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Product Optimization, Storage Quality and Sensory Acceptance of Low Calorie Beverage Developed from Bitter Gourd and Kiwifruit

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HIGHLIGHTS

- Low calorie Bitter gourd-Kiwifruit beverage has good antioxidant potential and sensory acceptability.
- Product optimization showed about 80% reduction in calorie value.
- Optimized beverage showed good storage stability at ambient conditions for a period of six months.

Abstract: Bitter gourd (*Momordica charantia* L.) fruit is good source of many nutraceutical compounds and possess antioxidant, anti-diabetic and hypoglycaemic activities. However, its utilization in the preparation of beverages is limited due to its bitter after taste. Therefore, to realize the functional and therapeutic benefits of bitter gourd, an attempt was made to optimize nutritious and low caloriebitter gourd based beverage by blending with kiwifruit (*Actinidia deliciosa*), a store house of bioactive compounds and substituting sugar with stevioside (steviol glycoside). The standard (sugar sweetened) bitter gourd (BG)-kiwifruit (K) blended beverage was developed by utilizing 30% fruit part of BG:K blended juice (80: 20) with 40ºB TSS and 1.3% acidity. Further, to develop the low calorie beverage, sucrose (table sugar) was replaced with 25, 50, 75 and 100% equi-sweetness level of stevioside (steviol glycoside). Results revealed that 75% substitution of sucrose with stevioside resulted in shelf stable beverage with identical taste, good antioxidant potential (68.80%) and strong antimicrobial activity (26 mm ZOI) with reduced calorie values (28.5 Kcal/100g) compared to the sugar sweetened control sample (150.60 Kcal/100g). Hence, the developed beverage can

be commercialized as low calorie beverage with additional health benefits of natural compounds of bitter gourd and kiwifruit with highest bioactivity.

Keywords: Bitter gourd; kiwifruit; low calorie beverages; stevia; stevioside.

INTRODUCTION

The changing human life style characterized by reduced levels of physical activities and increased consumption of high-calorie food products is becoming a health hazard [1]. Global health concerns have resulted in the health-conscious community to think about safe alternates to satisfy their desire for sugar rich foods with identical pleasant sensory characteristics and low percentage of sugar and calories [2]. The beverage market is continuously expanding and is expected to grow even faster in the coming decade. Fruit based beverages have drawn special attention in this category due to their enriched phytochemical profile. They have a thirst-quenching nature with additional advantages of disease prevention and play an important role in improvement of physical and mental well-being [3, 4]. Bitter gourd (*Momordica charantia*L.) is a good source of vitamin C, phosphorus and iron with low volume of naturalsugars [5]. It has been reported to be anti-diabetic, stimulant, stomachic, laxative, blood purifier and a source of many nutraceutical compounds with significant antioxidant and hypoglycemic activity [6, 7]. The nutraceutical value of bitter gourd has been associated to its high antioxidant properties due to the presence of terpenes, flavonoids, phenols, glucosinolates and anthroquinones, all of which confer a bitter after taste [6]. According to Indian medicinal system and *Ayurveda*, bitter gourd controls jaundice, fever, cure liver diseases and blood impurities however, due to the poor sensory profile of bitter gourd juice, there are limited opportunities to develop beverages or other related processed products. Hence, to address this problem blending bitter gourd juice with the juice/ pulp of suitable fruits is an effective alternative to develop nutritious and palatable beverage. Blending has become one of the most convenient alternatives and economical tools for the production of nutritious, palatable beverages [8,9]. Kiwifruit (*Actinidia deliciosa*) which is known for its high nutritive and medicinal values and contains exceptionally high amounts of vitamin C with an array of nutrients and biologically active compounds like chlorophyll, carotenoids, dietary fibre, potassium, vitamin E and B complex (B_1, B_2, B_3, B_6 and vitamin B_9) [10]. It also contains appreciable amounts of antioxidants, phytonutrients, enzymes and phenolics (syringic, chrysin and quercetin), which are known for lowering the risk of chronic health diseases like cardiovascular diseases, diabetesand cancer [11]. So, the idea of blending bitter gourd juice with kiwifruit pulp was conceptualized to cover up the bitter taste of bitter gourd with the tangy, sweet and sour taste of kiwifruit besides imparting unique flavor and functional enrichment to the resultant beverage.

