

# **Biochemical, nutritional and end use perspectives of Wheat grass as potential dietary supplement**

## **ABSTRACT**

Wheat grass being substantial cereal grass crop in the world; copious source of nutrients with noteworthy nutritional and therapeutic value. The research, wheat grass was grown in indoor trays and then used as powder and in juice. The objective were to use the wheat grass to formulate a suitable processing procedure for WGP enriched food products. Both raw WGP and cookies were analyzed for TDF, SDF, IDF, TPC and antioxidant activity. Juice was analyzed for pH, viscosity and TSS. WGP addition showed a significant effect on farinographic (water absorption, arrival time, DDT and MTI) and mixographic studies (peak height and peak time). The cookies prepared using different levels were generally accepted by sensory panelists but 3% substitution level of WGP have high acceptability. Moreover, on the basis of physicochemical and sensory attributes, 10% supplementation level of wheat grass in juice was best making it a nutrient enriched food source.

**Keywords:** Wheat grass, Biochemical evaluation, Dietary fibers, Rheology, Wheat grass cookies, Wheat grass drink

### **Practical Application:**

Wheat grass being the neglected and undermined, have great health potential and can be utilized in enriched food items. Wheat grass supplemented food products including confectionery and beverages having powder or extract can be introduced in market in order to divert the masses towards natural products.

WGP= Wheat grass powder

DDT= Dough development time

MTI= Mixing tolerance index

TDF= Total dietary fiber

SDF= Soluble dietary fiber

IDF= Insoluble dietary fiber

TPC= Total phenolic content

## **1. Introduction**

Health as a fundamental human right is also a worldwide social goal. It includes all humans, disregard of age. Poor food habits and sedentary self-indulgent lifestyle are the causes of perpetuation of diseases [1]. Now in recent times, trend towards functional and nutraceutical foods have been increasing. A functional food may be defined as, “any food that has a positive impact on an individual’s health, state of mind or physical performance in addition to its nutritive value” [2]. Nature can be regarded as an important storehouse of medicines. There are a large number of plants that are known for having therapeutic potential.

42 The integral source of phytochemicals and phyto-pharmaceuticals are plants. In various parts  
43 of the world plants are used as folk remedies [3].

44 Cereals and its ingredients are known for having functional and nutraceutical  
45 potential. As they provide energy, proteins, dietary fiber, minerals, vitamins and antioxidants  
46 that are needed for human health. Wheat grass is one of the cereal grasses that have numerous  
47 benefits. Grasses can be regarded as the introductory food for most of the land based life.  
48 After a long research conducted by various scientists in the 1930s, wheat grass was said to be  
49 the finest grass food of all [4]. Grasses have many health protecting and regenerating  
50 properties and wheat grass is one of the cereal grasses that have many nutritional benefits [5].  
51 It is one of the foods that is included in the category of green foods that are regarded as super  
52 natural nutrient source by health-conscious people [4].

53 Nutritional profile of wheat grass comprised on a vast list like vitamin A, B complex,  
54 C, D, E, F and K; choline and folic acid. Minerals like iron (Fe), calcium (Ca), potassium (K),  
55 sodium (Na), manganese (Mn), sulphur (S), phosphorus (P), cobalt (Co), magnesium (Mg),  
56 zinc (Zn) and amino acids like alanine, arginine, glycine, isoleucine, leucine, glycine, cysteine,  
57 tryptophan, threonine, glutamic acid, lysine, histidine, phenylalanine, methionine, aspartic  
58 acid, valine, proline and tyrosine are present in it. Superoxide dismutase(SOD), DNase,  
59 catalase, nitrogen oxyreductase, polyphenoloxidase, lipase, phospholipase, cytochrome  
60 oxidase, malic dehydrogenase, fatty acids transhydrogenase, phosphatase, RNase,  
61 peroxidase, protease, hexokinase and nitrate reductase are the enzymes that make it worthy  
62 [6].

63 Wheat grass is the power house of many nutrients like minerals and vitamins that are  
64 responsible for maintaining the human health. It can also be considered as a complete and  
65 whole protein which contains almost thirty enzymes and about seventy percent chlorophyll in  
66 crude form. One ounce (280ml) of wheat grass juice equals in nutrition to about 2.5 pounds  
67 (1.1 kg) of green leafy vegetables as it contains same quantity of amino acids, vitamins and  
68 minerals similar to that are present in 2.5 pounds of vegetables. Wheat grass juice, besides  
69 containing chlorophyll, has many therapeutic purposes [7].

70 Wheat grass may be converted into fresh juice or dried to make powder for the use of  
71 humans and animals; both the forms offer almost all the nutrients present in it i.e. minerals,  
72 enzymes, chlorophyll, vitamins and amino acids. Wheat grass juice can be defined as an  
73 extract squeezed from the mature sprouts of wheat seeds [8]. It has many potential benefits  
74 for people as it restores body balance, builds the blood, cleanses the lymph system, removes  
75 toxins of the body cells, provide nourishment to the kidney and liver and restores energy. It  
76 can be used as an effective and safe treatment for ailments such as obesity, high blood  
77 pressure, diabetes, asthma, ulcers, some cancers, gastritis, anemia and eczema [9].

78 Wheat grass juice has recently become the interest of many people as a vitality drink  
79 [10]. It can be taken orally having no side effects and regarded as a complete food. Wheat  
80 grass in the powder form is more palatable than fresh wheat grass juice due to many reasons  
81 by all age groups and its therapeutic efficacy is equal to that of fresh wheat grass. One  
82 teaspoon of wheat grass powder that is about 3g is equal in nutritional value to about 40g of  
83 fresh wheat grass [4].

84 Among foodstuff, biscuits are much liked and consumed by a wide range of  
85 population due to its longer shelf life, variety of taste and low cost. Many attempts are being  
86 made by adding nutritive ingredients to improve nutritional and functional value of biscuits,  
87 to be competitive in the market and to fulfill demand for healthy and functional products [11].  
88 With this background present work was undertaken to study nutritional, functional and end  
89 product quality of wheat grass both in juice and powder form.

## 90 **2. Materials and methods**

### 91 **2.1. Wheat grass**

92 Wheat grass was grown by planting wheat seeds according to the method of [10] with  
93 slight modifications. Wheat seeds were first soaked in water overnight. After one night  
94 soaking, steeped seeds were kept under moistened cloth for 24 hours to promote germination  
95 onset (cloth was sprayed with water to keep it moistened with 8 hour interval). Earthen pots  
96 were filled with 2.5 inches of soil. Soaked seeds were then spread evenly over the soil and  
97 seeds were again covered with a 0.5 inch layer of soil. For proper growth of wheat grass  
98 water in small quantity was evenly sprinkled over soil with 3-4 hours of indirect sunlight. On  
99 tenth day after germination of seeds, grass was about of 6 inches tall, it was cut from 0.5  
100 inches above soil surface.

