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Research Article

Nutritional and Bioactive Potential of Wheat Grass Juice Powder

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Abstract

Wheat grass cultivated at laboratory scale was processed into juice followed by freeze drying to obtain wheat grass juice powder. Wheat grass juice and its powder were investigated for proximate composition, total phenolic and flavonoid contents, chlorophyll content, vitamin C, and radical scavenging activity. The proximate composition of wheat grass juice powder revealed high levels of protein (26.02 ± 0.89 %) and ash content (20.62 ± 1.13 %). Wheat grass juice and its powder were found to possess excellent bioactive potential. Also, the freeze drying of juice to powder significantly ($p < 0.05$) enhanced both total phenolic and flavonoid contents, and corresponding radical scavenging activity. Besides, juice and powder contained ample amount of chlorophyll content and vitamin C and freeze drying exhibited negligible effect on such constituents. Overall, freeze drying retained or enhanced the quality attributes and was depicted as an effective technique in the development of quality products. The rich nutritional composition and high bioactive compounds content show the potential of wheat grass juice and its powder to develop various functional foods. The consumption of these functional foods can prevent the people from oxidative stress and related chronic disorders like atherosclerosis, cardiovascular disease and diabetes.

Keywords: wheat grass, freeze drying, bioactive potential, chlorophyll, vitamin C

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Introduction

Since ages, the humans are dependent on the plants and herbs to fulfill their dietary requirements as well as for their medicinal properties. Over the time, many plants and herbs have been cultivated at larger scale and processed into commercial products. In recent times, wheat grass has gained a lot of attention owing to its rich nutritional composition. Basically, the 7-10 days old sprouted wheat seeds are called as wheat grass [1]. Investigations have proved that, the plants tend to possess significant levels of vitamins, enzymes, amino acids and phytochemicals at this young stage [2].

Wheat grass is an ancient plant and has been used by the people since 5000 years ago in ancient Egypt. Also, it had been consumed by people of Mesopotamian civilizations [3]. Western world has started consumption of wheat grass juice since 1930's because of the experiment conducted by Charles Schnabel (agricultural chemist) who utilized wheat grass to cure the hens [4]. Wheat grass has gradually gained popularity as "a new age espresso" incorporated in salads, juices, smoothies and even in powders and tablets.

Wheat grass is said to be nature's finest medicine due to its rich bioactive composition as well as related antioxidant properties [5]. In such context, chlorophyll, which is also known as green blood, exists in abundance in the wheat grass. It provides wheat grass the ability to remove toxins from the body and act as a body detoxifier [6]. Moreover, the phenolics and flavonoids are present in considerable amounts which interact with reactive oxygen species i.e. free radicals, in order to stabilize them. Such free radicals can interact with proteins, amino acids and genetic material resulting in the harmful oxidative stress leading to various disorders like cardiovascular diseases, diabetes, cancer and atherosclerosis. Thus, the consumption of what grass and its processed products can prevent the body from such diseases. Wheat grass also contains good amount of minerals i.e. iron, magnesium, phosphorus, selenium and zinc, and vitamins [4].

Traditionally, wheat grass has played significant role as a herbal medicine for treating ailments, such as thalassemia and myelodysplastic syndrome [4]. Various studies have recommended wheat grass therapy for the patients of certain chronic disorders like diabetes, asthma, obesity, atherosclerosis, eczema, Parkinson's disease, insomnia, joint pains, constipation, bronchitis, flatulence, hypertension and hemorrhage. It is also effective for treating migraine, ear diseases, reproductive organs and gangrene [7].

Wheat grass is available in various forms like juice, powder, extract and capsules. It is also used as food supplements in the form of tablets, frozen turf water and herbal food capsules [8, 9]. Like wheat grass, its juice also has high nutritional value as well as therapeutic value. Wheat grass juice (WGJ) possesses high antioxidant potential which helps in neutralizing the free radicals and provide protection to the DNA and cell structure [10, 11]. It has been proved that WGJ enhances the speed of red blood cells formation after ingestion and lowers down the transfusion requirement in thalassemia patient. In addition, it prevents tooth decay and eliminates toothaches [12]. However, the major concern about WGJ is its shelf life, as the high moisture content of the juice causes it to deteriorate within 1-2 days. Thus, efforts are being made to produce some shelf stable forms of WGJ with the utilization of non-thermal techniques

Keeping in view the various aspects, a study had been planned in which wheat grass was cultivated at laboratory scale. After harvesting, the juice was extracted from wheat grass and freeze dried to develop wheat grass juice powder (WGJP). WGJ and its powder were analyzed for nutritional value and antioxidant potential, and the effect of freeze drying on these attributes was also observed.

