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Optimization of a process for microgreen and fruit-based functional beverage

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Abstract: Microgreen based functional juice blends containing fenugreek (*Trigonella foenum-graecum*), kinnow mandarin (*Citrus reticulate*) and aloe vera (*Aloe brobadensis*) in different ratios were blended with sorbitol and stevia. The different ratios of juice blends were analyzed for total soluble solids, sedimentation, viscosity and titrable acidity. They were also screened for total phenolic content, total carotenoid content and antioxidant properties such as DPPH, reducing power and metal chelating activity. The formulation with highest TPC, TCC and antioxidant property was selected to optimize a microgreen based functional juice. The optimized microgreen blend formulation had 20 ml 100^{-1} ml microgreen juice, 40 ml 100^{-1} ml kinnow juice, 8.5 ml 100^{-1} ml sorbitol, 1.78 g 100^{-1} ml stevia and 29.72 ml 100^{-1} ml aloe vera juice. It had high protein, minerals (sodium and potassium) and vitamin (vitamin C) content as well as good source beta-carotene, phenols and antioxidants. Antioxidant helps in reducing diabetic complications by reducing the oxidative stress and because of their protective action against reactive oxygen species.

Key words: Microgreens, functional juice, antioxidant properties, diabetes.

INTRODUCTION

Microgreens, frequently called "vegetable confetti", are exotic and emerging products that have gained popularity in the culinary practices in recent years. They are young and tender cotyledonary leafy greens that are harvested at the first true leaf stage. They are usually harvested after 7-21 days of germination when they grow to 1-3 inches in height with fully developed cotyledonary leaves (Treadwell et al. 2010). These have vivid colors, texture and intense flavors and thus used as an edible garnish. The concentration of phytonutrients is higher than the mature leaves. Thus, microgreens can be categorized as "functional foods". However, they have a short life span of 1-2 days when stored at ambient conditions (Xiao et al. 2012, 2014). Due to its high nutritive value, health promoting and disease preventing properties it is considered as a "functional food". For the last few years, they have begun to appear in a farmer's market, upscale markets and restaurants as the demand for microgreen increases. Its applications in the culinary preparation are so far immense, but little attention has been given to improve its application as a food ingredient in value added, fortified or enriched packaged products. Therefore, it is becoming important to study their utility in various commercial preparations that could help improve their shelf life and delivery of their valuable nutrients to the consumers (Mir et al. 2017, Xiao et al. 2014).

Fenugreek (*Trigonella foenum graecum*), commonly known as Methi, is an annual crop belonging to the family Fabaceae and native to Northern India as a culinary spice due to its strong spicy flavor (Olaiya & Soetan 2014). Fenugreek contains an anti-diabetic compounds namely, 4 Hydroxyisoleucin and trigonelline which helps in reducing blood glucose level (Dutta et al. 2014). Wheat flour when enriched with 10% fenugreek seed flour showed an increase in protein content, fiber, and total iron and calcium content (Khorshidian et al. 2016). Aloe vera (perennial succulent herb) belongs to Liliaceae family and its botanical name is *Aloe brobadensis* (Subramanian 2004). Aloe vera is known for its medicinal properties and both its gel and rind are used in pharmaceutical preparations. Aloe vera particularly helps in lowering blood glucose level (Rajasekaran et al. 2005). Its bioactive compounds are used as an anti-diabetic, anti-ulcer, antiseptic, anti-bacterial, anti-inflammatory, anti-oxidant and as an anticancer agents. It is also effective in treating stomach ailments, gestational problems, wound healing, skin disease, radiation injury, constipation, burns, dysentery, diarrhea etc. Aloe vera juice is bitter in taste due to the presence of high amount of polyphenolic compounds mainly aloin and an aloe-emodin (Joseph & Raj 2010) and thus, could be blended with other juices to improve acceptability. Kinnow mandarin (*Citrus reticulate*) has an attractive bright color, appealing taste and flavor and is one of the most popular citrus fruit. It is one of the richest sources of vitamin C and minerals such as magnesium and calcium and is used in preparation of value-added products like juice, nectar, squash and RTS drinks. Bioactive compounds present in kinnow helps to reduce the oxidative stress and scavenge the free radicals. Compounds like ascorbic acid, total phenolics and antioxidants helps in preventing cancer and cardiovascular diseases (Ahmed et al. 2007, Balaji & Prasad 2014, Khalid et al. 2016). Thus, the current project is aimed to prepare a ready to drink beverage using fenugreek microgreen and fruit base to improve the acceptability of fenugreek microgreen in the diet.

MATERIALS AND METHODS

Cultivation of microgreens

The fenugreek seeds for cultivation of micro greens, kinnows and aloe vera for preparation of the microgreen based functional juice were purchased from the local market of Jalandhar, India. The cultivation of micro greens was done under the optimum condition in the growth chamber. Soil and the coco peat mixture were used and the seeds were evenly spread over the soil after 2 hours of soaking in water for the cultivation of micro greens. Indirect and low air circulation was needed; 18 to 26 ºC temperature and 40 to 50 % relative humidity were required for the growth of micro greens.

Preparation and blending of different juices

The microgreen and aloe vera juice was extracted with the help of the blender and kinnow juice were extracted with the help of juicer mixer. After preparation of juices, they were mixed in different proportions and then the preliminary trails were done to estimate the values for the optimization of RSM (response surface methodology).