### 101 **2.2. Preparation of wheat grass juice (WGJ) and powder (WGP)**

102 The juice of wheat grass leaves was prepared as described by Shah, Sheth [9]. For  
103 preparation of wheatgrass juice, 100 gm. fresh wheat grass was crushed with little water and  
104 finally filtering it through four layers of muslin cloth to get the 100 mL filtrate.

105 The wheat grass leaves were washed with water and dried in dehydrator as described  
106 by Sharma, Velu [12] with slight modifications. The leaves are dried in dehydrator at 30-  
107 35°C for 24 hours. The dried leaves were coarsely powdered in a blender and powder was  
108 stored in polythene bags.

### 109 **2.3. Analysis of wheat grass powder**

#### 110 **2.3.1. Chemical characteristics**

111 Wheat grass powder (WGP) was evaluated for chemical composition i.e. moisture, ash, crude  
112 fat, crude fiber and crude protein and dietary fiber [13].

#### 113 **2.3.2. Phytochemical analysis of WGP**

114 Wheat grass powder was analyzed for the presence of reducing sugars, tannins and saponins  
115 as described by Tandon, Arora [14].

### 116 **2.4. Dough Characteristics**

117 Wheat flour blends with WGP were prepared by substituting it at 3, 5 and 7% level.  
118 Farinograph (E-380 Model, Brabender OHG, Duisburg, Germany) was used to determine the  
119 influence of WGP on dough rheology according to the AACC [13] standard methods.  
120 Characteristics of dough measured by farinograph were water absorption, arrival time,

departure time, dough development time, and mixing tolerance index and dough stability. Mixograph (National Mfg. Co, Lincoln, Nebraska, USA) was also used to study the extent of effect caused by WGP on dough rheology, according to AACC [13] standard methods.

## 2.5. Preparation of cookies

The formula used for preparation of cookies was as follows: 500g composite flour, 250g sugar, 300g shortening, 3.0g cardamom powder, 7.0g leavening agent and 2 eggs.

First of all creaming was done in 10 minutes by adding shortening and sugar. Then eggs were added and mixing was done for few minutes. Then composite flour and leavening agent was added. Cardamom powder was added after thoroughly mixing of this mixture for 5-6 minutes. Then molding was done and weight, height and diameter were measured. At the end baking was done at 180 °C for 10-12 minutes.

## 2.6. Analysis of cookies

### 2.6.1. Chemical characteristics of cookies

Cookies were evaluated for chemical composition i.e. moisture (method No. 44-15A), ash (method No. 08-01), crude fat (method No. 30-10), crude fiber (method No. 32-10), crude protein (method No. 46-10), soluble dietary fiber (Method No.32-07), insoluble dietary fiber (Method No.32-20) and total dietary fiber (method No.32-05) [13].

### 2.6.2. Preparation of extract

10 % (w/v) extract of wheat grass powder and cookies was prepared with 70% ethanol. The homogenate was centrifuged at 13500g for 30 minutes at temperature of 4°C and the resulting supernatants were analyzed for future experiment.

### 2.6.3. Measurement of total phenolic content

Total phenolic content was quantified by Folin-Ciocalteu reagent method as described by Kulkarni, Tilak [15] with slight modifications. In 50 µl of sample/standard, 3.5 ml of water was added followed by FC (Folin-Ciocalteu) reagent of 250 µl. Solution was mixed and incubated at room temperature for 8 minutes. 750 µl of 20% sodium carbonate was dissolved in mixture and incubated at room temperature. After incubation, UV-Vis spectrophotometer (IRMECO Germany) was used to take absorbance at 765 nm. Gallic acid's solution was prepared by dissolving its 25 mg in distilled water of 25 ml and its reading was taken as a standard at same 765 nm absorbance. Gallic acid concentration ranged from 0 to 450 µg/ mL. Standard curve of acid was used in total phenolic content's calculation of samples. Same procedure was followed for all other samples and preceded three replications.

TPC was calculated by the following formula

$$C=c \times V / m$$

Where:

C= total contents of phenolic compounds in mg GAE/g

c= concentration of Gallic acid mg/ml

V= the volume of extract

m= weight of extract in g

The total phenolic content (TPC) was expressed as mg Gallic Acid Equivalents/gram of dry extract.

#### **2.6.4. Radical scavenging activity by using DPPH method**

The antioxidant activity of plant methanol extract was determined based on the radical scavenging ability in reacting with a stable DPPH free radical by following the method of Brand-[14]. 1g of sample were extracted with 10 ml of 80% methanol (for 2 hours). Briefly, DPPH of 4 mg was dissolved in 100 ml methanol to prepare a solution, 50 µl methanol extract was added in 2 ml of this solution. Mixture was vigorously shaken and allowed to rest in the dark at room temperature. After a while reading was taken at 515 nm absorbance. The experiment was carried out in triplicate. Radical scavenging activity was calculated as.

Reduction of absorbance (%) =  $[(AB - AA) / AB] \times 100$

Where:

AB = absorbance of blank sample (t = 0 min)

AA = absorbance of tested extract solution (t = 15 min)

### **2.7. Evaluation of cookies**

#### **2.7.1. Physical characteristics**

Cookies width was measured by horizontally placing six cookies (edge to edge) and then rotating at 90° angles for replication.

Cookies thickness was measured by placing six cookies vertically on one another and replicate readings were taken.

Spread factor was calculated according to method no. “10-53” described in [13]. The spread factor was calculated according to the following formula

$$SF = \frac{W}{T} \times CF \times 10$$

Where CF = Correction factor at constant atmospheric pressure (1.0 in this case).

Texture analysis was done according to [16] by using texture analyzer (Mod. TA-XT2, stable micro system, Surrey, UK) interfaced with a computer, which controls the instrument and records the data. For the data treatment, the Texture Expert program version 1.21 was used. For every formulation, three repeated measurements were taken and mean values were reported

#### **2.7.2. Sensory evaluation of cookies**

Acceptability of product was determined by evaluating sensory characteristics of cookies incorporated with WGP. Cookies were evaluated for color, crispiness, taste, flavor, texture and overall acceptability on a 9-point hedonic scale [17].

### **2.8. Raw wheat grass juice analysis**

pH of the juice will be determined by digital pH meter as described by Houška, Strohaln [18]. Brix (%) was generally used as indicator for soluble solid content %. The total soluble solids of the juice samples were determined by using digital refractometer as described by Waghray, Gulla [19]. Juice viscosity was determined by using Brookfield DV-I

viscometer (LVDVE) as described by Kareem and Adebawale [20]. Apparent viscosity was measured at 25 °C temperature; sample was stirred for 40s before viscosity measurement. Spindle number 2 was used for this measurement rotated at 30 rpm. Viscometer reading was noted in centipoises (cp) units.

## **2.9. Preparation of juice**

The formula used for the preparation of juice was as follows: 500mL apple extract, 500mL water, 100g sugar, 1.5g malic acid, 1.0g CMC, 2-3 drops caramel color, 1mL apple flavor and 0.5g sodium benzoate. Wheat grass juice was replaced with water in the percentage of 5, 10 and 15.

