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Quality Evaluation and Consumer Acceptance Test of Functional Fruit-herb Beverages

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ABSTRACT

Background: Functional beverages are one of the kind that focuses solely on consumer interest in health and have distinctive formulations made up of different herbal ingredients along with fruits/vegetables blended together to meet the optimum outcomes. The purpose of the present paper is quality evaluation and organoleptic acceptability which is identified as a significant predictor.

Methods: The developed functional fruit-herb beverages produced from Wheatgrass, Pomelo fruit and Hibiscus flower in addition to one each from six treatments of different proportions were analyzed for consumer acceptance test along with chlorophyll, carotenoids, total Phenolic contents, flavonoids content and DPPH radical scavenging activity.

Result: The consumer's overall acceptability of the beverages one serving portion (200 ml) was significantly correlated to their taste and flavor and consumer preference on taste. Beverage (T2) was most preferred, but beverage T2 was favored by one group while beverage (T3) was chosen by the second group. This study examined the chemical characteristics of functional fruit-herb beverages and its conclusions imply that changing the proportions of the ingredients will change the optimal beverage blend's nutrient profile. Hibiscus juice in T5 and T2 at higher ratios greatly enhanced the formulation's antioxidant response. While the level of chlorophyll and carotenoids considerably rose in T6 and T3 as the ratio of wheatgrass juice increased.

Key words: Antioxidants, Bioactive compounds, Chlorophyll, Consumer acceptance, Functional beverages, Internal preference mapping.

INTRODUCTION

The main role of beverages and food in diet along with wellbeing are becoming increasingly more well-informed and also helps to inhibit the spread of oxidative stress-related decay diseases (Ogundele *et al*., 2016). Mostly, functional beverages are highly coveted for vegetables and fruit drinks which also have wide market recognition at the same time as for the milk drinks (Davoodi *et al*., 2013). The global market for functional foods certainly presents a big opportunity: sales are predicted to reach US$250 billion by 2020 (Darla *et al*., 2021). Most captivating ingredient for functional beverages is green juices containing cereal grass which are the young leaves of seedlings i.e. oat, rye, barley, alfalfa and wheatgrass are presently regarded as novel functional foods containing nutritious sources such as proteins, polyphenols, chlorophyll, minerals, vitamins etc. and are known to be super foods (Gruenwald, 2009). Wheatgrass (*Triticum aestivum*) the "green blood" is widely recognized for its high chlorophyll, 70% of which is a chemical part of it (Chauhan, 2014). Wheat grass, which can contain essential nutrients and vitamins, may be freshly juiced and dried into powder for use in individual's consumption. In the form of fresh juice, it plays a vital function in the anticancer progression, possessing high levels of chlorophyll, active enzymes such as superoxide dismutase, plant hormone abscisic acid or dormin (Salanta *et al*., 2016). Chlorophyll's molecular similarity to hemoglobin has been discovered to regenerate or supplement hemoglobin in cases of its deficiency. Wheat grass is used to treat thalassemia and hemolytic anaemia for this purpose (Padalia *et al*., 2010). Anti-allergic and anti-asthmatic therapy for wheat grass sage antioxidants such as vitamins C, E, zinc and β-carotene is the responsibility of bioflavonoids, whereas other health facilities, including inflammatory bowel disorders and as general detoxification (Chaudhary *et al*., 2021).

Hibiscus rosa-sinensis is commonly referred to as Hawaiian or Chinese hibiscus captivates anti-ovulteric, antitumor, spasma, anti-fertility, antipyretic, hypoglycemic, anti-inflammatory, antimicrobial and hypotensive function of *Hibiscus rosa-sinensis* and its chemical constituents apart from other therapeutic features are used throughout all its sections in curing diseases (Bahuguna and Vijayalaxmi, 2018). The whole plant can be used to prepare drinks such as hot beverages by using dried calyces or can be immersed in water for the preparation of amusing cold drinks (Mohamed *et al*., 2012). Whereas, Pomelo (*Citrus grandis*) a low-calorie underutilized fruit though, full of nutrients i.e.