Optimization by Response Surface Methodology (RSM)

The optimization of the product was performed using response surface methodology (RSM). The product variables included micro green juice (10-20 ml 100 ml^{-1}), kinnow juice (10-40 ml 100 ml^{-1}), sorbitol (5-10 ml 100 ml^{-1}), stevia (0-0.2 g 100 ml^{-1})

and aloe vera juice was added as to make up for 100 ml of total formulation. Thirty trials were carried out to optimize the microgreen based functional juice.

Moisture content

The moisture content was determined by AOAC (2005). Sample (5 g) was weighed accurately and taken on a petriplate. It was then heated at 110±2 ºC for 4 h in the hot air oven. The plates were removed. This was followed by cooling in a desiccator and weighed over one-hour interval until constant weight was attained. The moisture content was determined by the following equation:

$$\text{Moisture content (\%)} = W_2\text{-}W_1/W\times100$$

Where W_1 and W_2= weight of petriplates along with sample before and after drying, respectively, and W= weight of a sample.

Ash content

The ash content was determined by AOAC (2005). Sample (2 g) was taken in a silica dish and ignited over a low flame to char the organic matter. After completely charring, the dish was placed in the muffle furnace and heated at 550 ºC for 3-4 h, till grayish to off-white ash was obtained. The dish containing ash was cooled in a desiccator and weighted. Percentage ash was calculated as follows:

$$\text{Ash content (\%)} = \text{wt. of ash}/\ \text{wt. of sample}\times100$$

Protein content

The protein content was analyzed by the Kjeldahl method suggested by Ranganna (2014). Weighed sample (0.5 g) was digested along with concentrated sulfuric acid (20 mL) and 5 g digestion mixture (10 g potassium sulfate and 1 g copper sulfate) in a Kjeldahl digestion flask. The contents were cooled, diluted with small amount of distilled water and transferred into a 50 mL volumetric flask. The volume was made up to the mark with the addition of distilled water. A measured aliquot (5 mL) was taken in a distillation flask followed by 10 mL of 40% NaOH. Ammonia liberated was collected through a condenser in a flask containing 10 mL of 0.1 N HCl, prior to which methyl red indicator was added. The methyl red indicator solution containing liberated NH3 was titrated against 0.1 N NaOH. The amount of NaOH used to neutralize the indicator was recorded. One blank containing concentrated sulfuric acid and digestion mixture was also run along with the experimental samples.

By this method, the percentage of nitrogen present in the sample was calculated and then the crude protein content was calculated as follows:

$$\text{N (\%)} = ([A\text{-}B] \times 0.0014 \times \text{vol. of digest}) / (\text{Aliquot taken} \times S) \times 100$$

$$\text{Protein (\%)} = \text{N (\%)} \times 6.25$$

Where A = Sample titre (mL), B = Blank titre (mL), S = Weight of the sample taken.

Titratable acidity

Juice sample (5ml) was taken in the conical flask and a few drops of phenolphthalein indicator were added. The mixture was titrated against 0.1 N NaOH standard solutions till pink color was observed (endpoint) (Lugwisha 2014). Titrable acidity was estimated as below:

Titratable acidity (%) = (Titre volume × Normality of NaOH × Vol. make up × Equivalent weight of acid × 100)/ (Volume of sample taken × Volume of aliquot taken × 1000)

Mineral and ascorbic acid content

Sodium and potassium content was analyzed by flame photometery. The sample solution

(2.5 mL) was pipetted in 0.4 mL of NaOH (5 M) to maintain the pH between 4.0-4.5. Acetate buffer (0.7 mL, pH 4.5) was added along with 0.5 mL of Hydroquinone (25%). α1α1 Dipridyl 0.1% of 0.5 mL was added.Distilled water (0.35 mL) was used to make up volume up to 5 mL. The absorbance was taken against blank at 520 nm. Ascorbic acid content was measured by using dye, 2,6-dichlorophenol-indophenol titration method (Ranganna 2014).

TOTAL SOLUBLE SOLIDS (TSS)

Total soluble solid was measured by hand refractometer (Sinchaipanit and Kerr 2007). Clean the refractometer prism with distilled water and dry it with a soft tissue. The drop of microgreen based functional juice was placed on the prism of refractometer. The daylight plate was closed and in the scale where the boundary intercepts was read. The percentage scale of refractometer was shown in grams of sucrose or total soluble solids in 100 grams of aqueous solution and was equal to Brix number.

Estimation of antioxidant properties

The microgreen based functional juice formulations were analyzed for different antioxidant properties viz. DPPH (free radical scavenging activity), metal chelating activity and reducing power using spectrophotometer at different OD's.

