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Standardization of Technology for Development of Nutritious, Cost Effective and Antioxidant Rich Bitter Gourd- Kiwi Blended Functional Squash

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A B S T R A C T

In recent times, there has been growing recognition of the key role of foods and beverages in disease prevention and treatment. Thus, the production and consumption of fruits and vegetables is associated with reduction of occurrence of various degenerative diseases due to presence of antioxidants like Vitamin C and phenolics etc. in them. Bitter gourd fruit (*Momordica charantia* L.) has been reported to contain many nutraceutical compounds and possess antioxidant and hypoglycemic activity. Therefore, efforts have been made to formulate an unique antioxidant rich and palatable bitter gourd based functional squash by blending bitter gourd juice with different proportions of kiwi fruit (*Actinidia deliciosa)* juice (20, 40 and 50) using different levels of fruit part (30 and 35%) and TSS (40 and 45^{o}B). Among different formulations, the squash prepared using 30% blended juice of treatment T_3 having 80% *bitter gourd* juice and 20% *kiwi* juice*/pulp* with 45^{o}B TSS had recorded highest sensory scores for taste, bitterness acceptability and overall acceptability. The best formulation had been found to have good amount of ascorbic acid, phenolics, antioxidants and strong antimicrobial activity against *E coli* compared to control sample (100% *bitter gourd squash*). The unit cost of production of the developed product (Rs 69.75 per 750 ml PET bottle) was comparable to that of the standard bitter gourd squash (Rs 68.22 per 750 ml PET bottle) and thus its availability in the market will definitely benefit the health conscious people at reasonable prices.

Introduction

Presently, the growing demand for functional foods has been experienced world over because of their specific taste profile and disease preventing properties beyond general nutrition. *Functional beverages* are the drinks which are altered in such a way so to provide specific health benefits and disease preventing properties beyond general nutrition (Sharma, 2015). Bitter gourd (*Momordica charantia* L) is known to be a good source of vitamin C, phosphorus and iron while poor source of sugar. It is anti-diabetic, stimulant, stomachic, laxative, blood purifier and has been reported to contain many nutraceutical compounds and posses antioxidant and hypoglycemic activity (Anilakumar *et al.*, 2015).

Despite these tremendous nutritive and medicinal properties its utilization in beverage preparation has not been received much attention due to its bitter taste (Sharma and Tondon, 2015). To overcome this problem blending seems to be an effective alternative. Kiwi (*Actinidia deliciosa*) which is known for

its nutritive and medicinal values contained significant amount of biologically active compounds, including ascorbic acid, carotenoids and phenolics. Use of kiwifruit as a suitable option for blending with bitter gourd juice was considered due to its green colour, strong flavor, acidic nature and nutraceutical properties so as to develop a palatable and functional beverage. Therefore, in the present study blending of bitter gourd juice with kiwi fruit was attempted for the formulation of a unique delightful and delicious beverage with improved organoleptic and nutritive value.

Materials and Methods

Raw materials

Fresh and mature fruits of bitter gourd (*Momordica charantia L*) cv. Solan Hara were procured from local fruit and vegetable market, Solan (HP) and brought immediately to the fruit processing unit of Department of Food Science and Technology for the further studies. Whereas, Kiwifruits (*Actinidia deliciosa*) cv. Bruno were procured from the Kiwi Orchard, Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni Solan (HP).

Extraction of juice/pulp from bitter gourd and kiwifruit

Bitter gourd fruits (Cv *Solan Hara*) were sorted, washed thoroughly with water, cut into pieces and the juice was extracted in hydraulic press after grating the pieces. The extracted juice was heat preserved in glass bottles after lowering the pH to 3.5-4.0 by adding citric acid. The pulp of kiwi fruits (cv *Bruno)* was extracted by hot break pulping method followed by preservation with SO_2 @ 1000 ppm and storing at refrigerated temperature (4-7^oC) for later use.

Preparation of bitter gourd: kiwi blended beverage

Different combinations of bitter gourd juice and kiwi pulp were tried for optimization of a suitable combination for the preparation of palatable bitter gourd based functional squash. The beverages were prepared as per standard method (Fig. 1) and specifications of FSSA-2006 using 25, 30 and 35 per cent fruit part (blended), maintaining TSS and acidity between 40-45^oB and 1.2-1.3 per cent, respectively in all the treatments. Best combination/ blend was selected on the basis of sensory evaluation.