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niacin, ascorbic acid, potassium, phosphate, calcium, starch, sugar, cholesterol, magnesium, potassium, riboflavin and thiamine are supplied. Pomelo juice helps minimize damage and lose weight from other diseases (Sarkar *et al*., 2015). Citrus fruit like pomelo is a plant with high antioxidant content Dietary phenols, polyphenols, glucosinolates, flavonoids, isoflavones and terpenes present have many advantages in terms of medicine and health by reducing the risk of a number of diseases related to age including cancer, diabetes, cardiovascular and autoimmune disorders (Marzuki *et al*., 2018).

Herbal beverages mixed with fruit or vegetable juices have grown in popularity across health-conscious consumers and are well thought-out to be an outstanding intermediate for the supplementation of nutraceutical components. They have infiltrated an emerging niche market alongside other trendy beverages and a progressive increasing segment of population are opting it for attaining balanced diet which may improve the antioxidant and enhance the overall health status. It is always added expedient to consume a beverage possessing health benefits rather than ingesting vitamins or pills for the same health perquisite. The goal of this research study was to estimate certain bioactive compounds linked towards the health benefits of the functional beverage developed from Wheatgrass, Hibiscus flower and Pomelo fruit juice and Consumer adoption of functional fruit-herb beverages were examined and internal preference mapping established the preferred orientation of this customer category.

MATERIALS AND METHODS

Chemicals

Analytical-grade chemicals and reagents were obtained from Sigma Aldrich: Dimethyl sulphoxide (DMSO), Folin-Ciocalteu phenol reagent, sodium carbonate (20%), gallic acid, methanol, aluminum chloride, potassium acetate, Quercetin and ethanol.

Wheatgrass production and collection of samples

The seeds had been cleaned and dried for 24 hours in water. Floating seeds have been discharged. Then, for 12-48 hours, soaked seeds were covered with wet cloth to allow them to germinate. Sprouts have been put in the damp soil and sealed for 3 days with damp cheesecloth. They were eventually watered twice a day. After seven days, the wheat grass was collected; the harvest was made with soil cuts of around one inch. Pomelo was collected from Azadpur mandi, New Delhi and Hibiscus calyx from Khari baoli, New Delhi.

Preparation of beverages

Six treatments of beverages were prepared by adding different proportions of all juices from Wheatgrass (*Triticum aestivum*), Hibiscus (*Hibiscus rosa-sinensis*) and Pomelo (*Citrus grandis*). Pomelos were gathered at their optimal age and were sorted, washed, peeled and juice was extracted from them using hydraulic press (Jhonsoton Automation Co.). The Hibiscus calyces were cleaned with cold water, crushed and extracted using calyces immersed in hot water at ratio of 50g: 1000L for 15 minutes and filteration of the extract was carried out using a sterilized cheese cloth. Cleaned wheatgrass was homogenized for 5 minutes in 30% water in Philips blender (H17705/00700). Functional beverages were then prepared according to the design combinations (Fig 1) and adjusted at 12°Brix and 4-4.5 pH followed by pasteurization and permeated in 200 ml sterilized glass bottles which were immersed in hot water treatment plant (Sanco®) at 100°C. Before all chemical analysis and consumer acceptance tests, the sample beverages were held in cold storage (6-8°C) for a month.

Chemical analysis

Chlorophyll and carotenoid content

The contents of chlorophyll and carotenoid in all sample beverages were estimated using Arnon's method with certain modifications (1949). One millilitre of beverage sample and 7 millilitres of dimethyl sulphoxide (DMSO) were mixed thoroughly in a test tube and were kept in water bath at 65°C for one hour to facilitate the extraction of chlorophyll into the solution (Hiscox and Israelstam, 1979). The absorbance of the solution was measured at 645, 470 and 663 nm using double beam UV-Visible spectrophotometer (Thermo Fisher Scientific). The total chlorophyll and carotenoid content of the beverage sample was recorded as µg/200 ml (per serving) and was calculated by this equation:

$$\text{Total chlorophyll} = (20.2\times OD_{645})+(8.02\times OD_{663})\ V\times 1/1000\times W$$

$$\text{Total carotenoid} = (1000\times OD_{470})-(3.29\times Ca)-(104\times Cb)/198$$

Where,

V: Final volume of chlorophyll extracted.