Apples at commercial maturity were purchased from a local market and stored at 4°C until processed. Apples were water washed, each apple was cut in four pieces and seeds were removed carefully. Moulinex, Commercial Juice extractor was used to extract the juice.

Apple extract, water and wheat grass juice were mixed and heated to about 65°C. CMC was mixed with sugar and added in juice while continuously stirring the juice to avoid the clump formation and malic acid was also added in it. After that juice was cooled to room temperature and color, flavor and sodium benzoate added in juice. Juice was filled in bottles and stored at 4°C.

## **2.10. Analytical characterization of juice**

Juice was checked for pH, total soluble solids and viscosity as described in section 2.8.

## **2.11. Sensory evaluation of juice**

Juice was evaluated for appearance, taste, aroma, mouth feel and overall acceptability on a 9-point hedonic scale [17].

# **3. Results and Discussion**

## **3.1. Chemical composition of wheat grass powder**

Chemical composition of wheat grass powder was illustrated in Table 1 & Fig. 1. Wheat grass powder contains 4.06% moisture, 5.4% ash, 17.7% crude fiber, 0.29% crude fat, 21.6% protein and 50.95% nitrogen free extract. The results related to the proximate composition of wheat grass powder are similar to the previous findings [6]; [21].

The wheat grass found to contain 4.12% soluble dietary fiber, 23.07% insoluble dietary fiber and 27.19% total dietary fiber shown in Fig. 2. The results related to dietary fiber in wheat grass are related to the studies conducted by Chaturvedi, Sharma [21] and Svihus, Newman [22]. Total phenolic content in wheat grass powder was found to be 6.13 mg GAE/g. The results are in accordance with the previous findings of Tandon, Arora [14] who observed TPC in various extracts of wheat grass ranging from 2.44 to 6.48. The antioxidant activity (% Inhibition) of wheat grass powder was observed 51% as shown in Fig. 3. Present results are supported by previous findings [23].

### 3.2. Phytochemical Analysis of Wheat grass Powder

The results for phytochemical analysis are presented in Table 2. The present results are in accordance with the earlier findings of Tandon, Arora [14] and Kothari, Jain [24].

### 3.3. Influence of wheat grass powder on dough characteristics

Incorporation of WGP at 3, 5 and 7% levels showed significant difference in dough properties as shown in Table 3 and 4. Addition of WGP at different levels increased the water absorption from 62.5 (0%) to 67% (7%), arrival time from 1.4 to 2.1 min, DDT from 3.5 to 4.5 min and MTI from 35 to 84 BU while decreased departure time from 13.0 to 8.5 min and dough stability from 11.5 to 6.4 min. Increase in water absorption may be attributed to hydroxyl groups present in the fiber structure which interact with water through hydrogen bonds [25]. Dachana, Rajiv [26] also reported the same results that water absorption increases by increasing the amount of dried wheat grass powder in wheat flour.

The results are also in accordance with the earlier findings [27]; [28]. Sharma, Velu [12] observed the decrease in dough stability by the addition of dried *Tinospora cordifolia* leaves powder in wheat flour and reported that this decrease in dough strength is due to interaction between fiber and gluten. The findings of Ajila, Leelavathi [29] are also in accordance with the present results. Decrease in stability indicates the decrease in dough resistance, may have been caused by the fact that fiber diluted the gluten proteins of the flour [30]. Increasing of DDT may be due to the interaction of fiber with the gluten which is responsible for the prevention of protein hydration [25]. There is an inverse relationship between dough strength and MTI. As MTI was increased as the level of addition of dried wheat grass was increased that is the indication of decrease in dough strength [26]. Ashoush and Gadallah [31] also reported the increase in MTI due to dilution of gluten. Addition of WGP at different levels decreased the peak time and peak height as presented in Table 4. The present results are supported by earlier findings [32].

### 3.4. Influence of wheat grass powder on nutritional characteristics of cookies

The moisture and ash contents in cookies ranged from 3.85 to 5.28% and 0.77 to 1.2% respectively (Table 5). The protein content in control treatment was 7.98% and increased to 8.64%, 9.15% and 9.44% with 3, 5 and 7% addition of WGP respectively. The total dietary fiber content improved from 2.50 to 4.35% with rise in WGP from 0 to 7%. WGP incorporation significantly enhanced the total phenolic content of cookies. The total phenolic content increased from 48.3 to 90.0 mg GAE/100g and % inhibition increased from 23.2 to 41.5% (Table 6). The present results are in accordance with the findings of [33]; [34]; [35]; [29]; [26]; [31]; [36]; [37].

### 3.5. Influence of wheat grass powder on physical characteristics of cookies

With the increase in WGP from 0 to 7%, the diameter of cookies was decreased from 50.1 to 47.5mm and thickness was increased from 10.1 to 11.3mm while spread factor was decreased from 49.8 to 41.9. The hardness of cookies was increased and fracture ability was decreased with the addition of WGP. Results are supported by [26]; [12].

### 3.6. Influence of wheat grass powder on sensory characteristics of cookies

The sensory characteristics of WGP cookies are presented in Table 8 and Fig. 4. The sensory scores for color, flavor, taste, texture, crispiness and overall acceptability decreased with increasing WGP from 0-7%. The color of cookies steadily changed to green from golden brown. From these results it was concluded that cookies with 5% WGP can be consumed without any adverse effect.

### 3.7. Analytical characterization of WGJ

Wheat grass juice was analyzed for pH, total soluble solids and viscosity. The results have been presented in Table 9 and depicted in Fig. 5 that showed that wheat grass juice has a pH of 7.1 and the present results are similar to the previous findings [8]. Brix of wheat grass juice was found to be 3.63 which in accordance with the previous results [19]. Viscosity of wheat grass juice was recorded 3 centipoises. The results regarding viscosity of wheat grass juice are close to earlier findings [38].

### 3.8. Influence of wheat grass juice (WGJ) on characteristics of apple juice

As obvious from Table 10 and Fig. 6 the pH of apple juice was significantly affected by WGJ addition while WGJ has no significant effect on brix and viscosity of apple juice. The findings of the present study are in line with the earlier results [38]; [39]; [8]; [40].

### 3.9. Influence of wheat grass juice (WGJ) on sensory characteristics of apple juice

The sensory characteristics of juice are presented in Table 11 and Fig. 7. The sensory scores for appearance, aroma, taste, mouth feel and overall acceptability decreased with increase in WGJ from 0 to 15%. From these results it was concluded that juice with 10% WGJ can be consumed without any adverse effect.