Excess intake of sugar sweetened beverages (SSB) has been attributed to an increased risk of health problems like obesity, diabetes and cardiovascular diseases [1,2]. Hence, replacement of sugar with non-nutritive sweeteners has been suggested as a suitable alternative for developing thelow calorie beverages (LCB). Stevia is aperennial herbal from Asteraceae family and is also referred as '*madhupatra',* meaning sweet leaf in Sanskrit [12]. The leaf contains a mixture of sweet diterpene glycosides mainly stevioside and rebaudioside, which offer many therapeutic benefits including antihypertensive, antidiabetic, antiinflammatory, antitumor, antioxidant, antidiarrhoeal etc. [13]. Stevioside is 150 to 300 times sweeter than sucrose, stable at high temperature (<140ºC) and wide range of pH (2-10) which has favoured its usage in various food products [14,15]. Therefore, in the present study, an attempt was made to formulate bitter gourd based antioxidant rich, low calorie functional beverage by blending with kiwi fruit and substituting sugar sweetness with stevioside sweetness and to evaluate its physicochemical, nutritional and sensorialproperties during storage period up to 6 months.

MATERIAL AND METHODS

Procurement of raw materials

Fresh bitter gourd (*Momordica charantia L.* cv. Solan Hara) fruits harvested at optimum horticulture maturity were procured from the local vegetable grower and brought immediately to the laboratory for further studies. Whereas, kiwifruits (*Actinidia deliciosa L.*) cv. Bruno were procured from the Kiwi Orchard of YS Parmar University of Horticulture and Forestry, Solan, India. Stevioside (steviol glycoside) powder was purchased from Institute of Himalayan Bioresource Technology, Palampur, India. The chemicals used in the study were of analytical reagent grade (AR) and were procured from Qualigens Fine Chemicals, Mumbai, India.

Preparation of low calorie squash

Initially, the most suitable blend of bitter gourd and kiwi juice worked out by Sharma and co-workers [16] consisting of bitter gourd juice (80%), kiwi pulp (20%) with 30 % fruit part, 40ºB TSS and 1.3 % acidity was used to prepare control sample of bitter gourd (BG)-kiwi (K) blended beverage.To further improve the functionality of the developed beverage by reducing its sugar load, sucrose (table sugar) was replaced with equi-sweetness levels of stevioside. The sweetness potential of the stevioside powder used in the present study was 270 timeshigher compared with the table sugar. It means the sweetness imparted by 270gm sugar was replaced by 1 gm of stevioside powder. Various treatments for the replacement of sucrose constitutedT_1 (Sucrose 100%), T_2(Sucrose 75%+ Stevioside 25%), T_3 (50% Sucrose + 50% stevioside), T_4 (25% Sucrose + 75% stevioside), T_5 (15% Sucrose + 85% stevioside), T_6 (100% stevioside). The beverages were prepared as per the methodology detailed by Sharma and co-authors [9] and are shown in Figure 1.

Figure 1. Freshly prepared bitter gourd and kiwi juice based low calorie beverages by replacing the sweetness of sucrose with equi-sweetness level of stevioside

The prepared beverages were filled into pre-sterilized PET bottles (250 mL capacity) after adding suitable and recommended preservative (KMS @350ppm) and kept under ambient storage (20±2ºC & 66±5% RH) for periodic analysis to track important physicochemical and sensorial changes in the product.

Physico-chemical and nutritional attributes

The developed beverages were evaluated for potential changes in various physico-chemical and nutritional changes for 6 months of storage under ambient conditions. Total soluble solids TSS was determined by using Abbe's hand refractometer of 0–32% range at room temperature and expressed as degree Brix (ºB). The values obtained were corrected for temperature variation to 20ºC. The titratable acidity was analyzed by titrating known volume of sample against standard 0.1 N NaOH using phenolphthalein as an indicator following the analytical method described by Ranganna [17].Ascorbic acid was determined by 2,6-dichlorophenol-indophenol dye method [18], in which aliquot prepared in 3 per cent meta-phosphoric acid was titrated with dye to pink colour end point.