DPPH (Free Radical Scavenging Activity)

Free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhdrazyl radical (Devatkal et al. 2012). 2 ml of freshly prepared DPPH solution (0.0023 mg DPPH in 1000 ml of ethanol) was added in different concentrations of 2 ml micro green based functional juice. Absorbance was measured at 517 nm after incubating the test tubes in the dark at room temperature for 30 minutes with the help of UV- Visible spectrophotometer. The scavenging activity of different concentrations of functional juice was calculated by using the formula

Scavenging activity (% inhibition) = [(A-B) / A] * 100

Whereas A is absorbance of DPPH

B is absorbance of DPPH and micro green based functional juice

Reducing Power Ability (RPA)

The reducing power of microgreen based functional juice was determined with slight modifications (Arora & Kaur 2013). The functional juice (0.5 ml) of different concentrations was mixed with 1 ml of methanol in test tubes. Phosphate buffer (2.5 ml) of pH 6.6 and 2.5 ml of 1% potassium ferricyanide was added tothe above mixture. The test tubes were incubated in a water bath for 20 minutes at 50 ºC. After cooling the test tubes at room temperature 10 % trichloroacetic (2.5 ml) was added and the mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (0.5 ml) was mixed with 0.5 ml of distilled water and 1 ml of (0.1 %) ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. The experiments were conducted in triplicates and the results were averaged.

Metal chelating activity

Chelating activity (Fe^{2+}) was measured by using 2, 2' - bipyridyl competition assay (Bhanger et al. 2008) with some modification. $FeSO_4$ solution (1mM; 0.25 ml), 0.25 ml of sample was mixed in the test tubes. 1ml of Tris-HCl buffer (pH 7.4), 1 ml 2, 2' – bipyridyl solution (0.1% in 0.2 M HCl) and 2.5 ml of ethanol was added to the above mixture. The final volume is made up 6 ml with distilled water. The absorbance was measured with the help of spectrophotometer at 522 nm. The chelating activity is measured by the formula.

Chelating activity (%) = [(A-B) / A] * 100

Whereas A is absorbance of control

B is absorbance of microgreen based functional juice

Total phenolic content

The total phenolic content for microgreen based functional juice was calculated from the standard curve of Gallic acid (Rekha et al. 2012). In a test tube, 0.5 ml of juice was mixed with 2.5 ml of distilled water. Then 0.5 ml of 1:1 Folin-Ciocalteu reagent was added and incubated for 3 minutes. After adding 2ml of 20% sodium carbonate the test tubes were kept in the boiling water for 1 minute. Then the tubes were cooled at the room temperature and the absorbance was taken at 650 nm in a spectrophotometer with a reagent blank. Different concentrations of Gallic acid (standard 0-1000 μg/ml) were used for plotting a standard curve. The concentration of total phenolic content is measured as μg Gallic acid equivalents (GAE)/ml of juice.

Carotenoid content

The carotenoid content of functional juice was estimated spectrophotometrically by separating funnel method (Sharma et al. 2009) with some modifications. The juice was filtered with the help of filter paper in the conical flask. Then up to 3 ml of juice was poured in separating funnel and mixed with 10 ml of 3% petroleum ether. The mixture in the separating funnel was kept undisturbed for 30 minutes and the petroleum ether containing pigment was collected in a glass beaker. Again by using 3% petroleum ether the extraction was repeated until no more color was extractable. By diluting with acetone with water pigment were transferred to petroleum ether phase. For standard curve 25 mg beta-carotene was dissolved in 2.5 ml of chloroform and mixed with 250 ml of petroleum ether. Further concentrations were made and the color was measured at 452 nm using 3% acetone with petroleum ether as blank. The standard curve of absorbance against concentration was plotted.

Calculation

Carotene μg per 100 g = (Concentration of carotene in solution from a standard curve (μg per ml) × final volume × dilution × 100) / wt. of the sample

Estimation of sedimentation

The sedimentation was determined by using a centrifuge (Abedi et al. 2014). The 50 ml centrifuge tubes were filled with the different concentration of juices and centrifugation was performed at 3000 g for 15 minutes. The sedimentation index was measured by the formula.

Sedimentation index (%) = (HS/HD)*100

HS = Height of the sedimentations
HD = Height of the drinks

Estimation of viscosity

Viscosity of microgreen based functional juice was measured by using Ostwald Viscometer (Altan & Maskan 2005). The liquid is added to the Viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir, based on time of flow through a volumetric capillary. The viscosity of different concentration of juices was determined by using the following

Equation $\eta_1 = (\rho_1 t_1) / (\rho_2 t_2) \times \eta_2$

Where, ρ_1 =density of unknown liquid, ρ_2 =density of the liquids (water), t_1 =time of the other liquids, t 2 =time of the known liquid, η2 =viscosity of known liquid.

Sensory evaluation

The microgreen based functional juice formulations were subjected to sensory evaluation using 10 point hedonic scale which comprises color and appearance, aroma, taste, mouth feel, consistency and overall acceptability. The score of the judges was then calculated into total sensory score. The sensory evaluation was performed by a panel of 10 semi-trained judges (7 students and 3 teachers, aged 23-35 years) from the lovely professional university, Punjab (India). The sensory evaluation was carried out at 4, 25 and 37 °C.

RESULTS AND DISCUSSION

Optimization of process for microgreen and fruit based beverage

The experimental setup for optimization and values of sensory and physico-chemical parameters for the experiment are as shown in the Table I and II, respectively.