## LITERATURE CITED

1. Haripriya S, Premakumari S. Effect of wheat bran on diabetic subjects. *Indian Journal of Science and Technology*. 2010; 3(3):284-286.
2. Iyer U, Sharma M, Dhruv S, Mani UV. Glycemic and lipemic response of wheat grass incorporated recipes. *Journal of Herbal Medicine and Toxicology*. 2010; 4(1):161-164.
3. Kakkar A, Dubey PK, Dubey S, Khare P, Bias N, Netam R. Studies on synergistic antimicrobial potential of aloe-wheatgrass extract combination. *Asian J Pharm Life Sci*. 2012; 2(2):291-294.
4. Mishra A, Sharma A, Jhalani A, Sharma MS, Bhandari A. Wheatgrass: A new era of dietary supplements. *International Journal of Phytopharmacy Research*. 2011; 2:48-53.
5. Sagliano FS, Sagliano EA. Method for growing and preserving wheatgrass nutrients and products thereof. *Google Patents* 1998.
6. Pallavi K, Kumarswamy G, Shruthi. Pharmacognostic investigation and antibacterial activity of *Triticum aestivum*. *Journal of Pharmacy Research*. 2011; 4(10):3355-3359.
7. Alitheen NB, Oon CL, Keong YS, Chuan TK, Li HK, Yong HW. Cytotoxic effects of commercial wheatgrass and fiber towards human acute promyelocytic leukemia cells (HL60). *Pak J Pharm Sci*. 2011; 24(3):243-250.
8. Singhal VK, Singhal AK, Jagatheesh K, Padmavathi K, Elangoran N, Bangr OP, et al. Multifunctional role of green blood therapy to cure for many diseases. *Chronicles of Young Scientists*. 2012; 3(1):12.

- 316 9. Shah K, Sheth D, Tirgar P, Desai T. Anti-ulcer activity of *Triticum aestivum* on ethanol induced  
317 mucosal damage (cytoprotective activity) in wistar rats. *Pharmacologyonline*. 2011; 2:929-935.
- 318 10. Lai L-S, Wang D-J, Chang C-T, Wang C-H. Catalytic characteristics of peroxidase from wheat  
319 grass. *Journal of agricultural and food chemistry*. 2006; 54(22):8611-8616.
- 320 11. Tyagi S, Manikantan M, Oberoi HS, Kaur G. Effect of mustard flour incorporation on  
321 nutritional, textural and organoleptic characteristics of biscuits. *Journal of Food Engineering*. 2007;  
322 80(4):1043-1050.
- 323 12. Sharma P, Velu V, Indrani D, Singh R. Effect of dried guduchi (*Tinospora cordifolia*) leaf  
324 powder on rheological, organoleptic and nutritional characteristics of cookies. *Food research*  
325 *international*. 2013; 50(2):704-709.
- 326 13. AACC C. Approved methods of the American association of cereal chemists. *Methods*. 2000;  
327 54:21.
- 328 14. Tandon S, Arora A, Singh S, Monga J, Arora S. Antioxidant profiling of *Triticum aestivum*  
329 (wheatgrass) and its antiproliferative activity in MCF-7 breast cancer cell line. *Journal of Pharmacy*  
330 *Research*. 2011; 4(12):4601-4604.
- 331 15. Kulkarni SD, Tilak J, Acharya R, Rajurkar NS, Devasagayam T, Reddy A. Evaluation of the  
332 antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different  
333 conditions. *Phytotherapy Research*. 2006; 20(3):218-227.
- 334 16. Abdelghafor RFM. Quality of bread from composite flour of sorghum and hard white winter  
335 wheat: UOFK; 2015.
- 336 17. Lawless HT, Heymann H. *Sensory evaluation of food: principles and practices*: Springer  
337 Science & Business Media, 2010.
- 338 18. Houška M, Strohalm J, Kocurová K, Totušek J, Lefnerová D, Tříška J, et al. High pressure and  
339 foods—fruit/vegetable juices. *Journal of food engineering*. 2006; 77(3):386-398.
- 340 19. Waghay K, Gulla S, Kumar CS, Kumar MP, Kumar AA. Sensory Quality and Acceptability of  
341 Fresh Juices. 2012.
- 342 20. Kareem S, Adebawale A. Clarification of orange juice by crude fungal pectinase from citrus  
343 peel. 2007.
- 344 21. Chaturvedi N, Sharma P, Rohtagi S. Preliminary Phytochemical, Nutritional Potential of  
345 cereal Grass Powder Based Products for Effective Management of Diabetes. 2013.
- 346 22. Svihus B, Newman C, Newman R, Selmer-Olsen I. Changes in extract viscosity, amino acid  
347 content, and soluble and insoluble  $\beta$ -glucan and dietary fibre content of barley during different high  
348 moisture storage conditions. *Animal feed science and technology*. 1997; 64(2):257-272.
- 349 23. Randhir R, Kwon Y-I, Shetty K. Effect of thermal processing on phenolics, antioxidant activity  
350 and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science &*  
351 *Emerging Technologies*. 2008; 9(3):355-364.
- 352 24. Kothari S, Jain AK, Mehta SC, Tonpay SD. Hypolipidemic effect of fresh *Triticum aestivum*  
353 (wheat) grass juice in hypercholesterolemic rats. *Acta Pol Pharm*. 2011; 68(2):291-294.
- 354 25. Kohajdová Z, Karovičová J, Jurasová M. Influence of carrot pomace powder on the  
355 rheological characteristics of wheat flour dough and on wheat rolls quality. *Acta Scientiarum*  
356 *Polonorum, Technologia Alimentaria*. 2012; 11:381-387.
- 357 26. Dachana K, Rajiv J, Indrani D, Prakash J. Effect of dried moringa (*Moringa oleifera* lam) leaves  
358 on rheological, microstructural, nutritional, textural and organoleptic characteristics of cookies.  
359 *Journal of Food Quality*. 2010; 33(5):660-677.
- 360 27. Masoodi F, Chauhan G, Tyagi S, Kumbhar B, Kaur H. Effect of apple pomace incorporation on  
361 rheological characteristics of wheat flour. *International Journal of Food Properties*. 2001; 4(2):215-  
362 223.
- 363 28. Hwang J-Y, Sung W-C, Shyu Y-S. Effect of mulberry lees addition on dough mixing  
364 characteristics and the quality of mulberry toast. *J of Marine Sci and Technol*. 2008; 16:103-108.