Material and Methods

Raw materials

Wheat grains (HD 2967) were procured from grain market of Khanna, Punjab, India. PVC trays (1.5×2 feet) used for the cultivation of wheat grass were procured from local market.

Wheat grass cultivation

Grains were sorted and cleaned before soaking in normal water for 8-10 h. After soaking, they were rinsed with water 2-3 times and kept in damped cloth for 2 days to initiate the sprouting. After 2 days, sprouted grains were sowed in the soil bed prepared on PVC trays (**Figure 1**). Trays were kept in laboratory at 25-28 ^{0}C and water was sprayed at regular intervals to keep the soil damp. Wheat grass was harvested on the 8th day from sowing at the height of 7-8 inches as shown in **Figure 2**. After harvesting, grass was washed with tap water and kept under refrigerated conditions before final use.

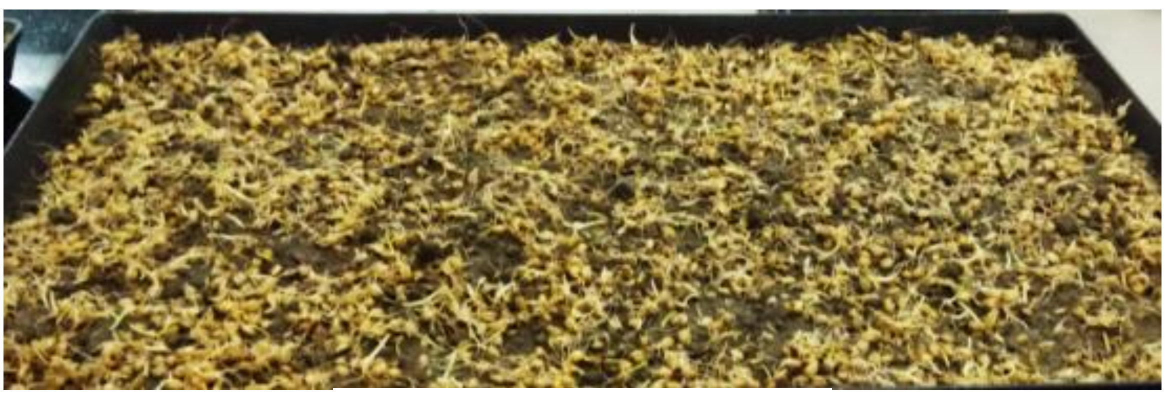

Figure 1 Sowing of sprouted wheat grains

Figure 2 Wheat grass at harvesting stage

Extraction of wheat grass juice

Manual fruit juice extractor was used for the extraction of juice at room temperature. Whatman No. 4 filter paper was utilized to filter the extracted juice. The filtrate was kept in amber glass container at 4 ^{0}C till used for chemical analysis and freeze drying.

Freeze drying of wheat grass juice

The technique of freeze dying was utilized to convert WGJ to powder. 150 ml of extracted juice was transferred into each round bottom flask and kept in deep freezer (-38 ^{0}C) for 6 hours. Thereafter, Mini-Lyodel freeze dryer was used at atmospheric temperature under vacuum to sublimize the frozen water for preparing WGJP.

Proximate analysis

Standard procedures were performed to measure the protein, fat, ash and fibre content of WGJ and WGJP [13]. The difference method was utilized to calculate total carbohydrates using the equation: 100 - (protein + fat + water + ash) g in 100 g.

Chlorophyll content

The method reported by Arnon [14] was utilized to measure the chlorophyll content. 1 g of WGJP was extracted with 80 % acetone and the contents were centrifuged at 3000 rpm for 10 min. Chlorophyll a and b, and total chlorophyll content of WGJ and WGJP were calculated using the following equations:

$$\text{Chlorophyll a} = 12.7\ (A_{663}) - 2.69\ (A_{645})$$
$$\text{Chlorophyll b} = 22.9\ (A_{645}) - 4.68\ (A_{663})$$
$$\text{Total Chlorophyll} = 20.2\ (A_{645}) + 8.02\ (A_{663})$$

Here, A_{645} and A_{663} are the absorbances of samples at 645 and 663 nm, respectively. Results were expressed as mg/g of sample on dry matter basis (dmb).