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value} \times \text{*Dye factor} \times \text{Volume made}}{\text{Aliquot taken} \times \text{Weight/Volume of sample}} \times 100$$

*Dye factor = 0.5/Titre value

Sugars were estimated according to Lane and Eynon method [17]. A known volume of sample was neutralized with NaOH and then kept as such for ten minutes after 2 mL of lead acetate. Excess of lead acetate was removed by adding 2 mL of potassium oxalate. After diluting it, filtrate was taken to estimate reducing sugars by titrating against a known quantity of Fehling's solutions using methylene blue as an indicator.

$$\text{Reducing sugars \%} = \frac{\text{*Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Volume of sample}} \times 100$$

*Factor = 0.05

Total sugar contents were also estimated by Lane and Eynon's volumetric method by titrating the prepared sample, after hydrolysis with citric acid, against the known quantity of Fehling's solution using methylene blue as an indicator. The end point was attained when a brick red precipitate appeared in the solution [17]. Total phenols were determined by extracting with 80 % ethanol, color development with Folin-Ciocalteau reagent and reading optical density at 675 nm [17].

Antimicrobial activity

The antimicrobial activity of the developed products against *E. coli* was measured by bell diffusion method [19]. The inoculum was spread with the help of swab uniformly on the plate and a standard cork borer of 7 mm diameter was used to cut uniform wells on the surface of solid medium. In each well, 100 µl of sample was loaded and the plates were then incubated at 37 ºC for 24 h. The antimicrobial activity was expressed in terms of mean diameter (mm) of the zones of inhibition measured.

Antioxidant activity

The antioxidant activity (Free radical scavenging activity) was measured following Brand –Williams method [20] using DPPH (2, 2 diphenyl-1-picrylhydrazyl) as a source of free radical. A quantity of 3.9 ml of 6×10^{-5} mol/L DPPH in methanol was put into cuvette with 0.1 ml of sample extract and decrease in absorbance was measured at 515 nm for 30 min. Methanol was used as blank and the % antioxidant activity was calculated using following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Energy value

Energy value was measured in bomb calorimeter (Model Toshiwal DT-100), which is based on the principle that the amount of heat produced by burning the sample must be equal to the amount of heat absorbed by calorimeter assembly. One gram of the dried sample was taken for estimation of energy value and expressed as Kcal/100g [18].

Stevioside (steviol glycoside)

The HPLC analysis of the samples and standard stevioside was performed with high performance liquid chromatograph (Waters) as per standard method [18]. The sample was extracted with methanol (sonicated in methanol for 10 min in triplicate at 50ºC) and the methanol fraction was collected, filtered and then evaporated by drying on rotavapour and then lyophilized. Known amount of sample was weighed and dissolved in known amount of mobile phase. The separation was achieved by a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm) and UV detector set at 210 nm. The mobile phase consisted of a mixture 32:68 (v/v) of acetonitrile and sodium-phosphate buffer (10 mmol/L, pH 2.6), set to a flow rate of 1.0 mL/min.

Sensory evaluation

Consumer preferences for the experimental samples were found out through sensory evaluation performed at monthly intervals. At least 15 panelists consisting of trained and semi trained staff members were given coded samples for three successive sessions consisting of different treatment combinations for giving their views on overall acceptability on the basis of appearance, body, flavour, bitterness acceptability and overall acceptability. The analysis was done in sensory laboratory at room temperature (20±2ºC). The evaluation was done by using the 9-point hedonic scale [21]. The evaluators allotted the scores to each sample from 9 (like extremely) to 1 (dislike extremely). Water was provided to rinse the palate before and after tasting the sample.

Statistical analysis

Results are presented as means of at least three independent determinations in form of data of two consecutive experiments. Statistical evaluation was performed by using two-way analysis of variance (ANOVA) of the IBM SPSS Statistics program (Somers, NY, USA) with a significance level $\alpha = 0.05$ and Tukey's test ($P < 0.05$) was used for mean separation during storage and shelf life periods under ambient holdings after periodic storage durations.

