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Vitamin C enriched bitter gourd based functional squash

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Abstract
Vitamin C enriched palatable bitter gourd based functional squash made by blending bitter gourd juice with aonla fruit (*Embilica officinalis)* juice in different ratios. The beverages were prepared by using different levels of fruit part or blended juice viz. 25, 30 and 35% and TSS (40 and 45ºB) and analyzed for their bio-chemical, antioxidant potential and sensory quality attributes. The squash prepared using 30 percent blended juice of the combination consisting of 70 percent bitter gourd and 30 percent aonla juice with 45ºB TSS had recorded highest sensory scores for colour, flavour and overall acceptability. Organoleptically best combination (T_7) had also been found to contain higher amounts of ascorbic acid and phenolics compared to bitter gourd squash. The unit cost of production of the developed product (Rs 66.57 per 750 ml PET bottle) was comparable to that of the standard bitter gourd squash (Rs 68.21 per 750 ml PET bottle) and Further, the developed product showed strong antimicrobial activity against *E. coli* and *Staphylococcus aureus* and good antioxidant potential indicating its health benefits and thus their availability in the market will definitely benefit the consumers.

Keywords: Bitter gourd, *Momordica charantia* L., aonla, functional beverages, blending, antioxidant activity

Introduction
Food provides not only essential nutrients needed for life but also other bioactive compounds which are useful in health promotion and disease prevention. Ancient Greek physician Hippocrates, the father of Western medicine has said "Let food be thy medicine and medicine be thy food" means food should be our medicine, not medicine be our food. Consumption of fruits and vegetables have strongly been associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts and age related macular degeneration. 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 as to provide specific health benefits and disease preventing properties beyond general nutrition (Sharma, 2015) [1]. 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) [2]. Despite these tremendous nutritive and medicinal properties its utilization in beverage preparation has not received much attention due to its bitter taste (Sharma and Tandon, 2015) [3]. To overcome this problem, blending seems to be an effective alternative. Aonla (*Emblica officinalis*) is highly nutritive and contains very high amounts of vitamin C (478.56 mg/100 ml) and is well known for its nutraceutical and pharmacological properties such as anti-sorbutic, laxative, anti-biotic, anti-viral, cardiotonic and hypoglycemic activity. Therefore, in the present study blending of bitter gourd juice with aonla fruit was attempted for the production of delightful and delicious beverage with improved organoleptic and nutritive value.

Methodology
Raw materials
Fresh and mature fruits of bitter gourd (*Momordica charantia L*) cv. 'Solan Hara' and aonla (*Emblica officinalis*) cv. 'Desi' 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. The fruits 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 [3]. Whereas, aonla fruits (cv. Desi) were sorted, washed thoroughly with water, manually destoned, grated and the juice was extracted by using hydraulic press followed by hot filling, bottling, pasteurization (90±2 ºC, 25 min) and stored at ambient temperature for further use.

Preparation of palatable bitter gourd: aonla blended squash

Different combinations of bitter gourd juice (BGJ) and aonla juice (AJ) 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 and specifications of FSSA-2006 using 25, 30 and 35 percent fruit part (FP) and maintaining TSS of 40 and 45ºB as per the detailed given below. The titratable acidity was kept between 1.2-1.3 percent in the treatments. Best combination/ blend was selected on the basis of sensory evaluation.

T_1= 100%BGJ, 25%FP and 40ºB TSS; T_2= 90% BGJ + 10%AJ, 30%FP and 40ºB TSS; T_3= 90% BGJ + 10%AJ, 30%FP and 45ºB TSS; T_4= 90% BGJ + 10%AJ, 35%FP and 40ºB TSS; T_5= 90% BGJ + 10%AJ, 35%FP and 45ºB TSS; T_6= 70% BGJ + 30%AJ, 30%FP and 40ºB TSS; T_7= 70% BGJ + 30%AJ, 30%FP and 45ºB TSS; T_8= 70% BGJ + 30%AJ, 35%FP and 40ºB TSS; T_9= 70% BGJ + 30%AJ, 35%FP and 45ºB TSS; T_{10}= 50% BGJ + 50%AJ, 30%FP and 40ºB TSS; T_{11}= 50% BGJ + 50%AJ, 30%FP and 45ºB TSS; T_{12}= 50% BGJ + 50%AJ, 35%FP and 40ºB TSS; T_{13}= 50% BGJ + 50%AJ, 35%FP and 45ºB 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, 2004) [4]. The antimicrobial activity of the developed beverages against *E. coli* and S. *aureus* was measured by well diffusion method (Aneja, 2003) [5]. 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 [6] 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 percent 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 proliferation during storage with respect to Total Plate Count (TPC) at ambient temperature. The total microbial load was calculated by standard palate count method (Aneja, 2003) [5].

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) [7]. 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 [8].

Results

Proximate composition of bitter gourd and aonla juice

The bitter gourd juice and aonla juice were analyzed for various physico-chemical parameters Table 1. The bitter gourd juice contained 5.50ºB total soluble solids (TSS), 0.29 percent titratable acidity (as % citric acid), 82.08 mg/100g ascorbic acid, 0.61 percent reducing sugars, 1.93 percent total sugars and 3.75 pH. Whereas, the total phenolic contents and antioxidant activity in bitter gourd juice were observed as 21.99 mg/100g and 68.86 percent, respectively. The yield of juice was found to be 41.56 percent when extracted with hydraulic press.