Vitamin C content

Direct calorimetric method was utilized to measure the vitamin C content of samples [15]. In this method, 1 g of WGJP was extracted using 2 % metaphosphoric acid. 1 ml of this extract (and 1 ml of WGJ) was taken in test tubes to which 9 ml of dye solution (2, 6 dichlorophenolindophenol dye and sodium bicarbonate) was added. The contents were incubated in dark for 10 min and absorbance was recorded at 515 nm. L-ascorbic acid was used as standard to express the results as mg Ascorbic acid equivalents (AAE)/g dmb.

Total phenolic content (TPC)

WGJP was refluxed with aqueous methanol (80 % methanol acidified with 0.1 % HCl) for the extraction of the bioactive compounds and the contents were centrifuged at 3000 rpm for 10 min. The final volume was made up to 50 ml with aqueous methanol at sample concentration of 20 mg/ml. Juice sample was analyzed without any refluxing and extraction.

The method given by Singleton *et al* [16] was used to determine the TPC. Specific volume of powder extract/ juice was diluted to 1 ml with aqueous methanol. Thereafter, freshly prepared Folin-Ciocalteu reagent (5 ml) and saturated sodium bicarbonate solution (4 ml) were added into reaction mixture followed by the incubation period of 2 h. Absorbance was read at 765 nm against reagent blank. The standard used was Gallic acid to express the results as mg Gallic acid equivalents (GAE)/g dmb.

Flavonoid content (FC)

The method explained by Dini *et al* [17] was used with slight modifications to analyze the FC of samples. Specific volume of previously prepared powder extract/juice was mixed with distilled water (2 ml) and then 5 % $NaNO_2$ solution (0.15 ml) was added. After 5 min, 10 % $AlCl_3$ solution (0.15 ml) was added followed by the incubation period of 6 min. Thereafter, 1M NaOH (1 ml) and 1.2 ml of distilled water were thoroughly mixed with reaction mixture. Absorbance was read at 510 nm against reagent blank. Catechin was used as standard to express the results as mg Catechin equivalents (CE)/g dmb.

DPPH radical scavenging activity

The standard procedure was utilized to measure the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity [18]. In this method, 0.1 ml of previously prepared powder extract/juice sample was mixed with 3.9 ml of DPPH followed by incubation period of 30 min under dark. Absorbance was recorded at 517 nm (A2). Solution containing aqueous methanol and DPPH was used as blank (A1). Calibration curve prepared using DPPH (5-30 ppm) was utilized to calculate the concentrations C1 and C2 from absorbance values A1 and A2. Antioxidant activity was evaluating using following equation:

$$\text{Antioxidant activity (mg DPPH/g dmb)} = \frac{(C1 - C2) \times v \times d}{w}$$

here, w is the sample weight dmb; v is the volume of sample solution and d is the dilution rate.

Statistical analysis

Experiments were performed in triplicates. The observed values were expressed as mean ± standard deviation (SD). Microsoft Excel was utilized to perform all the necessary calculations.

Results and Discussion

Proximate analysis

Proximate analysis depicts the basic nutritional composition of any product and it is responsible for the first impression of that product to the consumers. **Table 1** demonstrates the proximate composition of WGJ and WGJP. WGJP was found to contain excellent protein content which shows its potential for the development of various protein rich compositions. Also, the high value of ash content in WGJP shows the presence of important minerals required for normal functioning of the human body. In addition, the low fat content in the WGJ and WGJP make these products very suitable for the people suffering from health conditions like cardiovascular diseases and atherosclerosis.

Table 1 Proximate composition of wheat grass juice and wheat grass juice powder

Constituent	WGJ	WGJP
Moisture (%)	93.4 ± 1.07	7.9 ± 0.76
Protein (%)	2.96 ± 0.32	26.02 ± 0.89
Fat (%)	0.3 ± 0.11	2.49 ± 0.65
Ash (%)	0.89 ± 0.19	20.62 ± 1.13
Carbohydrates (%)	2.45 ± 0.55	42.97 ± 1.46
Crude fibre (%)	< 0.1	1.9 ± 0.37

Values are expressed as mean ± SD (n = 3).
WGJ, wheat grass juice; WGJP, wheat grass juice powder

