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Preparation and Quality Evaluation of Ready to Serve (RTS) Wheatgrass Juice

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Abstract

Wheatgrass is an inexpensive and efficient source to provide all the required nutrients and medicinal benefits for a healthy body. The major objective of this study is to formulate a health-beneficial beverage that could be organoleptically accepted by people of all age groups, to bring forward a new product from wheatgrass that has mostly remained unexplored. The wheatgrass harvested on the 9th day (optimized on the basis of chlorophyll, tannin, total polyphenols, flavonoids and DPPH radical scavenging activity) was taken for study. The wheat seeds (WK 1204 variety), collected from Agriculture Botany Division, Nepal Agriculture Research Council were used for the preparation of ready-to-serve juice. Eight different formulations of RTS were prepared with the help of Design-Expert®, version 7, keeping juice content 7-13%, TSS 9-16 °Bx and constant acidity at 0.3%. Response Surface Methodology, Doptimal design was adapted for the formulations. The optimization of the formulation was intended to be carried out based on sensory analysis. Proximate and phytochemical (Chlorophyll, Polyphenol, Flavonoids, Tannins and Antioxidant properties) composition of 9th days harvested wheatgrass, its extracted juice and the final product was also carried out. The statistical analysis (two-way ANOVA, no blocking) was done in order to evaluate the outcomes of sensory analysis. There was a significant difference for sensory attributes like flavor, taste, body and overall acceptability at p<0.05, whereas color wasn't found to be significantly different. Sensory analysis revealed that the beverage consisting of 12.8% Wheatgrass juice, 12.5°Bx TSS and 0.3% acidity was considered best among all the combinations.

Keywords: Phytonutrients, beneficial drink. ready to serve drink, beverage.

Introduction

Wheatgrass (*Triticum aestivum*), is an herb from the wheat family (Corleone, 2017). Wheatgrass is taken as a shoot of Triticum aestivum obtained from the cotyledons of the common wheat plant belonging to family *Graminea*. Triticum is a genus of annual and biennial grasses, yielding various types of wheat and is found in almost all parts the world (Mogra & Rathi, 2013). Triticum aestivum is mentioned as herbal system of medicine in Ayurveda and is known for its immune modulator, antioxidant, astringent, laxative, diuretic and antibacterial effects (Shirunde, 2011). Wheatgrass after harvesting could be freshly juiced or dried in powder form and could be consumed by both humans and animals in order to receive its extraordinary benefits. It is considered as a "living food". It is even considered as the "green blood" because of its high chlorophyll content. Wheatgrass juice is an abundant source of essential vitamins mainly Vitamin A, C, E and B complex. It contains a large number of vital minerals like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron, and molybdenum. Several of the enzymes like protease, amylase, lipase, cytochrome oxidase, transhydrogenase, superoxide dismutase is responsible for its pharmacological actions. The other salient feature of wheatgrass is its high proportion of amino acids such as aspartic acid. glutamic acid, arginine, alanine and serine. The antioxidant activity of the wheatgrass could be derived from its adequate amounts of bioflavonoids like apigenin, quercetin and luteolin. As per Padalia et al. (2010) therapeutic activity of wheatgrass is also contributed by its indole compounds like choline and laetrile Wheatgrass being an excellent source of several essential phytonutrients thus also exhibits an excellent anti-oxidant activity. RTS is defined as typical a fruit beverage generally prepared either from juice or pulp or both by mixing with the adequate amount of sweeteners (sugars), and acidulants (citric acid) whereas colourings and flavourings materials are optional. This type of beverages is not diluted before serving, hence called RTS (Ready-to-Serve). Ready-to-Serve beverages are both carbonated as well as non-carbonated. The use of plant based herbal products as dietary adjuncts has been increasing in food industry in recent years (Chandra et al., 2018). Dahiya et al. (2017) stated that the beverages comprising of chlorophyll are highly susceptible to degradation during the course of thermal processing because of isomerization resulting in color changes in the product. Therefore as stated by Pandey et al. (2020) such beverages could be preserved by the use of chemical preservatives.

Research Problem

Wheatgrass is an inexpensive and efficient source to provide all the required nutrients and medicinal benefits for a healthy and rejuvenating body. Wheatgrass products could be used to eradicate the malnutrition problems from developing and under developed countries as it is an inexpensive and complete source of nutrition. The raw material is cheap and available throughout the year (Singhal et al., 2012). The antioxidant and high chlorophyll content of wheatgrass is found to be effective against various degenerative disorders including thalassemia and hemolytic anemia (Padalia et al., 2010), reduce chemotherapy myelotoxicity (Dey, 2006), rheumatoid arthritis (Yadava, 2011), diuretic (Popovic, 2014), diabetes mellitus (Saravanan, 2011). Hence essential modification in the form of ready to serve beverage for the enhancement of its sensory attributes making it more palatable and popular among general and healthy public has also become equally essential.