T_1= 100%BGJ, 25%FP and 40^oB TSS; T_2= 80% BGJ + 20%KJ, 30%FP and 40^oB TSS; T_3= 80% BGJ + 20%KJ, 30%FP and 45^oB TSS; T_4= 80% BGJ + 20%KJ, 35%FP and 40^oB TSS; T_5= 80% BGJ + 20%KJ, 35%FP and 45^oB TSS; T_6= 60% BGJ + 40%KJ, 30%FP and 40^oB TSS; T_7= 60% BGJ + 40%KJ, 30%FP and 45^oB TSS; T_8= 60% BGJ + 40%KJ, 35%FP and 40^oB TSS; T_9= 60% BGJ + 40%KJ, 35%FP and 45^oB TSS; T_{10}= 50% BGJ + 50%KJ, 30%FP and 40^oB TSS; T_{11}= 50% BGJ + 50%KJ, 30%FP and 45^oB TSS; T_{12}= 50% BGJ + 50%KJ, 35%FP and 40^oB TSS; T_{13}= 50% BGJ + 50%KJ, 35%FP and 45^oB TSS.

Analysis

Physico-chemical analysis

All the beverages were evaluated for their physico-chemical characteristics viz. TSS, titratable acidity, total sugars, reducing sugars, total phenols and ascorbic acid as per standard analytical methods (Ranganna, 1986). The antimicrobial activity of the developed beverages against *E. coli* and S. *aureus* was measured by well diffusion method (Aneja, 2003). 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 hrs. The antimicrobial activity was expressed in terms of mean diameter of the zones of inhibition measured. Antioxidant activity (Free radical scavenging activity) was measured as per the method of Brand-Williams *et al.*, (1995) where DPPH (2, 2 diphenyl-1-picrylhydrazyl) was used as a source of free radical. A quantity of 3.9 ml of $6x10^{-5}$ 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 or until the absorbance becomes steady. Methanol was used as blank. The per cent antioxidant activity was calculated using the following equation:

$$\text{Antioxidant Activity (\%)} = \frac{Ab_{(B)} - Ab_{(S)}}{Ab_{(B)}} \times 100$$

Where $Ab_{(B)}$ = Absorbance of Blank; $Ab_{(S)}$ = Absorbance of sample

Microbiological studies

The prepared beverages were studied for microbial load during storage at ambient temperature. The total microbial load was calculated by standard palate count method (Aneja, 2003).

Sensory evaluation

Sensory evaluation of the products was conducted by a panel of 15 semi-trained judges using 9- point hedonic scale system for different parameters like appearance, body, flavor, bitterness acceptability and overall acceptability (Amerine *et al.*, 1965), whereas, the consumer acceptability study was conducted by serving the product to the masses (consumers) and obtaining their feedback on 9- point hedonic proforma.

Statistical analysis

All the analytical parameters were recorded in triplicates and the mean values of each parameter were described. The data of quantitative estimation of biochemical characteristics were assessed by factorial CRD whereas the data pertaining to sensory evaluation were analyzed by RBD as described by Cochran and Cox (1967).

Results and Discussion

Physico-chemical characteristics of fresh bitter gourd and kiwifruit pulp

The bitter gourd juice contained 5.50°B total soluble solids (TSS), 0.29 per cent titratable acidity (as % citric acid), 82.08 mg/100g ascorbic acid, 0.61 per cent reducing sugars, 1.93 per cent total sugars and 3.75 pH (Table 1). The results were closer to Satkar *et al.*, (2013) and Kaur and Aggarwal (2014) who reported that bitter gourd juice contained 3.20°B TSS, 0.04 per cent titratable acidity, 84.99 mg/100g ascorbic acid, 0.61 per cent reducing sugars and 1.93 per cent total sugars, respectively. Whereas, the total phenolic contents and antioxidant activity in bitter gourd juice were observed as 21.99 mg/100g and 52.86 per cent, respectively. The results were in conformity with those of Kaur and Aggarwal (2014).