RESULTS AND DISCUSSION

Sensory quality of stevioside sweetened low calorie bitter gourd-kiwi beverages

The variables optimized in the first part of the study conducted by the same group i.e. Sharma and co-workers [16], consisting of 80 % bitter gourd juice blended with 20 % kiwi fruit pulp accounting for 30 %fruit part with 40ºB TSSand 1.3 % acidity were used for producing low calorie beverages by substituting sucrose (table sugar), the sweetness of which was considered unity (1) with equi-sweetness of stevioside (270 times sweeter than sucrose). The replacement of sucrose with various stevioside levels significantly affected the sensory attributes (color, body, flavor, bitterness and overall acceptability) of the developed beverage (Table 1). The mean score of colour ($n=5$; $\alpha = 0.05$; $p< 0.05$) for different treatments varied from 7.46 to 7.89 with mean maximum score obtained for the beverage with 75 %replacement of the stevioside with sucrose (7.89). Similarly, highest body score (8.32) was recorded for the beverage with 75 %sucrose replacement. Results revealed non-significant differences amongst the treatment T_1, T_2 and T_3 while, they differ significantly ($p<0.05$) with the treatment T_4. At higher level of sucrose substitution (beyond 75%), the beverage reported a watery appearance which in turn had a negative effect on the color and body scores of the developed beverage. The flavour scores of the blend varied from 7.80 to 8.30 indicating that the developed beverage still had acceptable flavor in all the combinations. The typical aroma and bitterness imparted by stevioside at higher concentrations usually decreases the flavour and acceptability of the foods [22]. The present findings suggest the same results of adverse effects of stevioside above 75 % substitution of the sucrose. The bitterness (particularly the bitter after-taste) of stevioside at higher concentration (>60%) has also been reported earlier by Thandani and Subash [23]. Following the same pattern of the sensory attributes, the bitterness acceptability scores obtained for the blended squash was thehighest at 75% sucrose substitution level, beyond which it started to decrease. Further, as expected the peak score for overall acceptability (8.22) was recorded for the same formulation. Furthermore, it can be concluded that the replacement of sucrose up to 75 % level of sweetness with stevioside can be suggested for designing novel low calorie beverages without any significant effect on the quality and acceptability of the beverages.

Effect of storage period on sensory, physico-chemical quality and energy value

Sensory quality

The developed low calorie beverages were studied for storage stability at ambient temperature (20±2ºC) for 0, 3 and 6 months to trail vital biochemical and sensorial changes and thus define the keeping quality of beverage over the time. The mean organoleptic sensory scores obtained revealed that the colour, flavour, bitterness and overall acceptability scores reported a non-significant linear decline during 6 months of storage (Table1). During storage, the food products which are complex biomatrix may undergo co-polymerization, interaction between various coloring compounds like phenolics and degradation of colloidal particles and protein with concurrent generation of cation complexes with pectins. All these reactions ultimately results in the loss to the coloring matter [24]. The flavor values were usually highest for the beverage containing 100 % sucrose (7.75). Though, during storage, the flavour changes were non-significant and did not affect the quality of the beverage yet the small changes might have occurred due to the loss of volatile aromatic substances from the beverage [25]. The bitterness of the beverage on the other hand decreased slightly but non-significantly with progression in storage period. The losses of colour and flavour during storage were correlated to the declining trend of the overall acceptability of the product, while such reports are well reported in the literature [14,26]. Overall, the periodic sensory evaluation revealed that these non significant changes could not have any adverse effect on the acceptability scores which were still above 7.00 even after six months of storage under ambient conditions. Similar findings were also established by Balaswamy and coauthors [15] and Palamthodi and coauthors [27] in stevia sweetened RTS beverage and bottle gourd-jamun blended beverage, respectively.