W: Fresh weight.

$$Ca= [12.7\ (A663)-2.69\ (A645)] \times V/1000 \times W$$

$$Cb= [22.9\ (A645)-4.68\ (A663)] \times V/1000 \times W$$

Determination of total phenolic content (TPC) using spectrophotometric method

Total phenolic content (TPC) was determined using spectrophotometric method and was based on a slightly modified Folin-Ciocalteu protocol (Singelton and Rossi, 1965). In 25 ml volumetric flasks holding 9 ml of water and 1 ml Folin-Ciocalteu reagent, one milliliter of the sample

Fig 1: Six treatment of beverages.

was pipetted. After 5 minutes, ten milliliters of solution Na_2CO_3 were added to the residual amount and deionized water was filled in. Phenolic compounds are diminished to blue molybdenum and tungsten oxides through oxidation with FC. After 90 mins, absorbance from the sample composed of 10 ml of deionized water rather than distilled, against a blank (quantified at λ= 765 nm) utilizing UV Spectrophotometer (Shimadzu, UV-1800). The results were compared to a standardized curve of formulated Gallic acid solution (25-200 µg/ml) and the results were reported as mg of Gallic acid equivalents (GAE) per ml of beverages (T1-T6). Triplicates of each measurement were taken.

Determination of total flavonoids content (TFC) using colorimetric aluminum chloride method

Total flavonoids content was based on the colorimetric aluminium chloride process (Pal *et al*., 2013). Briefly, 0.5 ml of sample was combined separately with 1.5 ml of methanol, 10% aluminum chloride, 0.1 ml 1 M potassium acetate and 2.8 ml filtered water were used, all of which were kept at room temperature for 30 minutes per beverage sample. The absorbance of the reaction mixture was measured at 415 nm utilizing UV Spectrophotometer (Shimadzu, UV-1800). Total flavonoids contents were calculated as Quercetin from a calibration curve. By preparing Quercetin solutions at concentrations of 25 to 200 µg/ml in methanol the calibration curve was made.

Sensory evaluation

Eighty subjects (20-26 years) of ICAR-Indian Agricultural Research Institute, New Delhi, India were selected as a consumer sample group. All the female panelists were instructed to evaluate the sensory evaluation of beverages and put their preference record on color, taste, flavor and overall acceptability via 9-point hedonic scale as depicted in Fig 2 (Peryam and Pilgrim, 1957).

Statistical analysis

Completely randomized design was tested for outcomes of the chemical analysis (3 replications) while randomized complete block design was used as sensory tests. The Tukey HSD (honestly significant difference) study has performed mean comparisons. For the correlation test bivariate regression has been applied. External preference mapping has been used to classify user preferences from overall acceptability data. R version 3.2.1 was used for all statistical analysis (R Core Team, 2015).

RESULTS AND DISCUSSION

Chemical analysis

Chlorophyll, Carotenoids, total Phenols and flavonoids content of six treatments of Functional fruit-herb beverages are shown in Table 1. The chlorophyll and carotenoids content ranged between 10.46-125.34 µg/200 ml and 3.65-30.07 µg/200 ml, respectively as depicted in Fig 3 and 4. Functional fruit-herb beverage T6 and T3 containing 95% and 55% of Wheatgrass juice enclosed significantly higher ($p<0.05$) chlorophyll 125.34±0.28 and 99.60±0.43 µg/200 ml and carotenoids content 30.07±0.30 and 24.50±0.43 µg/200 ml, respectively when compared to T2>T5>T4>T1, respectively of all Functional fruit-herb beverages. The high Chlorophyll content in wheatgrass is responsible for about 70 percent of its total chemical components (Kumar *et al*., 2016) Wheatgrass juice's high chlorophyll content, are responsible for its therapeutic, stimulating and regenerative effects and for inhibiting carcinogen metabolic activation (Ogutu *et al*., 2017).