Kiwifruit pulp was found to contain 12.45°B average total soluble solids (TSS), 1.74 per cent titratable acidity (as % citric acid), 2.97 pH and 101.95 mg/100g ascorbic acid. While, according to other authors, kiwifruit pulp contained 12.40°B TSS, 1.68 per cent titratable acidity, 3.20 pH and 121.00

mg/100g ascorbic acid (Thakur and Barwal, 1988; Leahu *et al.,* 2013). The reducing sugars and total sugars were recorded as 3.58 per cent and 7.67 per cent, respectively which were lower than the findings of Sharma *et al.*, (2013) who reported that kiwi juice/pulp contained 12.07 per cent total sugars and 8.93 per cent reducing sugars. Total phenolic contents were found to be 131.66 mg/100g, while the antioxidant potential was observed to be 68.98 per cent. These findings were in the range of Park *et al.,* (2006) who reported the phenolic contents and antioxidant potential in kiwifruit pulp ranged between 131.08 mg/100g to 180.09 mg/100g and 57.10 per cent to 81.20 per cent, respectively. The pulp recovery of 60.67 per cent was recorded when extracted by hot break pulping method. The result was closer to the finding of Vaidya *et al.,* (2009) who reported 58.44 per cent juice /pulp yield.

Optimization of best formulation for the preparation of bitter gourd-kiwi blended squash

The effects of blending bitter gourd juice with different proportions of kiwi pulp on sensory attributes of the developed squash are presented in figure 2. It was observed that with the increase in the proportion of kiwi pulp up to 20% the appearance score increased, beyond which it decreased due to cloudy appearance of the beverage, whereas, the flavour (bitterness acceptability) score was increased gradually with the increase in proportion of kiwi pulp. However, at higher levels of kiwi pulp (≥40%), the flavour score decreased. The overall acceptability score followed the same trend as appearance and flavour. Results are in conformity with earlier studies conducted by Kausar *et al.,* (2012) and Raj (2013) in cucumber-melon functional drink and anti-oxidant rich blended sand pear-apple juice beverage. Among different combinations tried, the treatment T_3 (B_{80}: K_{20}; 80% bitter gourd juice + 20% kiwi pulp) with 30% fruit part and 45^o B TSS was adjudged the best in terms of better body, taste/aroma, bitterness acceptability and overall acceptability scores.

Physico-chemical, nutritional and sensory characteristics of the developed product

A comparison of data presented in table 2 revealed the effect of blending on various physico-chemical and nutritional attributes of the beverage. It was observed that addition of kiwi pulp has improved the nutritional quality of the squash as evident from its higher ascorbic acid (27.15 mg/100g) and total phenolics (25.22 mg/100g) compared to standard bitter gourd squash (18.25 mg/100g and 19.44 mg/100g), respectively. The comparison of nutritive value and antioxidant potential revealed that the bitter gourd:kiwi blended beverage had better nutritive as well as antioxidant potential compared to bitter gourd squash and also showed strong antimicrobial activity (15.00 mm inhibition zone) against *E. coli* (Fig. 3). Further, blending has also exerted positive effect on sensory attributes of the beverages as the blended squash had recorded higher score for appearance (7.40), flavour (bitterness acceptability (7.90) and overall acceptability (8.00) compared to bitter gourd squash (Table 2). Earlier, many workers have reported that two or more fruit pulp/juices blended in various proportions for making more palatable and nutritious beverages (Raj, 2013; Mohamed *et al.,* 2014; Sheela and Shruthi, 2014; Sharma *et al.,* 2016)

Microbiological studies

The microbial analysis of bitter gourd-kiwi blended squash was performed at initial day and after six months of storage at ambient temperature (12-25^{0}C). The product was found to be free from any microbial growth till the end of the storage period in terms of total plate count (TPC).

Cost of production of bitter gourd: kiwi blended functional squash

The cost of production of bitter gourd squash and bitter gourd: kiwi blended squash was calculated and given in table 3. The cost was calculated on the basis of current market prices of ingredients, nominal processing charges and reasonable profit margins.

Table.1 Physico-chemical characteristics of fresh bitter gourd and kiwifruit pulp

Parameters*	**Bitter gourd juice (Mean± SD)**	**Kiwi fruit pulp (Mean± SD)**
TSS (^{0}B)	5.50 ± 0.50	12.45± 1.00
Titratable acidity (%)	0.29 ± 0.01	1.74 ± 0.04
pH	3.75 ± 0.05	2.97 ± 0.04
Ascorbic acid (mg/100g)	82.08 ± 1.52	101.95 ± 3.03
Reducing sugars (%)	0.61 ± 0.08	3.58 ± 0.12
Total sugars (%)	1.93 ± 0.05	7.67 ± 0.22
Total phenolics (mg/100g)	21.99 ± 0.89	131.66 ± 3.78
Antioxidants potential (% free radical scavenging activity)	52.86 ± 1.23	68.98 ± 0.93