Research Objective

The general study of this research work is to prepare and evaluate the quality of ready to serve (RTS) wheatgrass juice. The specific objectives are to analyze the phytochemical content in wheat grass and its extracted juice; formulate RTS wheatgrass juice and to determine the best formulation based on the phytochemical content and sensory attributes.

Research Limitation

The mineral content and amino acid profile of wheatgrass could not be determined; preservation techniques couldn't be employed and the shelf life of the RTS could not be studied.

Significance of the Paper

The preparation of the RTS based on wheatgrass juice not only has high content of nutritional and bioactive component but also gives a diversification in the juice industries. The miraculous benefits of wheatgrass regarding its anti-oxidant functions, anti-carcinogenic as well as anti-diabetic function, bring it forward as a potentially important medicinal plant.

Methods and Materials

Raw Materials

Wheat (*Triticum aestivum*) of WK 1204 variety, collected from National Agricultural Research Institute, Agriculture Botany Division, Khumaltar, Kathmandu. Other additives like Sugar, Citric acid etc. were used in the product. Sugar was purchased from local market of Dharan. Citric acid of lab grade was collected from Central Campus of Technology, Dharan. Bottles used for filling were pre-used 250 ml bottles previously used for filling fruit beverages. They were thoroughly cleaned initially with cold water and KMS followed by hot water.

Cultivation of wheatgrass

Cultivation of wheat seeds (*Triticum aestivum*) of WK1204 variety, obtained from Nepal Agricultural Research Council, Khumaltar, Kathmandu were carried out by soil cultivation method according to Wigmore (1985). Wheat seeds were washed, soaked for 12 hours, and allowed to sprout by wrapping them in a moist muslin cloth for another 12 hours. Finally, the sprouted wheat seeds were sown in trays filled with soil and then was covered. The trays were uncovered after a couple of days, held in indirect light and watered daily at definite intervals. The grasses were harvested on 9thday as optimized by the study carried out by Adhikari et al. (2022) and the Total Polyphenol, Flavonoids, Tannins, Chlorophyll content and DPPH radical scavenging activity in wheatgrass extract and juice were carried out.

Preparation of Wheatgrass Extract

The extracts were prepared according to Ahmad et al. (2014) with slight modifications, by using the maceration technique. Briefly, 10 grams of wheatgrass was properly crushed using Mortar and Pestle, steeped in 100 ml of 80% Methanol for 12 hours at room temperature at nearly 20-25°C and filtered using Whatman No. 41 filter paper. The collected filtrate was transferred to 250 ml brown (coloured) glass bottles, sealed and stored at 4 ± 2 °C. This methanolic extract was used for phytochemical screening and quantitative determination. Chlorophyll was extracted as per the method given by Ranganna (1986) using 80 % acetone.

Juice Extraction

The harvested wheatgrass was properly trimmed, cleaned and washed in running tap water. The juice from wheatgrass was extracted using stainless steel manual screw juice extractor and the yield of the juice was determined.

Formulation of Ready to serve Wheatgrass Juice

Design-Expert®, version 7 software was used to develop different formulations of Ready to Serve drinks keeping juice content 7- 13%; TSS 9 -16°Bx and Acidity 0.3%. Eight different formulations of RTS were prepared (Table 1), bottled in sterilized PET bottles and stored at 4 ± 2 °C. Response Surface Methodology, D-optimal design was adapted for the formulations.

Table 1

	Eastan 1	Esster 2
Formulations	Factor 1	Factor 2
	Juice content (%)	TSS (®Bx)
А	8	10
В	12	10
С	8	15
D	12	15
E	7	12.5
F	12.8	12.5
G	10	9
Н	10	16

Different Formulations of Wheatgrass Ready-to-Serve Drink

Preliminary Phytochemical Screening of the Extracts

The phytochemical content of plant extract was analyzed according to the standard procedure as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative Analysis of Phytochemicals

Determination of Total Phenol Content

Determination of total phenol content in wheatgrass was carried out with Folin-Ciocalteau reagent as mentioned by Waterhouse (2002) with slight modifications. Briefly, 0.5 ml of methanolic extract was taken and 2.5 ml of 10% Folin-Ciocalteau reagent and 2.5 ml of 7.5% sodium Carbonate were added. Then it was incubated at 45°C for 45 minutes and finally, absorbance was measured in triplicates at 765 nm using UV single-beam spectrophotometer. Tot al phenol values were calculated using the standard curve equation and expressed in terms of Gallic Acid Equivalent i.e. (mg GAE/g) of dry mass.

