



# Development of Functional Ready-to-Serve Wheatgrass Beverage Blended with Aonla-ginger and their Physicochemical and Antioxidant Analysis

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## To Cite this Article

Neha Sahrawat, Neelam Chaturvedi and Tripti Singh. Development of Functional Ready-to-Serve Wheatgrass Beverage Blended with Aonla-ginger and their Physicochemical and Antioxidant Analysis. International Journal for Modern Trends in Science and Technology 2022, 8(04), pp. 45-52. <https://doi.org/10.46501/IJMTST0804010>

## Article Info

Received: 02 March 2022; Accepted: 28 March 2022; Published: 03 April 2022.

## ABSTRACT

Functional beverages have gained much importance as they deliver a health benefit beyond the basic nutritional functions. In view of the consumer need towards functional food category, the current study was carried out to assess the acceptance of Wheatgrass RTS beverage blended with Aonla-ginger by semi-trained panellists, as well as their physicochemical composition and antioxidant content. The data from the assessment forms were analysed using descriptive statistics and ANOVA. Another phase of the study involved evaluating the physicochemical properties, which have a strong influence on the sensory qualities of RTS beverages, as well as antioxidant content analysis, which included total phenols (mgGAE/100ml), total flavonoids (mgQE/100ml), and chlorophyll content (mg/100ml). Overall acceptability of sensory attributes of the five treatments of RTS beverages varies from 3.5 to 4.2. Sample T4 (38% Wheatgrass, 60% Aonla and 2% Ginger) beverage had insignificant value in terms of all sensory attributes. The results of physicochemical properties revealed that acidity, total sugar and reducing sugar were increased according to the addition of Aonla juice concentration in the wheatgrass beverages, whereas pH and chlorophyll were decreased accordingly. The antioxidant content in aqueous extract recorded higher amount of Total phenols which ranged from  $4.5 \pm 0.36$  to  $14.1 \pm 0.13$  mgGAE/100ml, Total flavonoids content from  $25.4 \pm 0.45$  to  $36.1 \pm 0.07$  mgQE/100ml and Ascorbic acid content from  $7.0 \pm 0.69$  to  $15.6 \pm 0.3$  mg/100ml respectively, which were significant at  $p \leq 0.05$  level with increased Aonla juice concentration in the wheatgrass beverages. The results indicate that Wheatgrass based RTS beverage could be successfully utilized to develop functional beverages with improved antioxidants and sensory quality.

**KEYWORDS:** Functional, Physicochemical, Sensory, Antioxidant, Ready-to-serve

## 1. INTRODUCTION

The growing demand of soft drinks in market offers an enormous opportunity to produce natural beverages that are nutrient-dense. These drinks provide customers with a minimum of synthetic chemicals and also have important advantages for health. The increased awareness of the health benefits of the goods consumed led to an exponential increase in food and beverage

demand from natural sources with nutraceuticals. In 2003, there were over 700 new products introduced into the categories of healthy drinks and fruit juices, an increase of 40% over the year 2002 (Anon, 2003). According to reports, the fastest growing category is functional beverages sector (Roberts, 2009). A promising segment in the food industry is a functional beverage made by mixing fruit, vegetables and medicinal

plants. Wheatgrass is being investigated as a possible ingredient in a variety of health foods and beverages due to its functional and nutraceutical properties (Rana *et al.*, 2011; Rebekic *et al.*, 2019). Green juices containing wheatgrass are high in nourishing nutrients, such as polyphenols, antioxidants, chlorophyll, minerals, vitamins, amino acids, active enzymes etc., are now considered new functional foods, superfood or specific foods. Consumer demand for high antioxidant foods is on the increase nowadays. Drinking high-antioxidants could help protect the population against chronic conditions during adulthood and ageing along with Alzheimer's disease (Nanasombat *et al.*, 2015). Alfalfa, barley grass and wheat grass are among these kinds of cereal grasses which are very good for a well-being and good health (Ashish *et al.*, 2012).