Name:				Product:		
Panelist No.:				Date:		
Instructions: ❖ Taste the given sample beverages, then place a mark (⍰) on the point in the scale which best describes your feeling.						
SCORE*	SAMPLE CODE					
	T1	T2	T3	T4	T5	T6
(9) Like extremely						
(8) Like very much						
(7) Like moderately						
(6) Like slightly						
(5) Neither like nor dislike						
(4) Dislike slightly						
(3) Dislike moderately						
(2) Dislike very much						
(1) Dislike extremely						

Fig 2: A 9-point Hedonic scale used for sensory evaluation.

Table 1: Treatments of beverages using *Triticum aestivum*, *Hibiscus rosa-sinensis* and *Citrus grandis.*

Treatments	*Triticum aestivum*	*Hibiscus rosa-sinensis*	*Citrus grandis*
T1	15%	25%	55%
T2	25%	55%	15%
T3	55%	15%	25%
T4	0%	0%	95%
T5	0%	95%	0%
T6	95%	0%	0%

Total Phenolic content also varied considerably ($p<0.05$) with increase in Hibiscus and pomelo juice ratio and ranged between 50.65-301.60 mgGAE/ml. Beverage T5 (301.60±0.26 mg GAE/ml) and T2 (166.23±0.44 mgGAE/ml) comprised significantly highest ($p<0.05$) Phenolic compounds than other six treatments of functional fruit-herb beverages T1>T4>T3>T6, respectively. The total flavonoids content levels also varied significantly ($p<0.05$) and ranged between 15.80-69.78 mg QE/ml, Beverages having higher content of Hibiscus extract *i.e*. T5 (69.78±0.40 mgQE/ml) and T2 (65.50±0.13 mgQE/ml) were rich in flavonoids followed by T6>T1>T4>T3, respectively. Fruit juices contain a high content of a different group of polyphenols, which have a potent antioxidant capacity (Mitic *et al*., 2011). These findings are consistent with previous research that estimated the phytochemical and antioxidant content of different pomelo species fruit juice and demonstrated that natural products synthesized by plants are responsible for plant antioxidant capability and are associated with total antioxidant capacity (Abudayeh *et al*., 2019). Also, Hibiscus flowers contain a significant amount of antioxidant compounds and the flowers possess a natural food colorant and preservative, making them suitable for use in novel functional beverages or nutraceutical applications (Mak *et al*., 2013). Reported by Biswas *et al.,* 2019 the additional herbs improved the beverage's therapeutic and nutritional qualities while also improving customer acceptance. This beverage contains a variety of important vitamins and minerals. Therefore, just one beverage would be sufficient to refill the body's needs rather than drinking numerous goods.

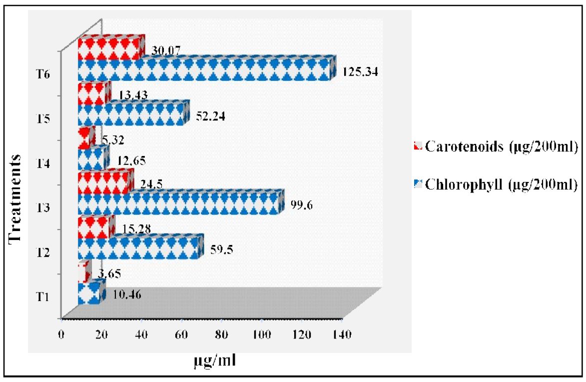

Fig 3: Chlorophyll and Carotenoids content of different treatments of Functional fruit-herb beverages.

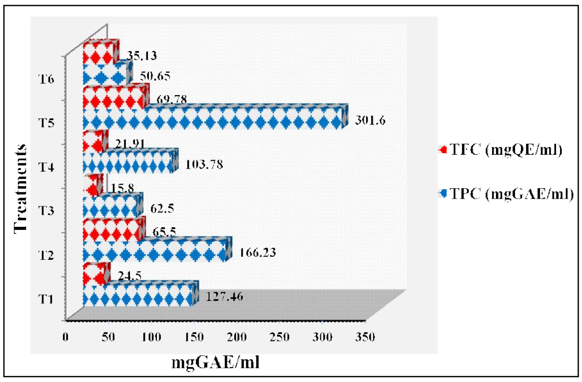

Fig 4: Total Phenolic (TPC) and total flavonoids content (TFC) of different treatment of Functional fruit-herb beverages.