Determination of Flavonoid

Total Flavonoid content was determined using Aluminium Chloride assay method as described by Barek et al. (2015) with slight modifications. Briefly, to 2 ml of the extract solution, 0.2 ml of 5% Sodium Nitrate was added and stood for 5 minutes. Then 0.2 ml of 5% Aluminium Chloride was added and again stood for 5 minutes. After that 2 ml of 1N Sodium Hydroxide was added and final volume was made up to 5 ml with distilled water. It was then incubated for 15 minutes at room temperature at 20-25°C and finally absorbance was measured at 510nm against blank in UV single beam spectrophotometer. Flavonoid contents were calculated using the standard curve equation and expressed in terms of Quercetin Equivalent i.e. (mg QE/g) of dry mass.

Determination of Tannins

Tannin was determined by method as described by Mythili et al. (2014) with slight modifications. Briefly, to 0.1 ml of extract7.5 ml of distilled water, 0.5 ml of 10% Folin-Ciocalteau reagent and 1 ml of 35% Sodium Carbonate were added. Then the final volume was made up to 10 ml with distilled water and mixed well. It was held for 30 minutes at room temperature i.e., 20-25°C. Then the absorbance was measured at 725 nm using UV single beam spectrophotometer. Tannin contents were calculated using the standard curve equation and expressed in terms of Gallic Acid Equivalent i.e. (mg GAE/g) of dry mass.

Determination of DPPH Free Radical Scavenging Activity

Free radical scavenging activity was determined by the method as described by Vignoli et al. (2011) with slight modifications. Briefly, to 1 ml of the extract, 2 ml of 0.1mlmethanolic DPPH solution was added, and then was incubated in dark for 30 minutes. After 30 minutes,

the absorbance was measured at 517 nm against control (1 ml of 80% methanol+2 ml of 0.1mM methanolic DPPH solution) in UV single beam spectrophotometer.

Percentage scavenging activity was calculated as the following formula:

% DPPH radical scavenging activity =
$$\left(\frac{Ac - As}{Ac}\right)X$$
 100%

Where, Ac=Absorbance of control; As=Absorbance of sample

Determination of Chlorophyll

Chlorophyll was determined as per the standard method given by Ranganna (1986). *Moisture Content*

It was determined by the hot-air oven method as per AOAC (2005).

Crude Protein

It was determined by Micro-Kjeldahl method as described in AOAC (2005).

Crude fat

It was determined by Soxhlet extraction method as cited in AOAC (2005).

Crude Fiber

It was determined by the method as cited in AOAC (2005).

Ash

It was determined by method according to AOAC (2005).

Sensory Evaluation

Sensory evaluation was performed according to the 9 points hedonic rating method (Ranganna, 1986).15 panelists were trained regarding the major sensory attributes of beverage mainly ready to serve drink like color, body, taste, flavor and overall acceptability of the beverage. After sensory evaluation the average mean scores between the samples and between the panelists were calculated for further statistical calculations.

Statistical Analysis

GenStat Discovery Program version 12.1 was used for the statistical analysis. For phytochemical analysis one- way ANOVA (No blocking) and for sensory evaluation two-way ANOVA (No blocking) was done. The means were compared using LSD method at 5% level of significance.

Results and Discussion

Analysis of Extract of Wheatgrass Harvested on 9th Days Qualitative Analysis for Phytochemicals

The qualitative analysis for bioactive components in methanolic extract of 9th day harvested wheatgrass revealed the results as shown in Table 2.

Table 2

Phytochemical Screening of Methanolic Extracts of Wheatgrass

Phytochemicals	Result	
Polyphenols	+ve	
Flavonoids	+ve	
Tannins	+ve	
Alkaloids	+ve	
Saponins	-ve	
Glycosides	+ve	
Steroids	+ve	
Terpenoids	+ve	

Similar results for preliminary phytochemical analysis was found in the research carried out by Suryavanthana et al. (2016) where extraction was carried out in different solvents and compared. Hence, from the preliminary phytochemical analysis, it was observed that wheatgrass is the natural food consisting of a wide range of essential Phyto-nutrients and has extensive benefit on human health.