The value addition to other medicinal plants such as Wheatgrass, Ginger and Aonla based RTS drinks can be an excellent way of providing consumers with these therapeutic benefits. The wheatgrass (*Triticum aestivum*) familiarly called young wheat herb that freshly juices or sweetened into powder for animal and in human consumption. Wheatgrass is a low-income weed which provides the human body with powerful nutrients and vitamins. It is high in minerals, active enzymes, chlorophyll and numerous vitamins in the form of fresh juice (Mujoriya and Bodla, 2011). The antioxidant activity of Wheatgrass has been proven to be high when the comparison was made with the standard drug ascorbic acid (Ashok, 2011). Because of the antioxidant content, Wheatgrass juice is a significant part of a macrobiotic diet when it comes to cancer treatment using a Complementary and Alternative Medicine (CAM) approach (Padalia *et al.*, 2010). Altering lifestyles of customers and patterns of food expenditure provides a great opportunity in the food sector for the development of functional beverages (Rezaei *et al.*, 2012). The Wheatgrass is also regarded as functional food which has a wide range of effects in thalassemia, haemorrhagic, haemolytic anaemia, asthma, cancer, inflammation, irritable bowel syndrome, and oxidative stress, according to reports from many studies. The chemical structure of Wheatgrass resembles to haemoglobin structure due to the presence of chlorophyll in it which thought to act as a "green blood." Eventually, wheat grass tends to be a possible herbal remedy for human blood plasma, and since it has a pH of about 7 (alkaline), it is readily

absorbed into the bloodstream and immensely detrimental (Mujoriya and Bodla, 2011); (Padalia *et al.*, 2010).

Aonla (*Embllica officinalis*) is a sub-tropical deciduous tree which is commonly known as Indian gooseberry has an abundant of antioxidants, phenols, dietary fibre and vitamin C in its both fresh and processed forms (Goraya and Bajwa, 2015). It is exceedingly nutritious, medicinally rich fruit that is high in phenols and tannins, such as elegendic acid or Gallic acid, which prevent vitamin C oxidation (Per 100 g pulp, Aonla contains 500 to 1500 mg ascorbic acid) (Singh and Bhatnagar, 2019). Aonla's medicinal properties are well-known for various diseases such as asthma, diabetes, bronchitis, scurvy, anaemia, tuberculosis, memory weakness, carcinoma, along with flu. The juice in Aonla is slightly astringent and acidic that is unpleasant to some if consumed as a fresh fruit (Goyal *et al.*, 2008). The fresh fruit and its juice refresh, quenches and boost the health and cater various nutritional needs of the body (Slavin and Lloyd, 2012).

Ginger (*Zingiber officinale*) is a common spice that is coveted for its aroma, flavour, and medicinal properties. It is often used for Ayurveda candy preparation, gastric enzymes, and anti-emetic to avoid nausea and vomiting apart from spice in cooking. The properties of Ginger for the treatment of rheumatoid arthritis, ulcers, heart attack and stroke have also been recognised. zingiberene, Zingiberol, linalool and phellandrene are volatile oils that make up the tuber's aroma (Mukherjee *et al.*, 2014). Ginger was previously reported as an antiviral, cancer and antifungal drug (Denyer *et al.*, 1994).

Since no previous comparable work in this field was found in the literature review, as a preliminary study it is necessary to perform such a research study that would be established further in the future. The creation of novel RTS blends is necessitated by the growing market demand, the need for sustained growth in the food processing industry, and the reduction of the unpalatable tastes of otherwise nutritionally superior ingredients. The use of astringent and acidic fruits like Aonla benefits from the mixing of two or more juices. The use of an appetizing RTS blend with mingled medicinal benefits of Wheat grass, Aonla, and Ginger could be helped by the use of a revitalizing and flavoursome ingredient.