Consumer acceptance evaluation

Table 2 shows the consumer acceptability test. The overall acceptability of the beverages was significantly associated with their taste, flavor and color, according to bivariate analysis (r = 0.813, 0.633 and 0.584, respectively, $p<0.05$). The flavor suggests a significant influence on the overall acceptability, as no additives in beverages were used. As depicted in Fig 5 the internal preference map shows most widely approved products were T2 and T3 out of all six Functional beverages ($p<0.05$), followed by T5, T1, T4 and T6 respectively based on their overall acceptability.

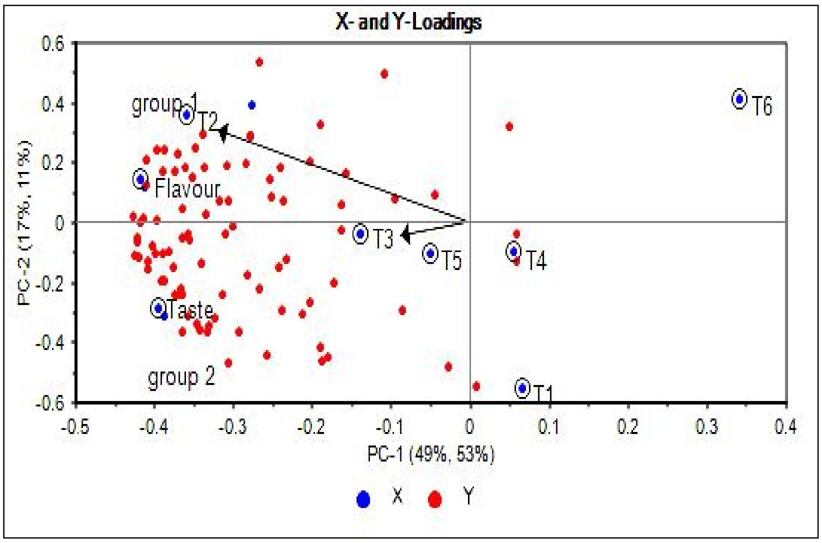

Fig 5: An internal preference map of 6 Functional fruit-herb beverages.

Internal preference mapping implies the design on the basis of acceptability data of a multidimensional product map. A main component analysis to produce preference dimensions extracted the most important source of variability in the preference data as the similar work is investigated in the study by Skapska *et al.,* 2020 The data used is a hedonic ranking for a certain consumer on a number of products. (Greenhoff and MacFie, 1999). For overall acceptability data of six Herbal functional beverages, three main components or choice dimensions have been developed and used to create an internal preference map as shown in the Fig 4. Position • of beverages expressed the difference in product adoption and role of customers • reflected the preference. The purpose of the cluster analysis is to delegate observers such that observations of variables or interest attributes within each group are identical and the groups themselves differ (Peter, 1997). The research analyzed the first two subgroups of 42 and 47 consumers near the suggested minimum values and identified two preferred paths. A minimum of 64 evaluators are recommended for the acceptance test (Wangcharoen *et al*., 2005) and 70 (Lyon *et al*., 1992). The flrst group (g1= 42) preferred beverage T2 than T3, T5, T1, T4 and T6, while the second group (g2 = 47) preferred beverage T3 than those of T2, T5, T4, T1 and T6 as revealed in Table 3. The difference in flavors and tastes in the beverages was apparent in two preferential directions of T2 and T3 which affected consumer acceptance of both the beverages. Functional beverage T5 was accepted by all consumers, while T1 and T4 was neither liked nor disliked but T6 beverage was disliked (Tables 3 and 4. These results explained the similarity of taste, flavour and color in T2, T3 and T5 and the non-similar with T1, T4 and T6. These results also comprehend the previous studies which reveal that the degree of change between consumers' sensory perception of reformulated products and their conventional counterpart seems to be regulated by information about nutritional properties of products and reformulation procedures (Reis *et al.,* 2017) and (Pereira *et al.,* 2019).

Table 2: Overall acceptability of different treatments of functional fruit-herb beverages (Mean±SD) by 2 groups of consumers.