Chlorophyll content

Chlorophyll, also referred to as green blood, is similar to the structure of hemoglobin. The only difference is the central atom, as the hemoglobin contains iron while magnesium is present in the chlorophyll. The similarity in the structures is responsible for the utilization of the chlorophyll in the treatment of many serious health conditions like anemia, thalassemia and other hemolytic disorders. The results obtained for WGJ and WGJP have been demonstrated in the **Table 2**. The processing of WGJ to WGJP had insignificant effect on the chlorophyll a, chlorophyll b and total chlorophyll content. This might be because processing had been carried out under lower temperature and vacuum conditions which maintained the integrity of the chlorophyll structure. Ali *et al* [19] compared the effect of thermal and non-thermal techniques on the various quality attributes of wheat grass juice and it was found that, the thermal processing has destructive effect on the total chlorophyll content. It was explained that, during the thermal processing, the Mg^{2+} present at the center gets displaced resulting in the conversion of chlorophyll to pheophytin which imparts lighter-reddish colour to the final product [19].

The results also depicted the WGJ and WGJP as excellent source of chlorophyll. Chomchan *et al* [2] and Thakur *et al* [20] have observed the similar results for WGJ and WGJP and associated the high chlorophyll content with the medicinal properties possessed by these products. It has also been reported that chlorophyll can interact with harmful free radicals to stabilize them for strengthening the immune system. Also, the superoxide dismutase present in chlorophyll helps in reducing the aging process by decomposing superoxide radicals from the body.

Table 2 Bioactive potential of wheat grass juice and wheat grass juice powder

Constituent	WGJ	WGJP
Chlorophyll a (mg/g dmb)	2.675 ± 0.16^{a}	3.095 ± 0.56^{a}
Chlorophyll b (mg/g dmb)	3.089 ± 0.34^{a}	3.132 ± 0.42^{a}
Total chlorophyll (mg/g dmb)	5.762 ± 0.29^{a}	6.225 ± 0.48^{a}
Vitamin C (mg AAE/g dmb)	15.937 ± 0.82^{a}	14.722 ± 0.73^{a}
Total phenolic content (mg GAE/g dmb)	7.073 ± 0.36^{b}	8.935 ± 0.53^{a}
Flavonoid content (mg CE/g dmb)	9.219 ± 0.79^{b}	10.816 ± 0.19^{a}

Values are expressed as mean ± SD (n = 3).
Means with different superscripts (a, b) indicate significant difference ($p < 0.05$) within a row.
WGJ, wheat grass juice; WGJP, wheat grass juice powder

Vitamin C content

Vitamin C is an important nutrient in the human diet as it is involved in the various body functions, for instance, repairing of tissues, production of certain neurotransmitters, etc. It is also considered as natural antioxidant which helps in preventing the human body from harmful reactive species. The results obtained for vitamin C content have been demonstrated in the Table 2. The results depicted the ability of freeze drying to retain the vitamin C content which is attributed to the lower processing temperature. Shofian *et al* [21] and Lin *et al* [22] observed similar results for mango, papaya, muskmelon, watermelon and carrot slices. Further, Lin *et al* [22] compared the different drying methods for the processing of carrots and the results revealed that, during air drying, significant loss of vitamin C took place which might be due to oxidation of ascorbic acid while no loss was observed during freeze drying of carrot slices.

The WGJ and WGJP prepared in present study were found to be excellent sources of vitamin C. The consumption of 1 g of WGJP can fulfil 32-33 % Recommended Daily Allowance (RDA) of vitamin C for school going children (9-13 years). Thus, the WGJP can be recommended as potential functional food for the people suffering from vitamin C deficiency and oxidative stress.

Total phenolic content (TPC)

In food products, phenolics are the primary compounds responsible for exhibiting antioxidant properties. Basically, the hydroxyl groups are present in the structure of phenolic compounds which can donate the hydrogen atoms to stabilize reactive oxygen species. Table 2 shows the results obtained for WGJ and WGJP and it had been observed that freeze drying process significantly ($p < 0.05$) improved the to TPC in the WGJP. The results obtained in present study agree with the findings of Das *et al* [23], Orphanides *et al* [24] and Valadez-Carmona *et al* [25]. Such studies explained that, the increment in TPC after freeze drying might due to the breakdown of cellular constituents which lead to the liberation of bound phenolics to free form. It is important to mention that these free phenolic compounds have better antioxidant properties as compared to bound phenolics. Another perspective is that, the active enzymes present in the juice sample tend to utilize the phenolic compounds which may lead to lower TPC, while in powder sample, the lower water activity causes the inactivation of these enzymes due to which the overall TPC remains unchanged [26].