Quantitative Analysis of Phytochemicals

The quantitative analysis for bioactive components in methanolic extract of 9th day harvested wheatgrass revealed the results as shown in Table 3.

Proximate Analysis

The moisture content, protein, fat, ash, crude fiber and carbohydrate content of the freshly 9th days harvested wheatgrass were 87.39 ± 0.23 , 29.66 ± 0.40 , 12.98 ± 0.11 , 24.48 ± 0.27 , 4.56 ± 0.15 and 28.32 ± 0.44 (db)% respectively (Table 3). These results were similar with the results reported by Chomchanet al. (2016). Slight variations might have occurred due to variation in variety, growing conditions and many more factors.

Table 3

Bioactive Characteristic and Proximate Composition of 9th days Freshly Harvested Wheatgrass

Parameter	Amount
Chlorophyll	16.26±0.076 mg/g
Total Polyphenols	371.33±1.53 mg/g
Flavonoids	183.64±33.49 mg/g
Tannin	9.65±0.35 mg/g
DPPH scavenging activity	92.27±1.92 %
Moisture	87.39±0.23 % db

Parameter	Amount
Crude protein	29.66±0.40 % db
Crude Fat	12.98±0.11 % db
Crude fiber	24.48±0.27 % db
Ash	4.56±0.15 % db
Total Carbohydrates	28.32±0.44 % db

Note. Values are means of triplicate, figures in the parentheses are the standard deviations.

Yield of Juice

Wheatgrass harvested on 9th day was subjected to juice extraction using manual spiral juice extractor. From 310.23 gm fresh wheatgrass, 227.58 gm of juice and 82.64 gm residual solids were obtained. Hence, the yield of the extracted juice was found to be 73.35%

Proximate Analysis of Wheatgrass Juice Harvested on 9th day

The physiochemical analysis of wheat grass juice harvested on 9th day was carried out and the result is shown in Table 4.

Table 4

Physiochemical Composition of Wheatgrass Juice

Para	meter	Mean
Total	Soluble Solids	3.3±0.057
Titra	table Acidity	0.35±0.036
pH		6.38±0.04
Asco	orbic acid (mg/ 100g)	135.12±2.26
Total	l Sugars (%)	1.53±0.057
Redu	cing Sugars (%)	0.9 ± 0.08

Note. The values are means of triplicate, figures in the parentheses are the standard deviation.

The parameters were similar to the findings obtained by Hasani (2016) where wheatgrass was being compared to apple juice and sour cherry/apple juice; Chomchan et al. (2016) where the sugar composition of the wheatgrass and rice-grass was being compared. Similarly, vitamin C of the fresh wheatgrass juice was slightly greater than that obtained by Sharmaet al. (2016). Slight differences might have occurred due to the variation in variety taken, growing conditions and analytical procedures.

Sensory Evaluation

Eight different formulations of ready to serve wheatgrass juice were prepared (Table 1) with the variation of juice content and total soluble solids, keeping acidity constant. There was significant difference for sensory attributes like flavor, taste, body and overall acceptability at p<0.05, whereas color wasn't found to be significantly different.

Color

The mean sensory scores of the samples A, B, C, D, E, F, G and H were 7.6, 7.1, 7.2, 7.4, 7.1, 7.3, 7.2, 7.2 respectively (Fig.1). LSD showed that all the eight samples were not significantly different (p>0.05) from one another in terms of color. Chlorophyll is the major pigment responsible for the visible greenish color in wheatgrass grown under light. Throughout all the formulations, the significant difference in color was not visibly distinct.

Figure 1

Mean Sensory Score for Color



Note. The values in the figure are the mean sensory score for color. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores.

Body

The mean sensory scores of the samples A, B, C, D, E, F, G and H were 5.9, 5.8, 7.8, 7.6, 6.9, 6.6, 5.1, 8.1 respectively (Fig.2). LSD revealed that samples C, D and H were not significantly (p>0.05) different with each other whereas sample F, E and A, B were not significantly (p>0.05) different among themselves but were found significantly (p<0.05) different with samples C, D and H. Sample G was found to be significantly (p<0.05) different with all other samples. The solid content can influence foods' texture, sweetness and flavor

(Reboucas, 2016). Hence, samples C, D, H might have received greater mean score due to high brix.

Figure 2

Mean Sensory Score for Body



Note. The values in the figure are the mean sensory score for body. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores.