Effects of juice blends on sensory properties

Table III shows the coefficient estimates for the sensory properties as a response. As evidenced from the Table, kinnow juice had a significant ($p < 0.01$) positive effect on appearance, aroma, taste, consistency, mouth feel and overall acceptability scores, while fenugreek juice shows negative effects($p < 0.01$) on these sensory parameters. As the concentration of fenugreek juice increases, appearance and overall acceptability of the juice blend decreases due to its dark green color. The brown color is due to the reaction of anthocyanins and tannins forming brown colored complexes and thus imparts dark color to the juice (Lawless et al. 2012) and a slight sour taste (Pimentel et al. 2015) decreasing the consumer acceptance. However, appearance and overall acceptability increases with increase in concentration of kinnow juice and it also gives good mouth feel and aroma. On the other hand, sorbitol also shows the positive effect on the taste, consistency, mouth feel and overall acceptability whereas stevia had a negative effect on the taste and mouth feel because of its bitter taste. Kinnow and fenugreek had an interactive significant effect ($p < 0.01$) on the consistency (Figure 1a) and mouthfeel (Figure 1b) of the juice blend.

Effect of juice blend on physicochemical properties

As evident from Table IV, fenugreek and kinnow juice showed interactive significant ($p < 0.01$) positive effect on the antioxidant properties. As the concentration of fenugreek juices in functional microgreen juice blend increases, the antioxidant properties are also increasing. Fenugreek juice had positive significant ($p < 0.01$) effect on reducing power (Figure 1c), metal chelation (Fig 1d), FRSA (Figure 1e) of juice blend (Table II). In case of kinnow juice, as it increases metal chelation, FRSA and reducing power also increases, while sorbitol and stevia had no significant effect on these properties of juice blend. The increase in antioxidant activity might be due to the presence of polyphenolic components present in fenugreek and kinnow juice (Nathiya et al. 2014). A significant correlation between polyphenolic content and its antioxidant activity was reported (Bahmani et al. 2016).The extracts of the plants can convert themselves in more stable products and terminate radical chain reaction by reacting with free radicals and they may also act as electron donors (Devatkal et al. 2012). This accounts for the increased antioxidant activity with the addition of fenugreek and kinnow juice.

Table I. **Sensory properties of microgreen based functional juice in response surface methodology.**

Runs	Fenugreek juice (%)	Kinnow juice (%)	Aloe vera juice (%)	Sorbitol (%)	Stevia (%)	Colour and appearance	Aroma	Taste	Mouth feel	Consistency	Overall acceptability
1	20	40	30	10	0	7.3	7.1	8	7.9	7.8	7.14
2	15	25	51.5	7.5	1	6.5	6.6	6.3	6.26	6.2	6.29
3	15	25	51.5	7.5	1	6.4	6.3	6.2	6.26	6.18	6.32
4	15	25	49.5	7.5	3	6.7	6.1	5.7	5.6	6.04	6.78
5	20	10	65	5	0	4	4.1	3.17	3.1	4.07	4.1
6	10	40	40	10	0	8.4	8.2	8.6	8.5	8.2	8.05
7	10	10	70	10	0	5.2	5	6.2	6.1	6.2	4.62
8	10	10	68	10	2	5.7	5.5	5.58	5.45	6.4	5.32
9	15	25	46.5	12.5	1	6.8	6.4	7.9	7.8	7.7	6.18
10	10	40	45	5	0	8	7.9	6.02	5.9	6.9	7.73
11	10	40	38	10	2	8.7	8.5	7.58	7.4	7.5	8.62
12	15	25	56.5	2.5	1	5.7	5.6	4	3.9	4.6	5.53
13	5	25	61.5	7.5	1	6.5	6	5.54	5.3	6.3	6.6
14	20	10	60	10	0	4.5	4.4	5.76	5.6	6.3	4.18
15	20	10	58	10	2	4.3	4.8	5.47	5.36	6.3	4.52
16	15	-5	71.5	7.5	1	3	3.4	3.26	5.15	4.1	3.32
17	10	10	75	5	0	5	5.4	3.56	3.4	4.17	4.73
18	15	25	51.5	7.5	1	6.1	6.3	6.18	6.04	6.11	6.38
19	10	10	73	5	2	5.5	5.1	3.58	3.4	4.5	5.14
20	15	25	53.5	7.5	-1	6.2	6.5	6.5	6.4	6.3	6.19
21	20	10	63	5	2	4.2	4	3.27	3.1	4.1	4.26
22	15	25	51.5	7.5	1	6.2	6.4	6.1	6.1	6.1	6.31
23	20	40	33	5	2	8.1	7.8	5.18	5.07	6	7.33
24	15	25	51.5	7.5	1	6	6.7	6.2	6.1	6.2	6.41
25	15	25	51.5	7.5	1	6.6	6.2	6.1	6.2	6.22	6.4
26	20	40	28	10	2	7.9	8	7.11	7	7	7.72
27	20	40	35	5	0	7	7.1	5.56	5.4	6.4	7.05
28	10	40	43	5	2	7.9	8.2	5.58	5.4	6.5	8.14
29	25	25	41.5	7.5	1	5.4	5.1	4.8	4.7	5.7	5.38
30	15	55	21.5	7.5	1	9	8.9	7.7	7.6	7.8	9.42

Fenugreek and kinnow juice had significant ($p < 0.01$) positive effect on TSS (Table IV). It might be due to high sugar content present in kinnow. TSS of the juice depends on the maturity of the fruits. So as kinnow moves towards the maturity, the TSS of the kinnow juice increases (Riaz et al. 2015). As the fruit is going towards maturity, starch is converted into sugars by the phenomenon of hydrolysis and it enhances the sweetness and flavor (Fawole et al. 2013). As the concentration of juice increases, the TSS of the juice blend also increases (Rebouças et al. 2016). The increase in TSS may also be due to the conversion of polysaccharides into oligosaccharides and monosaccharides (Wisal et al. 2013). The increase in TSS increases the overall acceptance of the product (Lawless et al. 2012). Sorbitol and stevia had no significant effect on TSS.