29. Ajila C, Leelavathi K, Rao UP. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of cereal science*. 2008; 48(2):319-326.
30. Turksoy S, Keskin S, Ozkaya B, Ozkaya H. Effect of black carrot (*Daucus carota* L. spp. sativus var. atropurpureus Alef.) fiber addition on the composition and quality characteristics of cookies. *Journal of Food, Agriculture and Environment*. 2011; 9:57-60.
31. Ashoush I, Gadallah M. Utilization of Mango Peels and Seed Kernels Powders as Sources of Phytochemicals in Biscuit. *World Journal of Dairy & Food Science*. 2011; 6(1):35-42.
32. Gajula H, Liu S, Alavi S, Herald T, Madl R, Bean SR, et al. Pre-cooked fiber-enriched wheat flour obtained by extrusion: rheological and functional properties. *International Journal of Food Properties*. 2009; 12(1):27-44.
33. Pasha I, Butt S, Anjum F, Shehzadi N. Effect of dietetic sweeteners on the quality of cookies. *Analysis*. 2002; 8(60):20.
34. Larrea M, Chang Y, Martinez-Bustos F. Some functional properties of extruded orange pulp and its effect on the quality of cookies. *LWT-Food Science and Technology*. 2005; 38(3):213-220.
35. Arshad MU, Anjum FM, Zahoor T. Nutritional assessment of cookies supplemented with defatted wheat germ. *Food chemistry*. 2007; 102(1):123-128.
36. Gupta M, Bawa AS, Abu-Ghannam N. Effect of barley flour and freeze-thaw cycles on textural nutritional and functional properties of cookies. *Food and bioprocess technology*. 2011; 8(4):520-527.
37. Škrbić B, Cvejanov J. The enrichment of wheat cookies with high-oleic sunflower seed and hull-less barley flour: Impact on nutritional composition, content of heavy elements and physical properties. *Food Chemistry*. 2011; 124(4):1416-1422.
38. Alvarez S, Riera F, Alvarez R, Coca J, Cuperus F, Bouwer ST, et al. A new integrated membrane process for producing clarified apple juice and apple juice aroma concentrate. *Journal of Food Engineering*. 2000; 46(2):109-125.
39. Kadakal Ç, Nas S, Ekinci R. Ergosterol as a new quality parameter together with patulin in raw apple juice produced from decayed apples. *Food Chemistry*. 2005; 90(1):95-100.
40. Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, et al. Effect of ultrasound on different quality parameters of apple juice. *Ultrasonics sonochemistry*. 2013; 20(5):1182-1187.