Physico-chemical quality

On the preparation day (0 day), total soluble solids (TSS) among different beverages varied significantly from 9.10 to 45.00, while the data on changes in TSS content revealed a non-significant increase during storage (Table 2) which could be due to hydrolysis of complex carbohydrates (polysaccharides) into simpler carbohydrate (monosaccharide and oligosaccharides) [15,16]. A slight reduction in titratable acidity was reported over the storage which might be due to loss of volatile acid and co-polymerization of organic acids with sugars and amino acids during storage [24,25]. Reducing sugars and total sugars among different treatments ranged between 3.15 to 12.45 % and 5.23 to 37.54 %, respectively with themaximum value reported for the 100 % sucrose sweetened beverage, whilethe lowest was reported in the beverage which contained 100 % stevioside. Possibly, the replacement of the sugar with stevioside which is free from carbohydrate did not contribute to the reducing sugar content [26,28]. However, the reducing sugars increased during storage of 6 months possibly due to the initiation of the inversion reactions under acidic conditions. Whereas, there was a marginal decrease in total sugars content of the product which might have resulted from the hydrolysis of insoluble polysaccharides into simpler forms at higher temperature. Our findings are alsoin line with the results obtained by Sharma and coauthors [9]. The quantity of total sugars remained directly proportional to the replacement of sucrose with equi-sweetness level of stevioside which is a non-sugar compound [25]. Among different treatments, statistically non-significant variation in phenolic content and antioxidant activity was noticed which could be attributed to same proportion of fruit part in all the treatments. Whereas, a significant decrease in the total phenolics and antioxidant activity was reported in all the treatments during storage which was attributed to the formation of complex polymeric compounds due to complexing reactions of protein and their subsequent precipitations leading to decrease in their volume [29].

Table 1. Effect of different proportions of stevioside and storage period on sensory attributes of low calorie bitter gourd: kiwi squash under ambient conditions.

Parameters	Storage period (Month)	T_1 (100 % Sucrose)	T_2 (75% Sucrose + 25% stevioside)	T_3 (50% Sucrose + 50% stevioside)	T_4 (25% Sucrose + 75% stevioside)	T_5 (15% Sucrose + 85% stevioside)	T_6 (100% stevioside)
Color	0	7.46^{aA}	7.56^{aA}	7.62^{aA}	7.89^{aC}	7.72^{aB}	7.70^{aB}
	3	7.32^{Aa}	7.43^{Aa}	7.51^{aA}	7.67^{bB}	7.60^{aB}	7.43^{bA}
	6	7.26^{bA}	7.32^{bA}	7.41^{bA}	7.53^{cB}	7.36^{bA}	7.23^{cA}
Body	0	8.00^{aA}	8.10^{aA}	8.17^{aA}	8.32^{aC}	7.90^{aB}	7.90^{aB}
	3	7.78^{aA}	8.04^{aB}	8.12^{aB}	8.24^{aC}	7.67^{aA}	7.65^{bA}
	6	7.40^{bA}	7.90^{aB}	7.90^{bB}	8.12^{bC}	7.50^{bA}	7.55^{cA}
Flavor	0	7.80^{aA}	7.93^{aA}	8.15^{aB}	8.30^{aC}	8.12^{aB}	7.96^{aA}
	3	7.71^{Aa}	7.87^{bB}	8.11^{aC}	8.20^{aC}	7.90^{bB}	7.81^{aB}
	6	7.50^{bA}	7.62^{cA}	8.00^{aB}	8.12^{aB}	7.67^{cA}	7.56^{bA}
Bitterness acceptability	0	7.90^{aA}	8.00^{bA}	8.20^{cB}	8.35^{bC}	8.10^{bA}	7.95^{aC}
	3	8.00^{bA}	8.05^{bA}	8.32^{bB}	8.39^{bB}	8.12^{bA}	8.05^{bA}
	6	8.12^{aA}	8.20^{aB}	8.40^{aC}	8.50^{aC}	8.24^{aB}	8.15^{aA}
Overall acceptability	0	7.78^{aA}	7.90^{aA}	8.07^{aB}	8.22^{aB}	7.96^{aA}	7.88^{aA}
	3	7.70^{aA}	7.87^{aA}	8.04^{aB}	8.13^{aB}	7.82^{aA}	7.74^{aA}
	6	7.56^{bA}	7.76^{bB}	7.94^{aC}	8.07^{aC}	7.69^{bB}	7.62^{aA}

Data presented in Table is the average mean data of three independent observations
Measurements of the stevioside sweetened bitter gourd: kiwi blended squash were made at 0 day and successively after 3 and 6 months of ambient storage
Values are mean ± SE. The values followed by the same lower case letter, in the same column and parameter and by the same upper case letter in the same row are not significantly different (Mean comparisons were performed using Tukey's least significance difference (LSD) test at $P < 0.05$).