## 2. MATERIALS AND METHODS

## 2.1. Juice extraction

The research work was performed and investigation was carried out in the Department of Home Science, Banasthali Vidyapith, Rajasthan. Wheatgrass was planted in experimental land to be cultivated plots at the 'jointing stage' the subsequent leaf develops after half of the first leaf at the joining point. All the preliminary operations like washing, cutting, slicing was carried out and the wheatgrass was then washed and immediately the juice was collected before being preserved at 6-7°C for sensory test which was executed the other day. The other ingredients like Aonla and ginger was purchased from local market of Banasthali Vidyapith and their respective juices were extracted through Philips juicer & mixer (HL1631) in

Department's Food Processing Lab followed by filtering through muslin cloth and storing separately for potential use in the refrigerator. For the bottling of juice, 200 ml of presterilized PET glass bottles were used.

## 2.2. Formulation of Beverages

Five treatments of Wheatgrass beverages blended with Aonla-ginger were formulated as per the flow chart prepared in Fig.1. The Wheatgrass RTS beverages were formulated in distinctive treatments such as T1- 88% Wheatgrass:10% Aonla: 2% Ginger, T2- 78% Wheatgrass: 20% Aonla: 2% Ginger, T3- 58% Wheatgrass: 40% Aonla: 2% Ginger, T4- 38% Wheatgrass: 60% Aonla: 2% Ginger, T5- 18% Wheatgrass: 80% Aonla: 2% Ginger. S- Standard orange juice as shown in Plate 1.

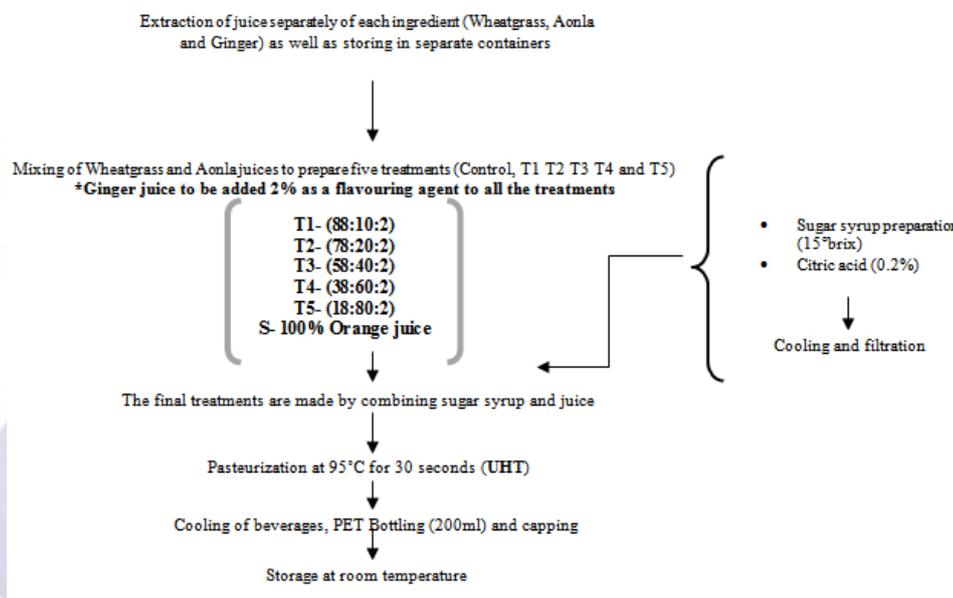


Fig. 1: Flowchart depicting the process of preparing Wheatgrass RTS beverages



Plate 1: Five Treatments of Wheatgrass RTS juice blended with Aonla and ginger

## 2.3. Physicochemical composition

An automated pH meter (Fisher Scientific Inc., MA, USA) was used to calculate the pH of the Wheatgrass RTS

beverages blended with Aonla-ginger. By titrating against 0.1 N NaOH, the acidity of various treatments was calculated and expressed as % citric acid using the A.O.A.C protocol. Lane and Eynon's method were used to calculate total sugars and reducing sugars % (Ranganna, 2001). The amount of ascorbic acid in the RTS beverage was measured using the titration method with 2, 6-dichlorophenol endophenol dye, as recommended by the (Ranganna, 2001).