**Figure 1: Chemical composition of wheat grass powder (WGP)**

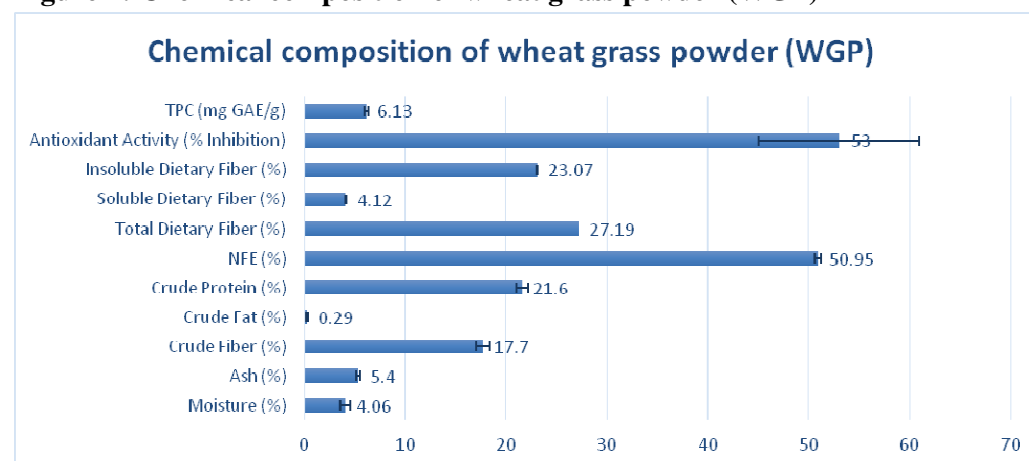


**Figure 2: Influence of wheat grass powder (WGP) on Dietary Fiber**

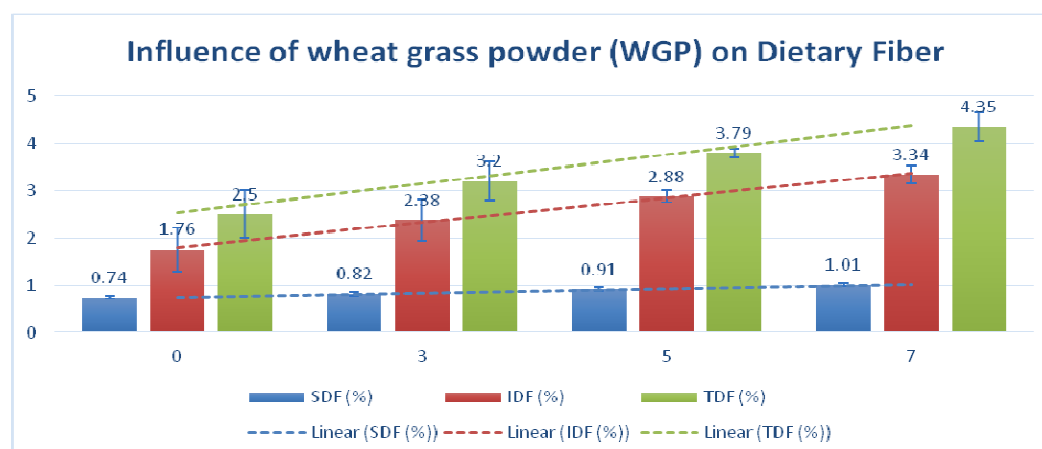


**Figure 3: Influence of wheat grass powder (WGP) on Total Phenolic content and Antioxidant potential of cookies**

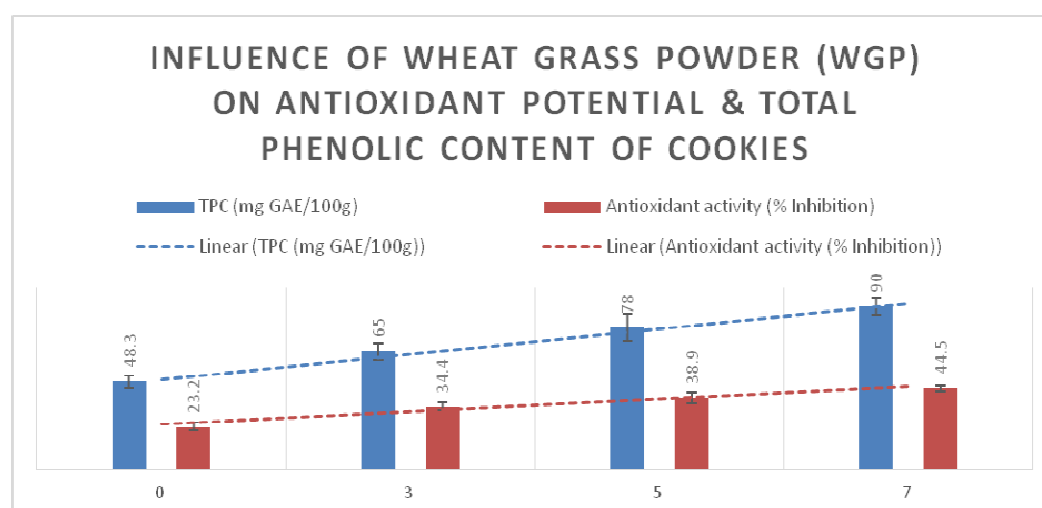


**Figure 4: Influence of wheat grass powder (WGP) on sensory acceptability of cookies**

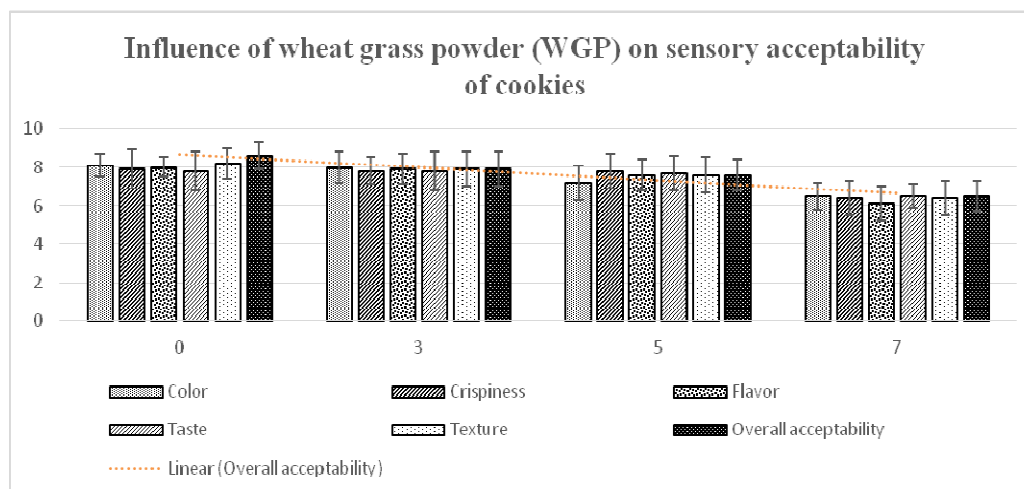


**Figure 5: Analytical characterization of wheat grass juice (WGJ)**

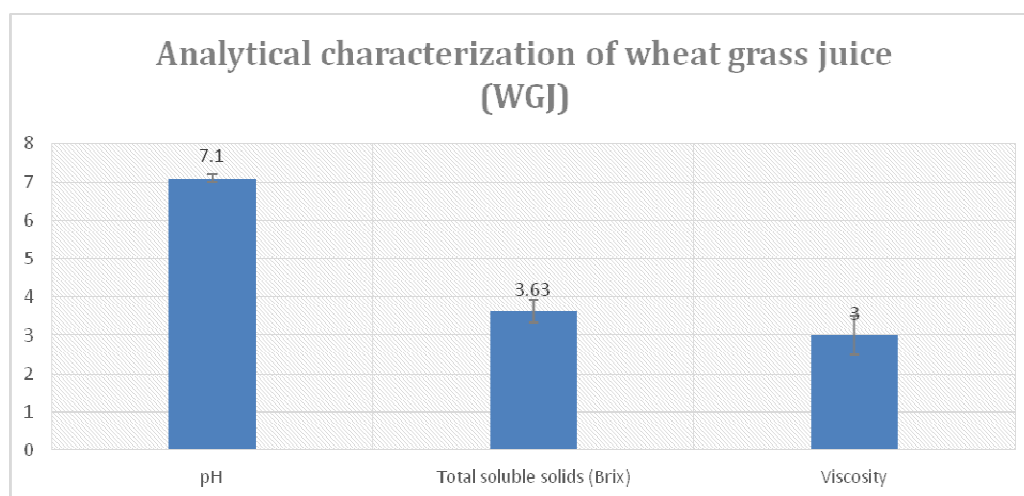


**Figure 6: Influence of wheat grass juice (WGJ) on characteristics of apple juice**

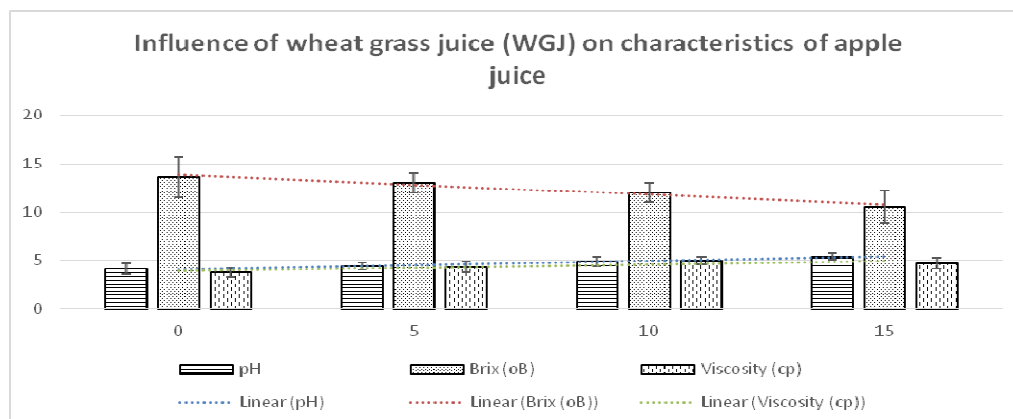


**Figure 7: Influence of wheat grass juice (WGJ) on sensory acceptability of apple juice**

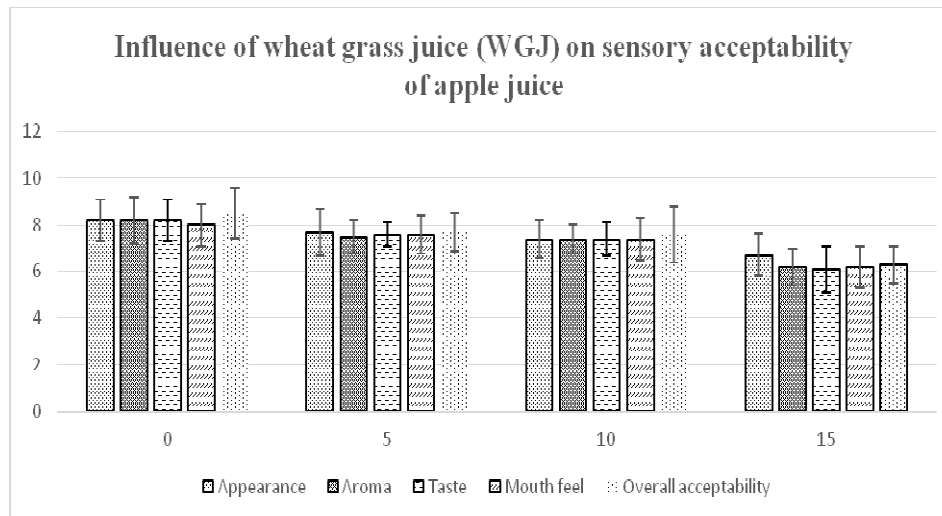


**Table 2: Chemical composition of wheat grass powder (WGP)**

| Parameter                           | Wheat grass Powder |
|-------------------------------------|--------------------|
| Moisture (%)                        | 4.06±0.5           |
| Ash (%)                             | 5.4±0.2            |
| Crude Fiber (%)                     | 17.7±0.7           |
| Crude Fat (%)                       | 0.29±0.1           |
| Crude Protein (%)                   | 21.6±0.6           |
| NFE (%)                             | 50.95±0.3          |
| Total Dietary Fiber (%)             | 27.19±0.03         |
| Soluble Dietary Fiber (%)           | 4.12±0.01          |
| Insoluble Dietary Fiber (%)         | 23.07±0.02         |
| Antioxidant Activity (% Inhibition) | 51±8               |
| TPC (mg GAE/g)                      | 6.13±0.2           |

\*All characteristics except moisture are expressed on dry matter basis.