Table II. **Physiochemical attributes of microgreen based functional juice in response surface methodology.**

Runs	Fenugreek juice (%)	Kinnow juice (%)	Aloe vera juice (%)	Sorbitol (%)	Stevia (%)	Reducing power (%)	Metal chelation (% inhibition)	DPPH (% inhibition)	TSS (ºBrix)	Titrable acidity (%)	Viscosity (Pa sec)	Sedimentation (%)
1	20	40	30	10	0	50.15	60.27	90.17	18.5	2.99	81.3	36.86
2	15	25	51.5	7.5	1	23.26	34.24	63.26	9	1.72	60.2	20
3	15	25	51.5	7.5	1	21.45	34.21	61.45	8.5	1.37	60.12	20.12
4	15	25	49.5	7.5	3	23.75	34.39	63.75	8	1.64	60.36	20.28
5	20	10	65	5	0	30.25	40.54	60.26	9	0.64	30.54	17.44
6	10	40	40	10	0	27.68	36.32	57.66	13	1.85	85.67	26.42
7	10	10	70	10	0	24.26	34.46	54.25	6.4	0.34	43.31	14.94
8	10	10	68	10	2	19.15	29.17	49.14	4.5	0.38	43.62	13.4
9	15	25	46.5	12.5	1	22.26	31.74	62.26	12.5	1.33	70.54	19.78
10	10	40	45	5	0	27.95	37.58	57.93	15.5	1.92	60.36	25.25
11	10	40	38	10	2	23.36	33.45	53.34	14	1.79	85.5	23.85
12	15	25	56.5	2.5	1	20.46	31.12	60.46	11	1.22	23.61	20.58
13	5	25	61.5	7.5	1	10.75	24.78	40.78	14	1.58	40.55	20.28
14	20	10	60	10	0	22.25	32.51	52.24	9.5	0.78	51.94	18.84
15	20	10	58	10	2	33.26	43.97	63.24	7.5	0.73	52.69	16.66
16	15	5	71.5	7.5	1	26.59	37.02	56.54	4.5	0.84	30.7	15.27
17	10	10	75	5	0	22.23	32.21	52.76	5.5	0.31	20.82	13.64
18	15	25	51.5	7.5	1	32.79	44.63	72.79	10	2.24	60.15	22.11
19	10	10	73	5	2	16.43	26.47	46.43	6	0.45	20.83	15.48
20	15	25	53.5	7.5	-1	22.28	32.22	62.28	9.5	1.47	60.07	19.90
21	20	10	63	5	2	33.35	45.15	63.35	8.4	0.97	31.02	19.12
22	15	25	51.5	7.5	1	22.49	32.44	62.49	8	1.41	60.15	21.01
23	20	40	33	5	2	48.26	58.27	88.26	19.4	2.69	57.11	34.36
24	15	25	51.5	7.5	1	24.49	34.65	64.59	8.5	1.87	60.16	20.43
25	15	25	51.5	7.5	1	22.58	34.14	62.58	9	1.67	6.14	20.92
26	20	40	28	10	2	56.28	66.41	96.27	18.9	2.78	81.6	30.89
27	20	40	35	5	0	48.71	58.37	88.75	20.5	2.81	57.15	32.46
28	10	40	43	5	2	25.39	35.87	55.37	12.6	1.63	59.88	27.47
29	25	25	41.5	7.5	1	50.12	60.34	90.17	17	2.54	46.46	25.16
30	15	55	21.5	7.5	1	58.17	69.25	98.14	22.5	3.71	98.95	40.54

Kinnow juice and sorbitol also had a significant ($p < 0.01$) positive effect on the viscosity of juice blend. Viscosity increases with increase in concentration of kinnow and microgreen juice (Table IV). With increase in concentration of the juice, the viscosity increases. Viscosity is directly proportional to the amount of pulp or solids present in the juice. This accounts for the increase in viscosity with an increase in kinnow juice (Kar & Kaya 2014). It was also observed that kinnow and fenugreek juice shows interactive significant ($p < 0.01$) positive effect on the sedimentation (Figure 1g). As the concentration of kinnow and microgreen juice increases the sedimentation of the micogreen based functional juice increases. Sedimentation increases with the increase in pectin content of the fruit (Altan & Maskan 2005) as in case of kinnow.

As shown in Figure 1f, kinnow and microgreen juice had interactive significant ($p < 0.01$) positive effect on the titrable acidity. With an increase in the concentration of kinnow juice titrable acidity increases because it belongs to the citrus fruit family and they are acidic in nature (Al-Juhaimi & Ghafoor 2013) whereas microgreen juice show slight increase in titrable acidity. Acidity increases due to the conversion of pectic acid into pectinic acid, which decreases the pH of the juice blend (Wisal et al. 2013). The increase in acidity decreases the consumer acceptance of juices (Rodbotten et al. 2009).