*Each value is average of 3 determinations; SD = Standard Deviation

Table.2 Comparison of physico-chemical, nutritional and sensory characteristics of bitter gourd squash and bitter gourd: kiwi blended squash

Parameters	**Mean ± SD**	
	Bitter gourd squash	**Bitter gourd: kiwi squash**
Physico-chemical and nutritional parameters		
TSS (^{0}Brix)	40.00 ± 0.99	44.83 ± 1.00
Titratable acidity (%)	1.20 ± 0.05	1.25 ± 0.05
pH	2.70 ± 0.05	2.68 ± 0.05
Reducing sugars (%)	21.71 ± 0.65	21.86 ± 0.65
Total sugars (%)	36.25 ± 0.85	37.54 ± 0.95
Sensory attributes		
Appearance	7.00± 0.80	7.40± 0.50
Flavour (Bitterness acceptability)	6.50± 0.20	7.90± 0.50
Overall acceptability	7.00± 0.60	8.00± 0.80

*Each value is average of 3 determinations; SD = Standard Deviation

Fig.1 Unit operations for the preparation of bitter gourd: kiwi blended functional squash

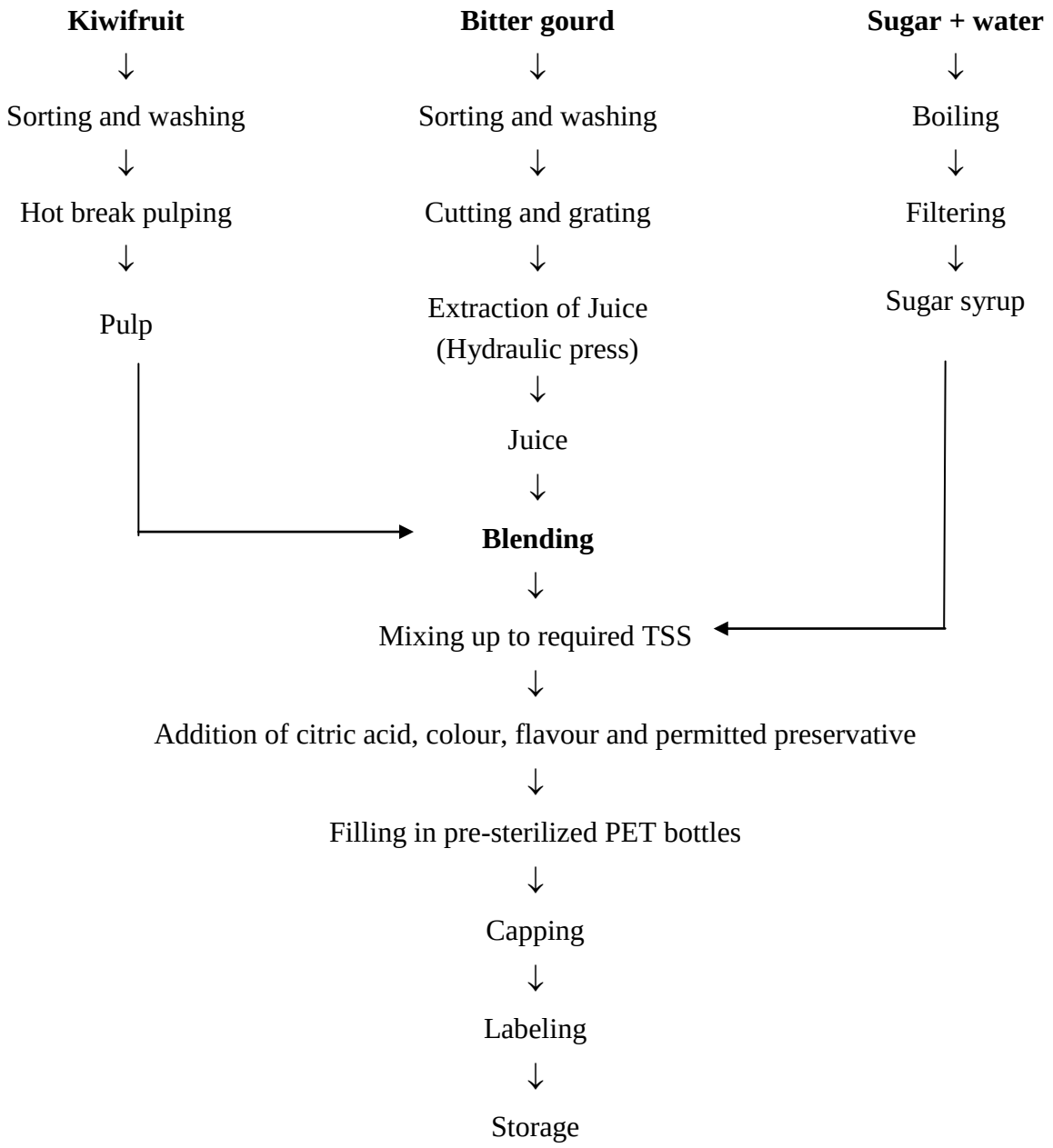


Table.3 Cost of production of bitter gourd and bitter gourd: kiwi blended squash

Particular	Rate per unit (Rs)	Bitter gourd squash		Bitter gourd: kiwi blended squash	
		Quantity	Amount (Rs)	Quantity	Amount (Rs)
Bitter gourd juice	65/L	3000 mL	195.00	2400 mL	156.00
Kiwi pulp	85/L	-	-	600 mL	51.00
Sugar	36/Kg	4500 g	162.00	4500 g	162.00
Citric acid	400/Kg	105 g	42.00	105 g	42.00
Sodium benzoate	285/Kg	7 g	2.00	7 g	2.00
PET Bottle (750mL)	13/bottle	13	169.00	13	169.00
Labels	1/label	13	13.00	13	13.00
Total cost of ingredient (Rs)	**-**	**-**	**583.00**	**-**	**595.00**
Processing cost @ 20%	-	-	116.60	-	119.0
Depreciation on machinery @ 10 %	-	-	58.30	-	59.00
Total preparation cost	**-**	**-**	**758.00**	**-**	**773.00**
Profit @ 20%	-	-	151.60	-	154.60
Total cost of product (10 L)	-	-	909.60	-	927.60
Sale price/bottle (750 ml)	**-**	**-**	**68.22**	**-**	**69.57**

Fig.2 Sensory evaluation of different formulations of bitter gourd: kiwi blended squash