Functional beverages	Group 1 (g1=42)	Group 2 (g2=47)
T1	5.26^{c}±1.23	4.82^{c}±1.52
T2	7.90^{a}±1.66	5.51^{b}±1.34
T3	6.21^{b}±1.00	7.14^{a}±1.59
T4	3.92^{d}±1.11	4.21^{d}±1.35
T5	5.35^{c}±1.52	5.31^{b}±1.33
T6	2.45^{e}±1.04	3.29^{e}±1.36

1= Dislike extremely, 9= Like extremely $^{a, b, c, d, e}$ Means with different superscripts in the same column are significantly different (p<0.05).

CONCLUSION

This paper showed the chemical properties of functional fruit-herb beverages and the findings suggest that altering the ingredient combination will alter the nutrient composition of the ideal beverage blend. Higher ratios of Hibiscus juice in T5 and T2 significantly improved the formulation's antioxidant response. Whereas, Chlorophyll and carotenoids content were significantly increased in T6 and T3 with rise in Wheatgrass juice ratio. Contrarily, fruit juices are blended

Table 3: Chlorophyll, Carotenoids, total Phenolic content (TPC) and total flavonoids content (TFC) of different treatments of Functional fruit-herb beverages.

Functional beverages	Chlorophyll (µg/200 ml)	Carotenoids (µg/200 ml)	TPC (mg GAE/ml)	TFC (mg QE/ml)
T1	10.46^{e}±0.03	3.65^{e}±0.03	127.46^{c}±0.14	24.50^{c}±0.35
T2	59.50^{c}±0.24	15.28^{b}±0.11	166.23^{b}±0.44	65.50^{a}±0.13
T3	99.60^{b}±0.43	24.50^{a}±0.43	62.50^{e}±0.28	15.80^{e}±0.29
T4	12.65^{e}±0.14	5.32^{d}±0.23	103.78^{d}±0.12	21.91^{d}±0.14
T5	52.24^{d}±0.36	13.43^{c}±0.22	301.60^{a}±0.26	69.78^{a}±0.40
T6	125.34^{a}±0.28	30.07^{a}±0.30	50.65^{e}±0.22	35.13^{b}±0.42

Values are means of triplicate determination. ^{a,b,c,d,e} means with different superscripts in the same column are signiûcantly different (p<0.05).

Table 4: Sensory evaluation of different Functional fruit-herb beverages (Mean ± SD) by consumer panel.

Functional beverages	Taste	Flavour	Colour	Overall acceptability
T1	7.6^{b}±2.38	6.4^{c}±2.40	5.9^{c}±2.42	7.8^{b}±2.33
T2	8.0^{a}±2.22	7.6^{a}±2.29	7.0^{a}±2.27	8.1^{a}±2.25
T3	8.0^{a}±2.43	7.0^{a}±2.21	6.5^{b}±2.33	8.0^{a}±2.39
T4	7.4^{c}±2.31	6.3^{d}±2.40	5.7^{d}±2.27	7.6^{c}±2.24
T5	7.7^{b}±2.29	6.8^{b}±2.33	6.0^{b}±2.30	7.9^{b}±2.26
T6	5.8^{d}±2.30	5.9^{e}±2.20	5.3^{e}±2.41	6.1^{d}±2.28

1=Dislike extremely, 9= Like extremely. $^{a,b,c,d, e}$ means with different superscripts in the same column are signiûcantly different (p<0.05).

to improve the acceptability and nutritional content of fruits that cannot be consumed raw, such as wheatgrass and pomelo, due to their bitter and sour taste and instead must be blended with other sweet extracts like hibiscus. Thus, blending of such seasonal juices is a more cost-effective way to fulfill everyday nutritional requirements. The preference mapping of beverages and the chemical analysis revealed the preference intake of functional fruit-herb beverages T2 and T3 out of all treatments. Functional Fruit-Herb beverages are potential sources of bioactive compounds that may aid in the reduction of disease risk and, as a result, in the management of Non-Communicable Diseases. However, while preclinical studies have revealed the bioactivities of some herbal beverages, further analytical and clinical research is needed to determine the bioactive compounds that reduce such effects and their mode of action in disease risk reduction and health promotion.

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Conflicts of Interest

No conflict of interest is declared by the authors.

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