Optimization of microgreen based blend

Based on the sensory scores of the juice blends, rheological properties and antioxidant properties, the optimum formulation for the development of the microgreen based functional juice was selected using RSM. The formulation containing 20 ml 100-1ml microgreen juice, 40 ml 100-1ml kinnow juice, 8.5 ml 100-1ml sorbitol, 1.78 g 100-1ml stevia and 29.72 ml 100-1ml aloe vera juice was taken as optimum formulation. The predicted and experimental values of the optimized product have been shown in Table V. The selected optimum formula had a total sensory score as for appearance and color, aroma, taste, consistency, mouth feel and overall acceptability 7.74, 7.3, 6.70, 6.75, 6.63 and 7.66 respectively; antioxidant properties as for reducing power, metal chelation and DPPH 53.56, 64.15 and 96.32 respectively and rheological properties as for TSS, titrable acidity, viscosity and sedimentation 18.14, 2.99, 76.91 and 32.07 respectively.

Table III. Coefficient estimates of sensory properties for microgreen based functional juice.

Factors	Appearance and colour	Aroma	Taste	Mouth feel	Consistency	Overall acceptability
Intercept	6.95	7.00	6.74	6.77	6.64	7.00
A	-0.7179*	-0.5504*	-0.3822*	-0.3376*	-0.3658*	-0.7785*
B	2.64*	2.59*	1.55*	1.41*	1.20*	2.69*
C	0.3137	0.2418	1.39*	1.31*	1.02*	0.1375
D	0.4143*	0.3148	-0.4428*	-0.5840*	-0.2069*	0.4297*
AB	0.3542	0.1875	-0.1417	-0.1333	-0.2917	-0.1146
AC	-0.1575	0.1225	-0.0105	-0.0280	0.1050	-0.0087
AD	0.0938	0.2062	0.1125	0.1462	-0.1125	-0.1369
BC	0.0437	-0.0437	-0.0700	-0.0700	-0.5338*	0.1269
BD	0.1406	0.2656	-0.3031	-0.3031*	-0.4469*	0.0359
CD	-0.0656	0.1969	-0.2782	-0.2704*	-0.1129	0.1221
A^2	-0.0702	-0.5187	-0.8837*	-1.30*	-0.0207	-0.2329
B^2	-0.3119	-0.2295	-0.7034*	-0.4019*	-0.2113	-0.2046
C^2	0.1126	-0.0337	-0.0508	-0.2220	0.0633*	-0.1803*
D^2	0.2418	0.1301	0.0260	-0.1705	0.0840	0.1474
Models	Significant	Significant	Significant	Significant	Significant	Significant

A Fenugreek juice, B Kinnow juice, C Sorbitol, D Stevia, AB Interactive term of fenugreek juice and kinnow juice, AC Interactive term of fenugreek juice and sorbitol, AD Interactive term of fenugreek juice and stevia, BC Interactive term of kinnow juice and sorbitol BD Interactive term of kinnow juice and stevia, CD Interactive term of sorbitol and stevia, A^2 Quadratic terms of fenugreek juice, B^2 Quadratic terms of kinnow juice, C^2 Quadratic terms of sorbitol D^2 Quadratic terms of stevia. The values within the column of each attribute denoted with * are significant at $p<0.01$

