a significant presence of flavonoids in the aqueous extract of wheatgrass. Moreover, the findings of the present investigation are in agreement with the finding of Irondi et al. (2013) where it was reported that freeze drying preserved the highest amount of saponins, tannins and total flavonoids in *Carica papaya* seeds. Zhou et al. (2011) also reported that flavonoids and total phenolics were better conserved in loquat flower by freeze drying methods than other drying methods. The antioxidant activity of shade, oven and freeze dried wheatgrass was found to be 50.13, 48.94 and 53.78%, respectively. Though the wheatgrass powder exhibited a good antioxidant activity, no significant difference in antioxidant activity of wheatgrass was found between the samples dried by the three methods. It was evident from the results that freeze drying is the most appropriate drying method of wheatgrass that retained the highest amount of most of the bioactive compounds analysed in the present study. Wheatgrass has been proved to be an effective radical scavenger as reported by Durairaj et al. (2014). The phytochemical analysis by Murali et al. (2016) revealed the presence of valuable primary and secondary metabolites such as tannins, steroids, terpenoids, alkaloids, flavonoids, cardiac glycosides, saponins, coumarins etc. in both the fresh and dried wheatgrass. This indicates that wheatgrass is a good source of bioactive compounds and hence suitable as therapeutic agent for various ailments. Antioxidant properties of shoot powder from wheatgrass were much higher as compared to pulse powder as reported by

Ghumman et al. (2017) thus highlighting the nutritional benefits of wheatgrass.

Conclusion

Drying methods influenced the proximate composition of wheatgrass except total ash content. Better nutrient retention was found in shade and freeze dried methods. Hence, either shade drying or freeze drying can be opted as processing methods when proximate composition is under consideration. The mineral content was found to be the highest in shade dried wheatgrass followed by oven dried wheatgrass and least in freeze dried wheatgrass except magnesium. Freeze drying was the best drying method of wheatgrass that preserved the maximum bioactive compounds and antioxidant activity.

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