Table 2. Effect of different proportions of stevioside and storage period on physico-chemical and nutritional characteristics of low calorie bitter gourd: kiwi squash under ambient conditions.

Parameters	Storage period (Month)	T_1 (100 % Sucrose)	T_2 (75% Sucrose + 25% stevioside)	T_3 (50% Sucrose + 50% stevioside)	T_4 (25% Sucrose + 75% stevioside)	T_5 (15% Sucrose + 85% stevioside)	T_6 (100% stevioside)
TSS (ºBrix)	0	45.00^{aA}	32.20^{aB}	27.25^{aC}	15.20^{aD}	13.26^{aE}	9.10^{aF}
	3	45.20^{aA}	32.35^{aB}	27.32^{aC}	15.26^{aD}	13.30^{aE}	9.17^{aF}
	6	45.31^{aA}	32.40^{aB}	27.40^{bC}	15.32^{aD}	13.35^{aE}	9.23^{bF}
Titratable Acidity (%)	0	1.27^{aA}	1.25^{aA}	1.22^{aB}	1.22^{aB}	1.22^{aB}	1.22^{aB}
	3	1.21^{aA}	1.19^{bA}	1.17^{aA}	1.17^{aA}	1.17^{aA}	1.17^{aA}
	6	1.15^{bA}	1.13^{bA}	1.10^{bB}	1.10^{bB}	1.10^{bB}	1.10^{bB}
Reducing Sugars (%)	0	12.45^{aA}	7.25^{aB}	5.76^{aC}	5.00^{aC}	4.28^{aD}	3.15^{aE}
	3	13.78^{bA}	7.85^{bB}	5.95^{aC}	5.12^{aD}	4.35^{aE}	3.24^{aF}
	6	13.86^{bA}	8.10^{cB}	6.15^{bC}	5.30^{aD}	4.52^{aE}	3.35^{aF}
Total Sugars (%)	0	37.54a	25.57a	20.18a	10.15a	7.65a	5.23a
	3	37.14a	25.45a	20.10a	10.10a	7.55a	5.20a
	6	37.02a	25.30a	20.00a	10.02a	7.48a	5.13a
Total Phenolics (mg/100g)	0	25.22^{aA}	25.26^{aA}	25.30^{aA}	25.32^{aA}	25.32^{aA}	25.32^{aA}
	3	23.65^{bA}	23.68^{bA}	23.72^{bA}	23.72^{bA}	23.72^{bA}	23.72^{bA}
	6	20.32^{cA}	20.40^{cA}	20.42^{cA}	20.42^{cA}	20.42^{cA}	20.42^{cA}
Antioxidant activity (%)	0	68.29^{aA}	68.37^{aA}	68.55^{aA}	68.70^{aA}	68.80^{aA}	68.80^{aA}
	3	65.20^{aA}	65.32^{bA}	65.38^{aA}	65.45^{bA}	65.45^{bA}	65.45^{bA}
	6	65.00^{bA}	65.10^{bA}	65.15^{Aa}	65.17^{bA}	65.17^{bA}	65.17^{bA}

Data presented in Table is the average mean data of three independent observations
Measurements of the stevioside sweetened bitter gourd: kiwi blended squash were made at 0 day and successively after 3 and 6 months of ambient storage
Values are mean ± SE. The values followed by the same lower case letter, in the same column and parameter and by the same upper case letter in the same row are not significantly different (Mean comparisons were performed using Tukey's least significance difference (LSD) test at $P < 0.05$)

Antimicrobial activity

Antimicrobial activity of beverages has been recognized as one of the most important functional attributes while product optimization [30]. Figure 2 represents that, stevioside sweetened low calorie (LC BG-Kiwi) squash had reported maximum antimicrobial activity against *E. coli* (26 mm inhibition zone) in comparison to bitter gourd and bitter gourd-kiwi blended beverages. Increase in antimicrobial activity has also been reported earlier in stevia sweetened *Aloe vera*: aonla squash [9]. Further, antimicrobial activity of the beverages was not affected by the storage duration, indicating their storage stability up to a period of six months.