## 2.4. Antioxidant and chlorophyll content

Extracts from RTS beverages were diluted 100 times and were used for evaluation of functional components and properties by centrifugation at 5000 rpm for 15 minutes. By construction of slight modifications in

Folin-Ciocalteu method (Dewantoet *al.*, 2002), Total phenols were determined: 2.5 ml of Folin-Ciocalteu reagent was added in 0.5 ml sodium carbonate (100 g/L), with an incubation during 1 hour in darkness. Absorbance was measured at 760 nm and the results of Gallic acid equivalents were expressed (mgGAE/100ml). The updated  $AlCl_3$  method was used to calculate total flavonoids content (Zhishenet *al.*, 1999). Briefly, a 1 mL aliquot of beverage extract was blended with 0.3 mL of 5 percent sodium nitrite and dissolved in 5 mL of distilled water. After 5 minutes, 10% of  $AlCl_3$  (3 ml) was added, followed by 1 M NaOH (2 ml) to finish the reaction. The absorbance was estimated at 510 nm, and the findings were expressed in Quercetin equivalents (mgQE/100ml). To assess the net chlorophyll content, chlorophyll was measured in Wheatgrass RTS beverages blended with Aonla-ginger (Ranganna, 2001).

### 2.5. Sensory evaluation

34 semi-trained female panelists used a 5-point acceptance score (1= disliked extremely; 5= extremely liked) to determine the Overall acceptability of beverages. Samples were also evaluated for colour, appearance, flavour, taste, aroma (Chen *et al.*, 2010). The panelists were graduate and post-graduate students aged 20 to 30 years old, chosen in such a way that they all had prior understanding of customer needs. Individually refrigerated (7°C) samples were served in coded identical cups. The panelists were divided into two groups, having 20 and 14 panel members in each group for control effect.

### 2.6. Statistical analysis

The results were expressed as mean values  $\pm$  standard deviations from three replications for different parameters. SPSS was used to conduct statistical research (Statistical software Student Version 20.0, Chicago, IL, USA). ANOVA and descriptive statistics were used to interpret the data. Significant differences were described as those with a  $p < 0.05$  (95% confidence level) difference. General statistical methods were used to measure descriptive statistics for the evaluation of organoleptic evaluation of Wheatgrass-based RTS beverages, including mean acceptance, standard deviation, and coefficient of variation. A paired t-test comparison was used to see whether there was a statistical difference in product organoleptic assessment between the two groups of panel members. The analysis of variance  $p < 0.05$  was used to see whether there were any statistically significant differences in the organoleptic assessment

scores of the five RTS beverage samples used in this study for the 5 attributes mentioned above.

## 3. RESULTS AND DISCUSSION

Before blending, wheatgrass, Aonla, and ginger juices were analysed and their properties were registered. Wheatgrass, Aonla, and ginger juices had pH values of  $6.7 \pm 0.03$ ,  $1.97 \pm 0.01$  and  $4.72 \pm 0.22$ , respectively. Wheatgrass, Aonla, and ginger juices had acidity values of  $0.009 \pm 0.01$ ,  $2.34 \pm 0.15$  and  $0.17 \pm 0.05$  %, respectively, when expressed as % citric acid. Wheatgrass, Aonla, and ginger juices were found to have antioxidant activity of  $0.360 \pm 0.01$ ,  $22.95 \pm 1.02$  and  $3.12 \pm 0.42$  mg/100ml, respectively. Table 1 shows, however, that combining wheatgrass juice with aonla found to boost the antioxidant ability of the beverages.

The results obtained from physicochemical analysis for six treatments of Wheatgrass beverages blended with Aonla-ginger are tabulated in Table 1. The observations reveal that when the concentration of Aonla juice was increased there was decrease in the pH value whereas there was an increment in acidity %, total sugar % and reducing sugar %.