Taste

The mean sensory scores of the samples A, B, C, D, E, F, G and H were 6.2, 6.4, 5.3, 5.5, 6.5, 7.4, 6.1 and 5.5 respectively (Fig.3). LSD revealed that the samples A, B, E and G were not significantly (p>0.05) different with each other in terms of taste. Samples C, D and H were not significantly (p>0.05) different with each other but were significantly (p<0.05) different with other samples A, B, E and F. Sample G was significantly (p<0.05) different with samples A, B, C, E and F. Sample F was superior in terms of taste and was significantly (p<0.05) different with all other samples. The lowest score might be due to its predominantly sweet taste. Similar research findings were obtained by Ramachandran and Nagarajan (2014) in aloe gel and papaya beverage.

Figure 3

Mean Sensory Score for Taste



Note. The values in the figure are the mean sensory score for taste. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores.

Flavor

The mean sensory scores of the samples A, B, C, D, E, F, G and H were 6, 6.6, 4.5, 5.9, 4.4, 7.5, 6.8 and 5.2 respectively (Fig. 4). LSD revealed that samples E and C were not significantly (p>0.05) different with each other but were significantly (p<0.05) different with all other samples. Samples H and D were significantly (p<0.05) different with each other. Sample A was not significantly (p>0.05) different with sample B and D. Sample G was not significantly (p>0.05) different with sample B but was significantly different (p<0.05) with all other samples. Sample F was superior which might be due to its balanced effect of astringency, acidity and sweetness and was significantly (p<0.05) different among all the others. According to Satkar et al. (2013), the taste of bitter gourd RTS was greatly attributed to the appropriate sugar-acid blend in the product. The low scores might be due to the relatively higher astringency of wheatgrass as compared to the sweetness and acidity. Similar findings were also observed by Jain and Jain (2014) and Ganjyal et al. (2015) where muffins and cookies incorporated with wheatgrass received lower sensory scores as the proportion of wheatgrass went on increasing. Mixed reviews were obtained regarding the acidity of the product.

Figure 4

Mean Sensory Score for Flavor



Note. The values in the figure are the mean sensory score for flavor. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores.

Overall Acceptance

The mean sensory scores of the samples A, B, C, D, E, F, G and H were found to be 6.7, 6.7, 6.9, 6.9, 6.1, 7.8, 6 and 6.5 respectively (Fig.5). In overall acceptance, LSD revealed that samples A, B, C, D were not significantly (p>0.05) different with each other but were significantly (p<0.05) different with samples E, F, G and H. Similarly, samples G, E, H were also not significantly (p>0.05) different with each other. Sample F was superior in terms of overall acceptability and was significantly (p<0.05) different with all other samples. Dahiya et al. (2017) concluded that the score for sensory parameters increased as level of wheat grass decreased.

Figure 5

Mean Sensory Score for Overall Acceptance



Note. The values in the figure are the mean sensory score for overall acceptance. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores.

Analysis of Final Product

According to sensory score the selected proportion of optimized beverage was 12.8% of wheatgrass juice, TSS 12.5°Bx and acidity 0.3%. The final product was further analyzed for its physiochemical (Table 5) and phytochemical (Table 6) composition.

Table 5

Physicochemical Composition of Optimized Wheatgrass RTS

Parameter	Amount
Total Soluble Solids (°Bx)	12.5 ± 0.1
Titratable acidity (%)	0.32 ± 0.036
pH	3.48 ± 0.01
Ascorbic acid (mg/ 100g)	14.85 ± 0.01
Total Sugars (%)	13.01 ± 1.08
Reducing Sugars (%)	0.09 ± 0.01

Note. The values are means of triplicate, figures in the parentheses are the standard deviation.

Table 6

Phytochemical Composition of Optimized Wheatgrass RTS

Parameter Amount	
Chlorophyll	$0.0939 \pm 0.002 \text{ mg/g}$
Total Polyphenol	$30.18 \pm 3.08 \text{ mg/g}$
Flavonoids	$9.78 \pm 0.004 \text{ mg/g}$
Tannin	$0.6 \pm 0.004 \text{ mg/g}$
DPPH scavenging activity	13.33 ± 1.84 %

Note. The values are means of triplicate, figures in the parentheses are the standard deviation.

Conclusion

As per the optimization carried out in this research, A functional RTS having the formulations as 12.8% Wheatgrass juice, TSS of 12.5°Bx and an acidity of 0.3% was found to be superior in terms of sensory quality. Thus, prepared ready-to –serve beverage will also exhibit excellent health benefits.

Implication

This finding suggests that the formulation optimized for wheat grass r can be utilized for the preparation of ready to serve juice high in bioactive components with overall acceptable sensory attributes.

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