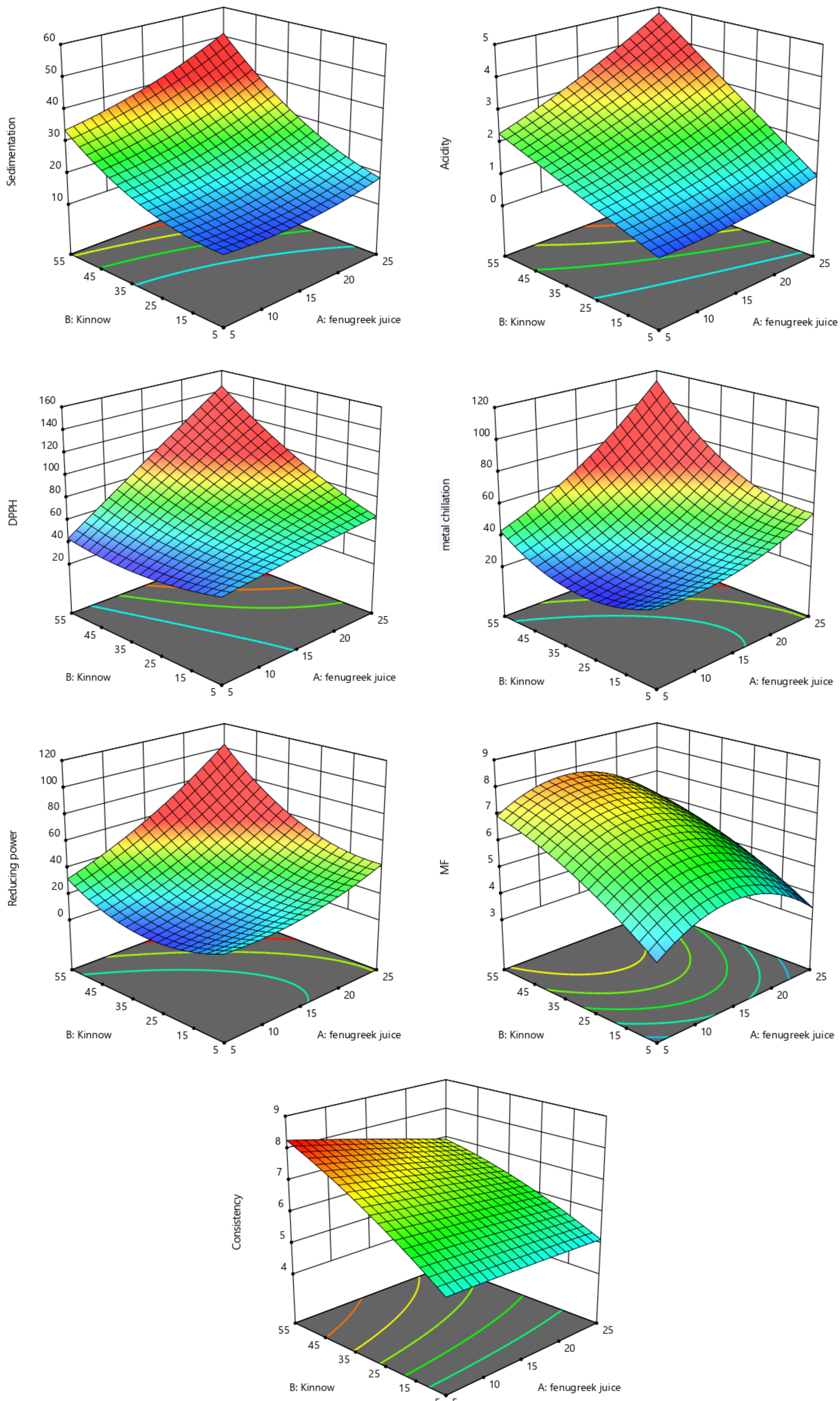

Figure 1. Response surface plots for (a) Consistency (b) Mouthfeel (c) Reducing power (d) Metal chelation (e) DPPH (f) Acidity (g) Sedimentation.

Table IV. Coefficient estimates of physiochemical properties for microgreen based functional juice.

Factors	Reducing power (%)	Metal chelation (%)	DPPH (% inhibition)	TSS (ºBrix)	Titrable acidity (%)	Sedimentation (%)	Viscosity (Pa sec)
Intercept	27.62	38.63	68.05	10.42	1.98	22.70	62.34
A	22.78*	22.44*	29.49*	3.71*	0.7518*	5.24*	0.6596
B	17.21*	16.82*	19.29*	8.92*	1.42*	12.83*	32.89*
C	0.7101	0.0007	-0.0092	0.6361	-0.1526	-1.26*	15.83*
D	0.4699	0.3328	-0.5020	-0.6365	-0.1742	-1.30*	3.69
AB	12.91*	12.55*	21.38*	2.13	0.5083*	3.54*	-10.80
AC	-0.1890	-0.0770	-0.1050	-0.2100	0.0210	0.5425	-0.7875
AD	7.05*	7.07*	7.13*	0.0000	0.0225	-0.8475	0.3412
BC	1.53	1.55	1.61	-0.3792	0.0729	0.0467	1.64
BD	-0.6875	-0.5594	-0.6125	0.2187	-0.1781	-0.6594	-0.3031
CD	1.76	1.62	1.83	0.2100	-0.0446	-2.61*	0.1601
A^2	6.87*	7.40*	-0.6679	6.38*	0.1495	2.14*	-7.11
B^2	15.97*	15.31*	7.75*	2.60*	-0.0001	4.83*	8.79
C^2	-1.08	-1.83	-2.34	1.29*	-0.3114*	-0.1978	-1.73
D^2	-0.3069	-1.05	-1.76	-0.2103	-0.2000	-0.2777	5.40
Models	Significant	Significant	Significant	Significant	Significant	Significant	Significant

A Fenugreek juice, B Kinnow juice, C Sorbitol, D Stevia, AB Interactive term of fenugreek juice and kinnow juice, AC Interactive term of fenugreek juice and sorbitol, AD Interactive term of fenugreek juice and stevia, BC Interactive term of kinnow juice and sorbitol BD Interactive term of kinnow juice and stevia, CD Interactive term of sorbitol and stevia, A^2 Quadratic terms of fenugreek juice, B^2 Quadratic terms of kinnow juice, C^2 Quadratic terms of sorbitol, D^2 Quadratic terms of stevia. The values within the column of each attribute denoted with * are significant at $p<0.01$

Table V. Optimization of micro green based blend.