Values in the row with the same letter in superscript are not significantly different from each other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.

**Table 3: Phytochemical screening of wheat grass powder**

| Compound        | Observation                                        | Results |
|-----------------|----------------------------------------------------|---------|
| Reducing sugars | Fehling A solution turned to brick red precipitate | +ve     |
| Tannins         | Appearance of Bluish green colour                  | +ve     |
| Saponins        | Appearance of thick persistent                     | +ve     |

**Table 4: Effect of wheat grass powder (WGP) on farinographic characteristics**

| WGP (%)     | WA (%)                       | AT (min)                    | DT (min)                    | DS (min)                    | DDT (min)                    | MTI (BU)                  |
|-------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|---------------------------|
| 0 (Control) | 62.5 <sup>b</sup> $\pm$ 1.5  | 1.4 <sup>c</sup> $\pm$ 0.1  | 13.0 <sup>a</sup> $\pm$ 0.6 | 11.5 <sup>a</sup> $\pm$ 0.1 | 3.5 <sup>b</sup> $\pm$ 0.3   | 35.0 <sup>d</sup> $\pm$ 1 |
| 3           | 64.0 <sup>b</sup> $\pm$ 1.6  | 1.7 <sup>b</sup> $\pm$ 0.1  | 10.9 <sup>b</sup> $\pm$ 0.4 | 9.2 <sup>b</sup> $\pm$ 0.1  | 3.90 <sup>b</sup> $\pm$ 0.3  | 52.0 <sup>c</sup> $\pm$ 1 |
| 5           | 64.9 <sup>ab</sup> $\pm$ 1.7 | 1.9 <sup>ab</sup> $\pm$ 0.2 | 9.0 <sup>c</sup> $\pm$ 0.6  | 7.1 <sup>c</sup> $\pm$ 0.2  | 3.97 <sup>ab</sup> $\pm$ 0.4 | 67.0 <sup>b</sup> $\pm$ 1 |
| 7           | 67.0 <sup>a</sup> $\pm$ 2.16 | 2.1 <sup>a</sup> $\pm$ 0.2  | 8.5 <sup>c</sup> $\pm$ 0.4  | 6.4 <sup>d</sup> $\pm$ 0.1  | 4.5 <sup>a</sup> $\pm$ 0.2   | 84.0 <sup>a</sup> $\pm$ 2 |

Values in the row with the same letter in superscript are not significantly different from each other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.

WA, water absorption; AT, arrival time; DT, departure time; DDT, dough development time; DS, dough stability; MTI, mixing tolerance index.

**Table 5: Effect of wheat grass powder (WGP) on mixographic characteristics**

| WGP (%)     | Peak Time (min)            | Peak Height (%)          |
|-------------|----------------------------|--------------------------|
| 0 (Control) | 4.7 <sup>a</sup> $\pm$ 0.3 | 62 <sup>a</sup> $\pm$ 2  |
| 3           | 4.1 <sup>b</sup> $\pm$ 0.3 | 59 <sup>ab</sup> $\pm$ 3 |
| 5           | 4 <sup>b</sup> $\pm$ 0.4   | 55 <sup>bc</sup> $\pm$ 4 |
| 7           | 3.8 <sup>b</sup> $\pm$ 0.2 | 51 <sup>c</sup> $\pm$ 4  |

Values in the row with the same letter in superscript are not significantly different from each other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.

**Table 6: Influence of wheat grass powder (WGP) on the proximate composition\* of cookies**

| WGP (%)     | Moisture (%)            | Ash (%)                 | Crude fat (%)           | Crude fiber (%)          | Crude Protein (%)       | NFE (%)                |
|-------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|------------------------|
| 0 (Control) | 3.85 <sup>c</sup> ±0.14 | 0.77 <sup>c</sup> ±0.05 | 22.5 <sup>a</sup> ±1.1  | 1.20 <sup>c</sup> ±0.06  | 7.98 <sup>c</sup> ±0.4  | 63.7 <sup>a</sup> ±1.5 |
| 3           | 4.14 <sup>c</sup> ±0.14 | 0.94 <sup>b</sup> ±0.01 | 21.9 <sup>ab</sup> ±0.9 | 1.63 <sup>bc</sup> ±0.06 | 8.64 <sup>b</sup> ±0.2  | 58.7 <sup>b</sup> ±1   |
| 5           | 4.54 <sup>b</sup> ±0.22 | 1.04 <sup>b</sup> ±0.02 | 20.1 <sup>bc</sup> ±1   | 2.01 <sup>ab</sup> ±0.02 | 9.15 <sup>ab</sup> ±0.4 | 55.2 <sup>c</sup> ±1.4 |
| 7           | 5.28 <sup>a</sup> ±0.14 | 1.2 <sup>a</sup> ±0.1   | 19.2 <sup>c</sup> ±1    | 2.39 <sup>a</sup> ±0.03  | 9.44 <sup>a</sup> ±0.2  | 50.5 <sup>d</sup> ±0.8 |

\*All characteristics except moisture are expressed on dry matter basis.

Values in the row with the same letter in superscript are not significantly different from each other at P≤0.05. Values are means of three replicate ± standard deviation.

**Table 7: Influence of wheat grass powder (WGP) on dietary fiber, antioxidant activity and total phenolic content of cookies**

| WGP (%)     | SDF (%)                 | IDF (%)                  | TDF (%)                 | TPC (mg GAE/100g)      | Antioxidant activity (% Inhibition) |
|-------------|-------------------------|--------------------------|-------------------------|------------------------|-------------------------------------|
| 0 (Control) | 0.74 <sup>d</sup> ±0.04 | 1.76 <sup>c</sup> ±0.46  | 2.50 <sup>c</sup> ±0.5  | 48.3 <sup>d</sup> ±3.5 | 23.2 <sup>d</sup> ±2.2              |
| 3           | 0.82 <sup>c</sup> ±0.04 | 2.38 <sup>b</sup> ±0.44  | 3.20 <sup>b</sup> ±0.4  | 65.0 <sup>c</sup> ±4.6 | 34.4 <sup>b</sup> ±2.3              |
| 5           | 0.91 <sup>b</sup> ±0.04 | 2.88 <sup>ab</sup> ±0.13 | 3.79 <sup>ab</sup> ±0.1 | 78.0 <sup>b</sup> ±7.2 | 38.9 <sup>b</sup> ±3.1              |
| 7           | 1.01 <sup>a</sup> ±0.04 | 3.34 <sup>a</sup> ±0.18  | 4.35 <sup>a</sup> ±0.3  | 90.0 <sup>a</sup> ±5   | 44.5 <sup>a</sup> ±1.6              |

Values in the row with the same letter in superscript are not significantly different from each other at P≤0.05. Values are means of three replicate ± standard deviation.