The pH value of the beverage is influenced by the presence of free hydrogen ions and the buffering ability of the juices (Shubhangini, 2002). The results showed that the pH of beverage T1 having superior composition of Wheatgrass juice had high pH value of  $7.0 \pm 0.49$  whereas T5 had lowest pH value ( $2.6 \pm 0.52$ ) due to higher content of citric acid present in Aonla juice. The data on acidity percent shows the % rise in Aonla juice in all five treatments of Wheatgrass beverages blended with Aonla-ginger resulted in a substantial increase in acidity %. The acidity of the formulations T1 through T5 increased by  $1.4 \pm 0.06$  to  $4.2 \pm 0.02$  %, respectively. The explanation may be due to the Aonla juice's inherent acidity; a similar pattern was stated by Daramola and Asunni, (2007). Increases in acidity percent can be due to the degradation of polyphenols in ginger and Aonla, as well as the rapid conversion of proteins to amino acids in Wheatgrass beverages containing Aonla-ginger. The findings are corroborated by results reported by (Yadav *et al.*, 2013).

There was an insignificant percentage difference between the total sugars and reducing sugars of Wheatgrass beverages blended with Aonla-ginger. Wheatgrass, Aonla, and ginger juices are predicted to be well

balanced and compatible for blending or mixing and preparing therapeutic RTS beverages with different physicochemical characteristics. Vitamin C is a vital aspect in any functional beverage. Table 1 indicates the variations in vitamin C content during the blending of Wheatgrass: Aonla: Ginger juice. Since Aonla juice has higher vitamin C content than Wheatgrass and ginger

juice, the vitamin C content increased from  $7.0 \pm 0.69$  to  $20.6 \pm 0.31$  mg/100ml with an increase in the concentration of Aonla juice from 10 to 80 % in the RTS beverage. This exponential rise while blending two separate fruit juices is consistent with the findings of (Nidhi et al., 2008), who found the same trend in their analysis of a bael-guava blended beverage.

**Table 1:** Physicochemical composition of different treatments of Wheatgrass RTS beverages blended with Aonla-ginger

Physicochemical Composition	T1	T2	T3	T4	T5
pH	$7.0 \pm 0.49$	$6.0 \pm 0.50$	$5.6 \pm 0.30$	$3.9 \pm 0.80$	$2.6 \pm 0.52$
Acidity (%)	$1.4 \pm 0.06$	$2.2 \pm 0.10$	$3.0 \pm 0.01$	$3.5 \pm 0.01$	$4.2 \pm 0.02$
Total sugar (%)	$6.3 \pm 0.17$	$7.3 \pm 0.20$	$8.3 \pm 0.30$	$8.7 \pm 0.24$	$9.8 \pm 0.30$
Reducing sugar (%)	$3.6 \pm 0.98$	$4.2 \pm 0.10$	$4.3 \pm 0.43$	$4.6 \pm 0.83$	$4.9 \pm 0.66$
Ascorbic acid (mg/100ml)	$7.0 \pm 0.69$	$10.2 \pm 0.68$	$15.2 \pm 0.57$	$18.7 \pm 0.11$	$20.6 \pm 0.31$

The Mean±Standard deviation is used to express the data. The ratio of Wheatgrass: Aonla: Ginger in the five treatments were (88:10:2), (78:20:2), (58:40:2), (38:60:2) and (18:80:2) for T1, T2, T3, T4 and T5 respectively

Total polyphenols such as Phenolic acids, flavonoids and other polyphenolic compounds are considered chief suppliers of vegetables and fruits antioxidant activity. It was determined that the total Phenolic and flavonoids contents of five treatments of Wheatgrass beverages blended with Aonla-ginger. In Table 2 the result showed, a significant variation in Total Phenols Content (TPC) which ranged from  $4.5 \pm 0.36$  to  $14.1 \pm 0.13$  mg Gallic acid equivalents (mgGAE/100ml) in all five treatments. It was observed that T5 had the highest level of TPC and T1 had the lowest level of TPC. Similar results were noted in Total Flavonoids Content (TFC) which ranged from  $25.4 \pm 0.45$  to  $36.1 \pm 0.07$  Quercetin equivalents