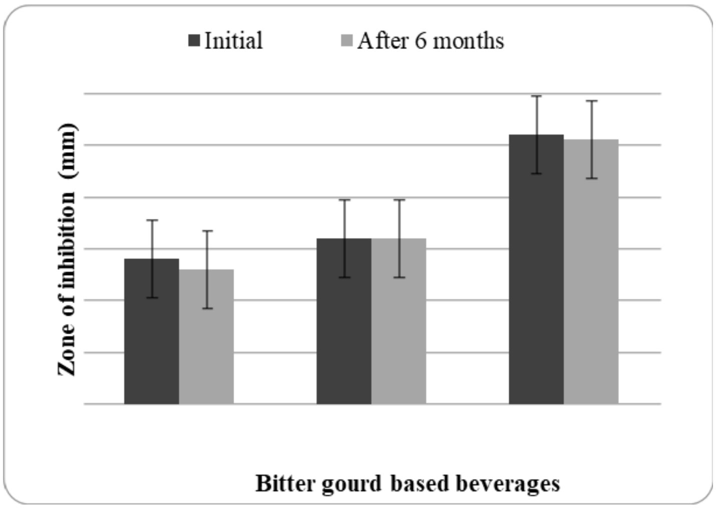


Figure 2. Antimicrobial activity of bitter gourd based beverages against *E coli*. BG- Bitter gourd, LC- low calorie.

Energy value

Data in Figure 3 provides the energy values of BG:K blended squash which ranged from 22.45 to 150.60 Kcal/100g. The control sample containing 100% sucrose recorded the highest energy while, the lowest value of 22.45 Kcal/100g was recorded in the treatment where 100 % replacement of the sucrose was done. The decrease in energy values might be due to replacement of sucrose sweetness with equi-sweetness level of steviside which are well recognized for their non caloric nature. Further, the energy value in all the beverages decreased during storage, which might be related to the decrease in the content of the total sugars over a period of time. Reduction in energy value during storage has also been observed by Sharma and coauthors [9] in *Aloe vera* based low calorie beverage.

Stevioside content

Total six samples were analyzed for the quantification of stevioside by using the pre- optimized method. The analytes were separated from the unidentified peaks of the matrix as are depicted in Figures 4 (A&B) showing typical chromatograms of the developed product. Stevioside content of the blended squash remained stable and unaffected throughout the storage period (0.42 mg/mL) ambient storage. Further, Figure 4 also showed occurrence of stevioside peak in the developed beverage with same retention time compared with standard stevioside.