**Table 8: Influence of wheat grass powder (WGP) on physical characteristics of cookies**

| WGP (%) | Diameter (mm) | Thickness (mm) | Spread factor | Hardness (g) | Fracturability (mm) |
|---------|---------------|----------------|---------------|--------------|---------------------|
|---------|---------------|----------------|---------------|--------------|---------------------|

|             |                         |                         |                        |                           |                         |
|-------------|-------------------------|-------------------------|------------------------|---------------------------|-------------------------|
| 0 (Control) | 50.1 <sup>a</sup> ±1    | 10.1 <sup>c</sup> ±0.5  | 49.8 <sup>a</sup> ±2.9 | 1742.3 <sup>d</sup> ±50   | 80.5 <sup>a</sup> ±1.1  |
| 3           | 49.1 <sup>ab</sup> ±0.2 | 10.4 <sup>bc</sup> ±0.2 | 47.4 <sup>ab</sup> ±1  | 1891.3 <sup>c</sup> ±27.5 | 80.3 <sup>a</sup> ±0.7  |
| 5           | 48.3 <sup>bc</sup> ±0.9 | 10.8 <sup>ab</sup> ±0.3 | 44.6 <sup>bc</sup> ±2  | 1994.9 <sup>b</sup> ±23   | 78.4 <sup>ab</sup> ±2.3 |
| 7           | 47.5 <sup>c</sup> ±0.7  | 11.3 <sup>a</sup> ±0.4  | 41.9 <sup>c</sup> ±0.9 | 2325.6 <sup>a</sup> ±65.6 | 76.1 <sup>b</sup> ±1.3  |

Values in the row with the same letter in superscript are not significantly different from each other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.

**Table 9: Influence of wheat grass powder (WGP) on sensory acceptability of cookies**

| WGP (%)     | Color                 | Crispiness            | Flavor                | Taste                 | Texture               | Overall acceptability |
|-------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| 0 (Control) | 8.1 <sup>a</sup> ±0.6 | 7.9 <sup>a</sup> ±1   | 8.0 <sup>a</sup> ±0.5 | 7.8 <sup>a</sup> ±1   | 8.2 <sup>a</sup> ±0.8 | 8.6 <sup>a</sup> ±0.7 |
| 3           | 8.0 <sup>a</sup> ±0.8 | 7.8 <sup>a</sup> ±0.7 | 7.9 <sup>a</sup> ±0.8 | 7.8 <sup>a</sup> ±1   | 7.9 <sup>b</sup> ±0.9 | 7.9 <sup>b</sup> ±0.9 |
| 5           | 7.2 <sup>b</sup> ±0.9 | 7.8 <sup>a</sup> ±0.9 | 7.6 <sup>a</sup> ±0.8 | 7.7 <sup>a</sup> ±0.9 | 7.6 <sup>c</sup> ±0.9 | 7.6 <sup>b</sup> ±0.8 |
| 7           | 6.5 <sup>c</sup> ±0.7 | 6.4 <sup>b</sup> ±0.9 | 6.1 <sup>b</sup> ±0.9 | 6.5 <sup>b</sup> ±0.6 | 6.4 <sup>d</sup> ±0.9 | 6.5 <sup>c</sup> ±0.8 |

Values in the row with the same letter in superscript are not significantly different from each other at  $P \leq 0.05$ .

**Table 10: Analytical characterization of wheat grass juice (WGJ)**

| Parameter                   | WGJ      |
|-----------------------------|----------|
| pH                          | 7.1±0.1  |
| Total soluble solids (Brix) | 3.63±0.3 |
| Viscosity                   | 3±1      |

**Table 11: Influence of wheat grass juice (WGJ) on characteristics of apple juice**

| WGJ (%)     | pH                     | Brix (°B)              | Viscosity (cp)          |
|-------------|------------------------|------------------------|-------------------------|
| 0 (Control) | 4.1 <sup>b</sup> ±0.6  | 13.6 <sup>a</sup> ±2.1 | 3.73 <sup>b</sup> ±0.5  |
| 5           | 4.4 <sup>b</sup> ±0.4  | 13 <sup>a</sup> ±1     | 4.30 <sup>ab</sup> ±0.6 |
| 10          | 4.9 <sup>ab</sup> ±0.5 | 12 <sup>a</sup> ±1     | 4.97 <sup>a</sup> ±0.4  |
| 15          | 5.4 <sup>a</sup> ±0.3  | 10.5 <sup>a</sup> ±1.7 | 4.70 <sup>ab</sup> ±0.6 |

467 Values in the row with the same letter in superscript are not significantly different from each  
468 other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.

469

470 **Table 12: Influence of wheat grass juice (WGJ) on sensory acceptability of apple juice**

| WGJ (%)     | Appearance                 | Aroma                      | Taste                       | Mouth feel                 | Overall acceptability      |
|-------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|
| 0 (Control) | 8.2 <sup>a</sup> $\pm$ 0.9 | 8.2 <sup>a</sup> $\pm$ 1   | 8.2 <sup>a</sup> $\pm$ 0.9  | 8.0 <sup>a</sup> $\pm$ 0.9 | 8.5 <sup>a</sup> $\pm$ 1.1 |
| 5           | 7.7 <sup>ab</sup> $\pm$ 1  | 7.5 <sup>b</sup> $\pm$ 0.7 | 7.6 <sup>ab</sup> $\pm$ 0.5 | 7.6 <sup>a</sup> $\pm$ 0.8 | 7.7 <sup>b</sup> $\pm$ 0.8 |
| 10          | 7.4 <sup>b</sup> $\pm$ 0.8 | 7.4 <sup>b</sup> $\pm$ 0.6 | 7.4 <sup>b</sup> $\pm$ 0.7  | 7.4 <sup>a</sup> $\pm$ 0.9 | 7.6 <sup>b</sup> $\pm$ 1.2 |
| 15          | 6.7 <sup>c</sup> $\pm$ 0.9 | 6.2 <sup>c</sup> $\pm$ 0.8 | 6.1 <sup>c</sup> $\pm$ 1    | 6.2 <sup>b</sup> $\pm$ 0.9 | 6.3 <sup>c</sup> $\pm$ 0.8 |

471 Values in the row with the same letter in superscript are not significantly different from each  
472 other at  $P \leq 0.05$ . Values are means of three replicate  $\pm$  standard deviation.