(mgQE/ml) in all five treatments. T5 had the highest level of TPC and T1 had the lowest value of TPC. Chlorophyll can neutralize the polluting elements, strengthens the cell, protect us from carcinogens and detoxifies the liver (Rana et al., 2011). The chlorophyll contents of five Wheatgrass beverage blended with Aonla-ginger were ranged from  $4.7 \pm 0.76$  to  $13.9 \pm 0.30$  mg/100ml. RTS beverage T1 contained high chlorophyll content than T2, T3, T4 and T5 respectively due to the high ratio of Wheatgrass juice in each beverage, which has been used for haemoglobin deficiency treatment (Padalia et al., 2010).

**Table 2:** Antioxidant contents of different treatments of Wheatgrass RTS beverages blended with Aonla-ginger

Antioxidant content	T1	T2	T3	T4	T5
Total Phenols Content (mgGAE/100ml)	$4.5 \pm 0.36$	$6.3 \pm 0.12$	$8.0 \pm 0.05$	$12.4 \pm 0.23$	$14.1 \pm 0.13$
Total Flavonoids Content (mgQE/100ml)	$25.4 \pm 0.45$	$27.3 \pm 0.25$	$29.0 \pm 0.04$	$32.7 \pm 0.53$	$36.1 \pm 0.07$
Chlorophyll (mg/100ml)	$13.9 \pm 0.30$	$11.2 \pm 0.25$	$8.5 \pm 0.43$	$6.2 \pm 0.22$	$4.7 \pm 0.76$

The Mean±Standard deviation is used to express the data. The ratio of Wheatgrass: Aonla: Ginger in the five treatments were (88:10:2), (78:20:2), (58:40:2), (38:60:2) and (18:80:2) for T1, T2, T3, T4 and T5 respectively

Table 3 shows the average sensory scores for fresh Wheatgrass beverages infused with Aonla-ginger the

sensory characteristics of RTS beverages are critical because they assess the juice's acceptability and

marketability. Colour, appearance, flavour, taste, aroma, and overall acceptability were assessed using 34 qualified female panellists divided into two groups of 20 and 14 members respectively. For each attribute, including colour, appearance, flavour, taste, aroma, and overall acceptability, Mean scores, Standard deviations, and coefficients of variation were determined for all panelists. These values along with the mean, SD, and CV parameters are shown in following Table 3. The best accepted RTS beverage by consumers was T4 (38% Wheatgrass, 60% Aonla and 2% Ginger) with the mean value of overall acceptability  $4.2\pm 0.78$  and CV 0.075. The less accepted with a mean value of overall acceptability  $3.5\pm 0.81$ , and CV 0.055 was T5 (18% Wheatgrass, 80% Aonla and 2% Ginger). The result clearly indicates that T4 scored higher with respect to flavour, taste, after taste and overall acceptability. The ginger juice was added as flavouring agent and had a positive impact on sensory

attributes of Wheatgrass beverages. Wheatgrass has a bitter-astringent flavour, but the addition of Aonla-ginger juice enhanced the sensory qualities of functional beverages. In terms of overall acceptability, the result suggests that variation T4 was the best formulation. Among the comments and suggestions, it was discovered that sweetening the taste, improving the aroma of fruit juices, and improving the consistency by eliminating the precipitation issue were all necessary. The following Figure 1. depicts the organoleptic assessment of each formulation for colour, appearance, flavour, taste, aroma, and overall acceptability. Standard sample (orange juice) was provided to both groups of female panelists for control effect purposes, as previously mentioned. The standard sample and RTS beverages were compared using a T-test pair comparison to see whether there was a statistical difference in product attributes assessment by 5-point composite score.