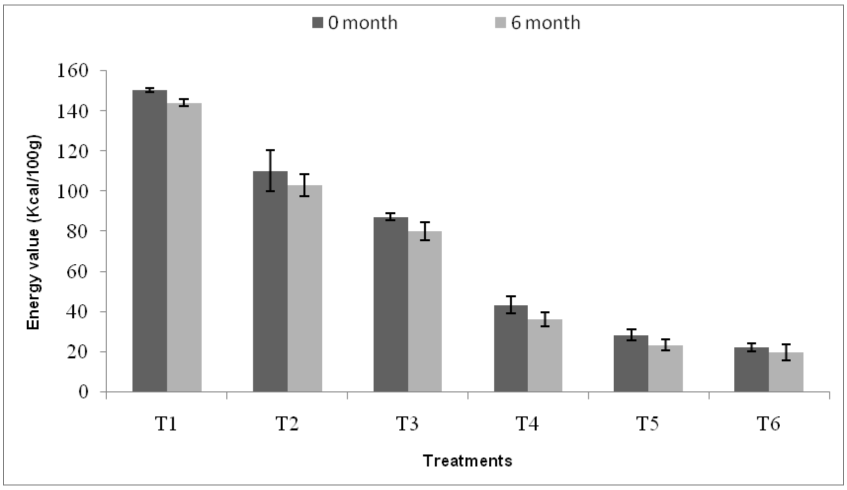
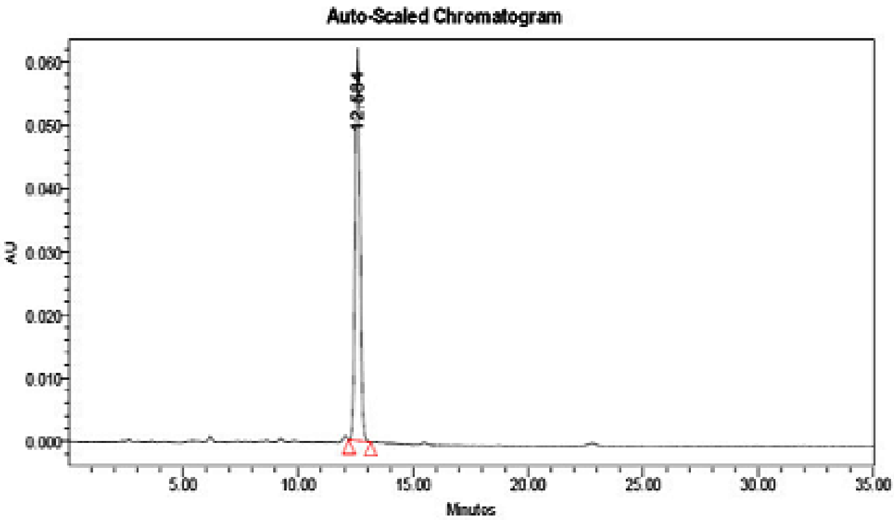
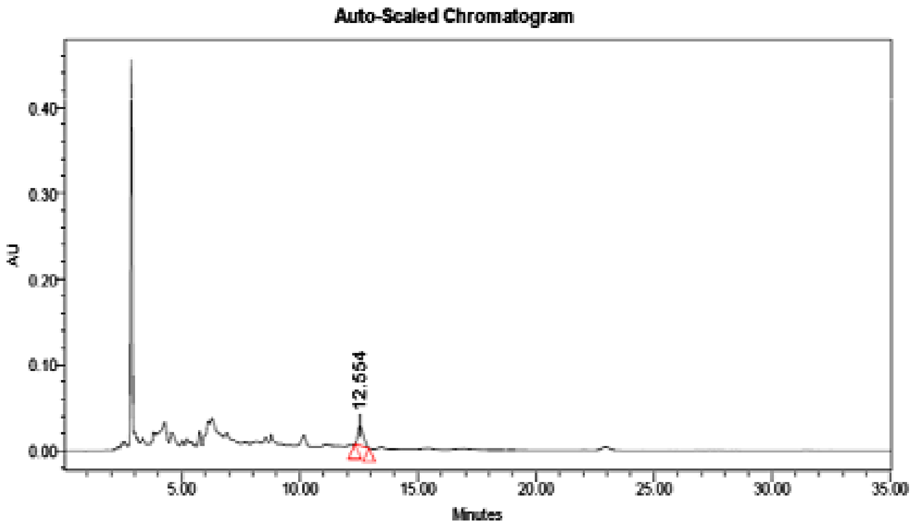


Figure 3. Effect of different proportions of stevioside on energy value of bitter gourd: kiwi blended squash during storage at ambient temperature.



A) Stevioside standard peak



B) Bitter gourd: kiwi blended squash showing stevioside peak

Figure 4 (A-B). HPLC Chromatograms of low calorie bitter gourd-kiwi blended squash sweetened with stevioside.

CONCLUSION

In the present investigation, a nutritious beverage with a shelf stable quality and storage life was developed by blending of kiwifruit pulp in bitter gourd juice to overcome the bitterness of bitter gourd with the strong flavor of kiwi fruit while keeping intact the nutritious quality. Blending not only enhanced the sensory quality but also improved the nutritional quality by significant enhancement in ascorbic acid content, antioxidant and total phenolics. Further, low calorie squash prepared by replacing the sweetness of sucrose with 75% sweetness level of stevioside proved to be the best without adversely affecting the sensory quality. The developed product was shelf stable for a period of 6 months under ambient conditions with non-significant changes in the biochemical and sensorial quality of the product. Conclusively, the developed product has better taste, palatability and nutritive value, besides reduced energy value, hence can benefit the '*at risk*' as well as health conscious people who are continuously seeking the alternate functional food products to keep well.

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