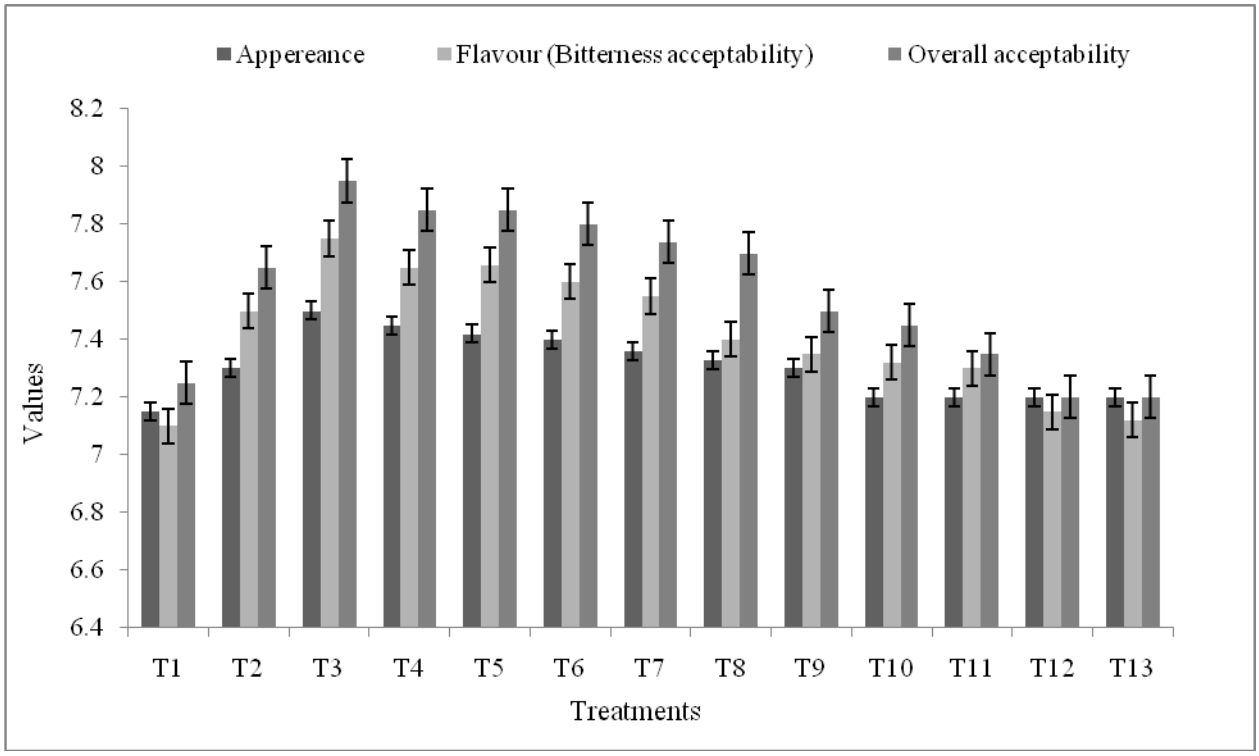
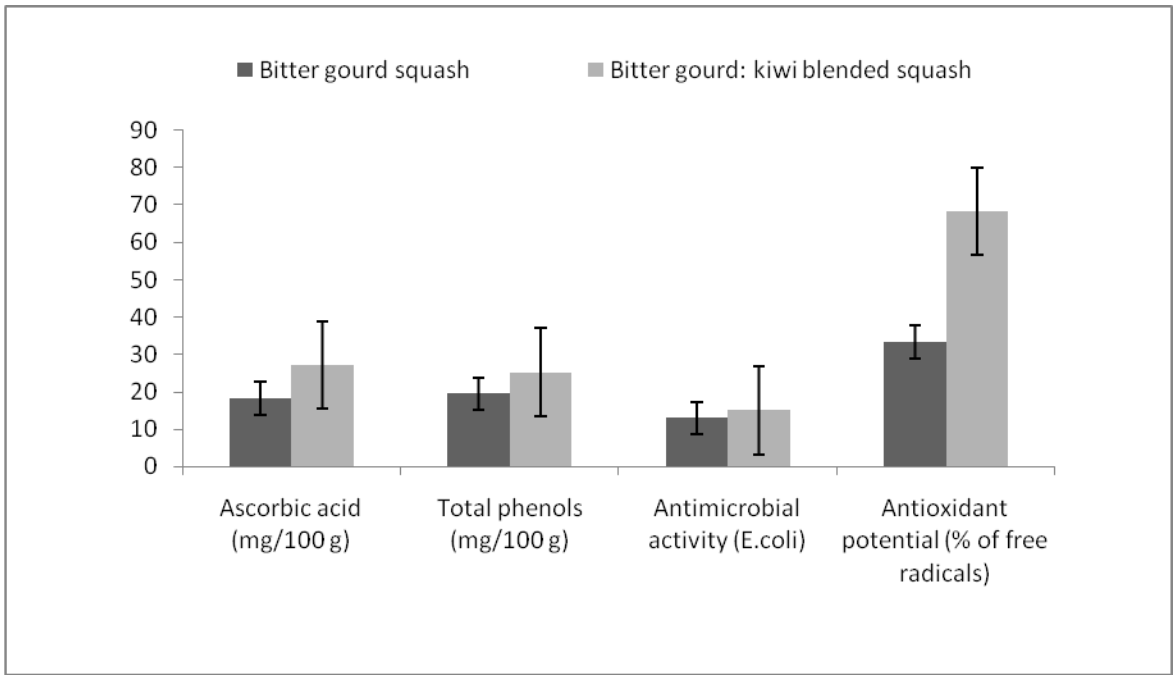


Fig.3 Nutritive, antioxidant potential and antimicrobial activity of bitter gourd and bitter gourd: kiwi blended squash



The cost of 100 percent bitter gourd squash was calculated to be Rs 68.22 per 750ml PET bottle. Whereas, it was Rs 69.57 per 750ml PET bottle for bitter gourd (80%): kiwi (20%) blended squash. It is clear from the data that there was no much difference in the unit cost of production as well as sale price of the developed blended squash than that of standard bitter gourd squash. Thus, its availability in the market will definitely benefit the health conscious people at reasonable prices.

In conclusion, in the present investigation, it emerge that, use of kiwi fruit pulp/juice helped in developing a palatable beverage having combined health benefits of bitter gourd and kiwifruit. The use of kiwifruit pulp/juice @ 20 per cent gave higher sensory score (*Liked very much*) for appearance, flavour (bitterness acceptability) and overall acceptability to the resultant beverages. The blended squash also contained higher amount of ascorbic acid, total phenolics and had also shown higher antioxidant as well as antimicrobial activity compared to the control sample. Besides improving the taste profile as well as nutritional value, blending of bitter gourd juice with kiwi pulp has not affected the cost of production to a greater extent. Hence, commercial production of the developed product seems to be a profitable proposition.

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