**Table 3:** Sensory evaluation of Wheatgrass RTS beverages blended with Aonla-ginger

Attributes	Standard	T1	T2	T3	T4	T5
Colour	4.9±0.63	3.1±0.63	3.4±0.81	3.8±0.75	3.8±0.56	3.6±0.56
Appearance	4.9±0.81	3.5±0.83	3.9±0.79	3.6±0.81	3.8±0.86	3.6±0.82
Flavour	4.9±0.63	3.0±0.99	3.3±0.51	3.8±0.77	4.4±0.91	3.5±0.91
Taste	5.0±0.01	2.9±0.96	3.2±0.94	3.6±0.81	4.5±0.96	3.8±0.91
Aroma	4.5±0.77	3.7±0.77	3.4±0.64	3.3±0.72	4.4±0.98	3.2±0.92
Overall acceptability	4.5±0.79	3.9±0.76	3.7±0.75	4.0±0.69	4.2±0.78	3.5±0.81
Mean CV	0.047	0.122	0.078	0.065	0.075	0.055

The Mean±Standard deviation is used to express the data and coefficient of variation (CV) for all samples involved in sensory analysis. The ratio of Wheatgrass: Aonla: Ginger in the five treatments were (88:10:2), (78:20:2), (58:40:2), (38:60:2) and (18:80:2) for T1, T2, T3, T4 and T5 respectively

The results showed statistical difference, analysis of variance ( $p < 0.05$ ) in characteristics including taste ( $p$ -value 0.039,  $F$ -value=3.867) and appearance ( $p$ -value 0.038,  $F$ -value= 2.168) from the sensory attributes on sample T1. As for sample T2, On the basis of attributes there are various assessments such as Aroma ( $p$ -value 0.015,  $F$ -value=4.082) and overall acceptability ( $p$ -value 0.073,  $F$ -value=4.048). The panelists seem to have various opinions on the attributes. No statistical difference was

observed for sample T3 and T4 among the attribute evaluation. For sample T5, there were different evaluations among the sensory attribute for the taste ( $p$ -value 0.056,  $F$ -value=5.232). For overall acceptability, a high correlation value (0.780) was observed followed by colour (0.693), Taste (0.671), flavour (0.625), Appearance (0.208) and the lowest correlation obtained was Aroma (-0.336).

**Table 4:**T-test pair comparisons for evaluation of control sample and Wheatgrass RTS beverage blended with Aonla-ginger among two groups of panelists

T-test Pair	Comparison of Control sample with the attributes	Mean	t	df	Sig. (2-tailed)	Correlation
Pair 2	Colour	-.866	-2.70	15	.042	-.201
Pair 3	Appearance	.750	2.82	15	.037	-.027
Pair 4	Flavour	.816	2.38	15	.063	-.091
Pair 5	Taste	-.800	-2.30	15	.070	.076
Pair 6	Aroma	.883	3.27	15	.022	.065
Pair 1	Overall acceptability	.641	2.66	15	.045	-.337

## CONCLUSION

Wheatgrass juice in its pure form is unpalatable and challenging to consume. Therefore, the sour taste of the juice obtained, unique flavour and blending it with other juices could be a way out for enhancing its taste and making it acceptable for consumers. From the result it is concluded that out of all developed RTS beverages with different treatments, Wheatgrass blended Aonla-ginger RTS beverage (T4) was well accepted with regards to sensory characteristics. Physicochemical characteristics of pH, acidity, total sugar, and reducing sugar were increased significantly with increased in percentage of Aonla juice whereas pH and chlorophyll was decreased accordingly. Antioxidant analysis showed Total phenols content, total flavonoids content and ascorbic acid was high in (T5) with increased in percentage of Aonla juice.

## ACKNOWLEDGEMENTS

The authors wish to show appreciation to the Department of Food Science and Nutrition, Banasthali Vidyapith, India for supporting this research.

## Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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