Experiments	Predicted values	Experimental values
Color and Appearance (%)	7.74[a]	7.50±0.50[a]
Aroma (%)	7.73[a]	7.76±0.25[a]
Taste (%)	6.70[a]	6.83±0.28[a]
Consistency (%)	6.75[a]	6.83±0.28[a]
Mouth feel (%)	6.63[a]	6.66±0.28[a]
Over all acceptability (%)	7.66[a]	7.50±0.50[a]
TSS (º Brix)	18.14[a]	18.00±0.57[a]
Titrable acidity (%)	2.99[b]	3.46±0.05[a]
Metal chelation (%)	64.15[a]	62.25±0.54[b]
Reducing power (%)	53.56[a]	51.44±1.11[b]
Sedimentation (%)	32.07[a]	31.33±0.50[b]
DPPH (% inhibition)	96.32[a]	93.75±0.35[b]

The experimental values are presented as Mean ± Standard deviation. The values represented with different superscripts in row, differ significantly at $p < 0.05$.

Nutritional composition of microgreen based beverage

The nutritional composition of the optimized microgreen based beverage is shown in Table VI. The values of all the nutrients present in kinnnow and aloe vera juice were found to be in accordance with the values obtained by various other authors (Ahmed & Hussain 2013, Kour et al. 2014, Safdar et al. 2017, Singh et al. 2016, Touati et al. 2013). The moisture content of the microgreen blend was found to be less as compared to the individual values of fenugreek microgreen, kinnow and aloe vera. At the same time, the values of different properties such as reducing power, metal chelation activity and free radical scavenging activity were found to be higher as compared to the ones found for fenugreek microgreen, kinnow and aloe vera. The antioxidant properties of fenugreek microgreen based functional juice blended with kinnow and aloe vera were 97.75±0.35, 54.44±1.11 and 64.25±0.54 for DPPH, reducing power and metal chelating activity respectively. The antioxidant values of functional juice were highly contributed by the kinnow, aloe vera and microgreens. Antioxidant helps in reducing the diabetic complications by reducing the oxidative stress and because of their protective action against reactive oxygen species (Dixit et al. 2005). The nutritional values such as total phenols, beta-carotene, protein, minerals (potassium and sodium) and vitamin C were found to be higher than the individual components. The vitamin-C value of functional juice (59.49±0.26) was highly contributed by the microgreen and kinnow juice (45.49±0.21 and 66.34±0.44 respectively). According to the study, microgreens are rich in phytonutrients (such as β-carotene, α-tocopherol and ascorbic acid) and excellent source of vitamins and carotenoids (Xiao et al. 2015). The higher nutritional value of the optimized microgreen based functional juice might be due to the combination of the microgreens of the fenugreek, kinnow and aloe vera. The blending three juices enhance

Table VI. Nutritional values of fenugreek microgreens, kinnow, aloe vera and microgreen blend.

Nutrients	Fenugreek Microgreen	Kinnow	Aloe vera	Microgreen blend
Protein (%)	5.79±0.31^{b}	0.70±0.16^{d}	8.73±0.23^{a}	3.79±0.33^{c}
Moisture (%)	89.46±0.25^{c}	93.35±1.27^{b}	97.43±0.45^{a}	84.45±0.32^{d}
Ash (%)	4.85±0.19^{c}	4.59±0.36^{d}	16.71±0.41^{a}	7.81±0.33^{b}
Potassium (mg)	58.50±0.42^{a}	4.72±0.90^{c}	4.50±0.37^{d}	14.93±0.33^{b}
Sodium (mg)	72.49±0.15^{a}	1.59±0.37^{d}	3.24±0.72^{c}	16.09±0.43^{b}
Total phenols (GAE g^{-1})	25.55±0.39^{d}	82.63±0.67^{a}	29.32±0.48^{c}	46.61±0.32^{b}
Total carotenoids (μg100g^{-1})	42.47±0.18^{b}	75.03±0.03^{a}	1.39±0.31^{d}	38.82±0.25^{c}
Vitamin C (mg)	45.49±0.21^{b}	66.34±0.44^{a}	0.73±0.003^{d}	35.62±0.26^{c}
Reducing Power (%)	41.19±0.34^{c}	45.44±0.12^{b}	15.70±0.26^{d}	56.65±1.11^{a}
Metal chelation activity (% inhibition)	55.45±0.30^{b}	45.55±0.39^{c}	40.02±0.24^{d}	65.31±0.54^{a}
Free radical scavenging activity (% inhibition)	72.50±0.38^{a}	55.79±0.83^{c}	38.21±0.071^{d}	69.09±0.35^{b}

The values are presented as Mean ± Standard deviation. The values represented with different superscripts in row, differ significantly at $p < 0.05$.

nutraceutical properties and also consumer acceptance of the blend (Lawless et al. 2012).

CONCLUSIONS

Fenugreek microgreens are highly rich in bioactive compounds, minerals (sodium and potassium) and vitamin (vitamin C) content as well as good source beta-carotene, phenols and antioxidants as compared to mature leaves. Fenugreek contains an anti-diabetic compounds 4 Hydroxyisoleucin and trigonelline which helps in reducing blood glucose level Due to these properties, they can be used in the formulation of microgreen juice blend optimized by applying RSM. The juice blend with acceptable sensory and physicochemical properties could be beneficial for reducing the diabetic complications by reducing the oxidative stress. The optimum conditions for preparation of microgreen and fruit based beverage on sensory and physicochemical basis are 20 ml 100^{-1} ml microgreen juice, 40 ml 100^{-1} ml kinnow juice, 8.5 ml 100^{-1} ml sorbitol, 1.78 g 100^{-1} ml stevia and 29.72 ml 100^{-1} ml aloe vera juice.

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