Flavonoid content (FC)

Like phenolic compounds, flavonoids do contribute to the antioxidant properties of the food matrix. They have the ability to inhibit the toxic radical reactions which prevent the body from oxidative stress. The results obtained for FC content have been demonstrated in Table 2. It had been observed that, the freeze drying significantly ($p < 0.05$) improved the flavonoid content in WGJP. Das *et al* [23] and Pérez-Gregorio *et al* [27] have reported the similar results for wheat grass and onion. According to these studies, the significant increment in the flavonoid content after freeze drying might be due to the alteration in the tissue structure which eases the liberation of flavonoid compounds. Contrary results have also been reported in some investigations which might be due to disparity in the molecular composition of different food matrices. Also, the solubility of polyphenols varied according to the different types of solvents which might also contribute to variation in the results.

DPPH radical scavenging activity

DPPH is a stable free radical compound which gets reduced in the presence of antioxidants. The result obtained for radical scavenging activity has been demonstrated in **Figure 3**. WGJP exhibited better antioxidant capacity as compared to WGJ. The increment in antioxidant capacity might be due to enhanced TPC and FC. As explained, the structure of phenolic compounds contains hydroxyl groups which can donate the hydrogen atoms to reduce free radicals and convert them into stabilize forms. Das *et al* [23] and Ibrahim *et al* [28] have reported similar results for wheat grass and *Streblus asper* leaves and explained that, the higher levels of antioxidant activity in freeze dried samples were strongly correlated with the concentration of phenolics. The excellent antioxidant activities of WGJ and WGJP makes them potential choice as functional foods.

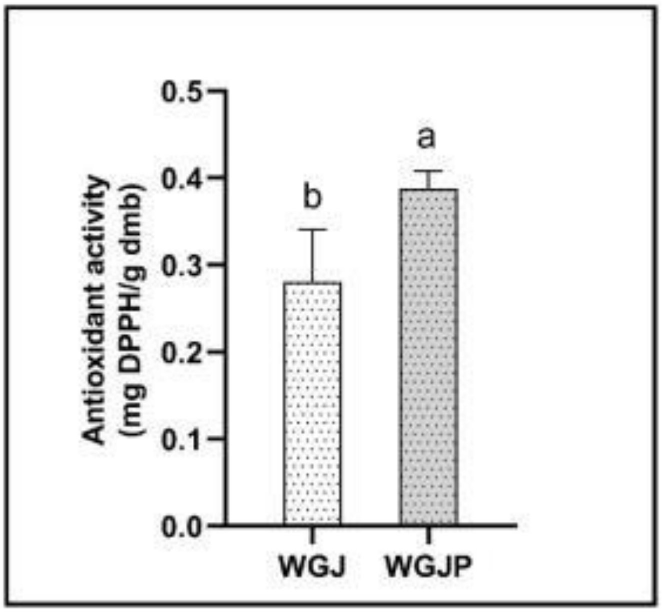

Figure 3 DPPH radical scavenging activity. Error bars represent the standard errors of the means of three separate replicates. Different letters (a, b) on bars indicate significant difference ($p < 0.05$). WGJ, wheat grass juice; WGJP, wheat grass juice powder

Conclusion

Wheat grass juice is gaining great attention from consumers as well as food processors owing to its rich nutritional composition as well as bioactive potential. The major concern is its high moisture content which limits its shelf life. In the present study, wheat grass was cultivated at laboratory scale and extracted into juice which was further processed to powder using freeze dryer. WGJ and WGJP were analyzed for nutritional composition and bioactive potential. After freeze drying, the significant improvement was recorded in the TPC, FC and radical inhibition activity which was attributed to the ability of freeze drying to alter the tissue structure which results in the liberation of phenolic compounds. In addition, WGJ and WGJP were found to contain ample amount of vitamin C and chlorophyll content, and the freeze drying process successfully retain these constituents. Thus, the results depicted that, the freeze drying is an effective technique in the development of high-quality products. Also, the rich nutritional composition as well as high bioactive potential make the WGJ and WGJP as potential functional foods.

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