Table 1: Physico-chemical characteristics of fresh bitter gourd and aonla juice Mean ±SD

Parameters*	Bitter gourd juice	Aonla juice
TSS (^{0}B)	5.50 ± 0.50	14.50 ± 0.50
Titratable acidity (%)	0.29 ± 0.01	2.58 ± 0.05
pH	3.75 ± 0.05	3.17 ± 0.02
Ascorbic acid (mg/100g)	82.08 ± 1.52	440.67 ± 6.51
Reducing sugars (%)	0.61 ± 0.08	4.26 ± 0.69
Total sugars (%)	1.93 ± 0.05	7.98 ± 0.30
Total phenolics (mg/100g)	21.99 ± 0.89	168.33 ± 5.13
Antioxidants potential (% free radical scavenging activity)	68.86 ± 1.23	82.82 ± 1.97

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

On the other hand, the aonla juice was found to contain 14.50ºB mean total soluble solids (TSS), 2.58 percent titratable acidity (as % citric acid), 3.17 pH and 440.67 mg/100g ascorbic acid. Whereas, the reducing sugars and total sugars were observed as 4.26 percent and 7.98 percent, respectively. While, the total phenolic contents and antioxidant potential were estimated to be 168.33 mg/100g and 82.82 percent, respectively. The juice recovery of 48.92 percent was recorded when extracted by hydraulic pressing of the grated material.

Optimization of best combination

It was observed from the Fig 1that with the increase in proportion of aonla juice up to 30 percent and corresponding decrease in bitter gourd juice up to 70 percent, the appereance score of prepared squash increased significantly. Whereas, in case of flavour (bitterness acceptability) it was observed that addition of aonla juice beyond 30 percent had successfully reduced the bitter taste of the product and it increased when aonla juice was used at higher concentration of 50 percent. It might be due to the fact that at higher levels, aonla juice gave its own astringent flavour which failed to mask the bitter taste of bitter gourd juice. Among different combinations tried, the treatment T_7 (70% bitter gourd juice + 30% aonla juice) with 30% fruit part and 45° B TSS was adjudged the best in terms of better appearance, flavour (bitterness acceptability) and overall acceptability scores.

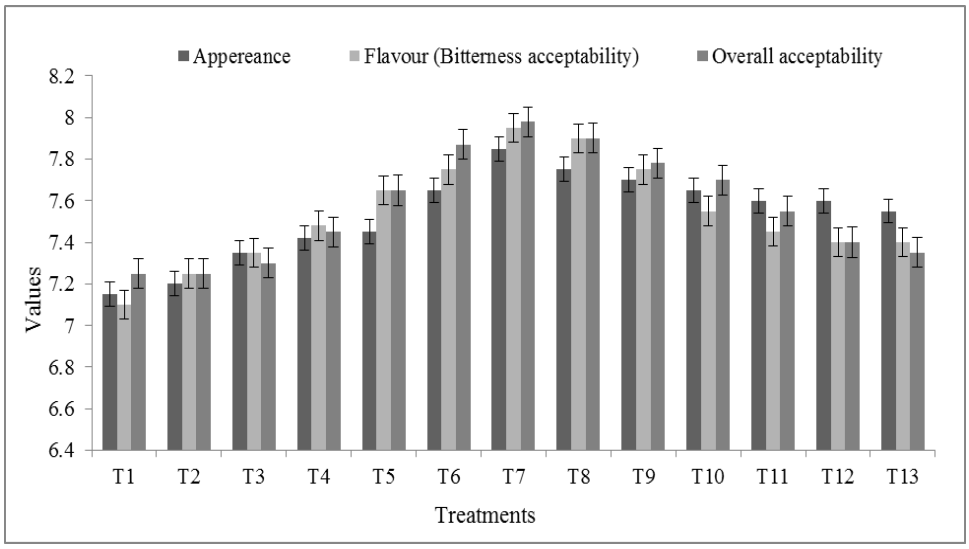

Fig 1: Sensory attributes of bitter gourd squash and bitter gourd: aonla blended squash

Physico-chemical, nutritional and sensory characteristics

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 aonla juice has improved the nutritional quality of the squash as evident from its higher ascorbic acid (39.75 mg/100g) and total phenolics (33.11mg/100g) compared to standard bitter gourd squash (18.25 mg/100g and 19.44 mg/100g), respectively. The blended squash was found to have higher antioxidant potential (74.19%) and also showed strong antimicrobial activity (Table 2 and Fig 2) against *S. aureus* (18.00 mm inhibition zone) as well as *E. coli* (16.00 mm inhibition zone). Further, blending has also exerted positive effect on sensory attributes of the beverages as the blended squash had recorded higher score for appearance (7.80), body (8.00), flavour (7.80), bitterness acceptability (7.92) and overall acceptability (8.00) compared to bitter gourd squash (Fig 3).

Table 2: Physico-chemical, nutritional and sensory characteristics of bitter gourd squash and bitter gourd: aonla blended squash

Parameters	Mean ±SD	
	Bitter gourd squash (100%)	**Bitter gourd: aonla squash (70% + 30%)**
TSS (^{0}Brix)	40.00 ± 0.99	45.00 ± 0.60
Titratable acidity (%)	1.20 ± 0.05	1.27 ± 0.01
pH	2.70 ± 0.05	2.68 ± 0.05
Ascorbic acid (mg/100g)	18.25 ± 0.50	39.75 ± 1.48
Reducing sugars (%)	21.71 ± 0.65	21.98 ± 1.02
Total sugars (%)	36.25 ± 0.85	36.74 ± 1.26
Total phenolics (mg/100g)	19.44 ± 0.50	33.11 ± 1.10
Antioxidants potential (%)	33.32 ± 0.75	74.19 ± 3.14

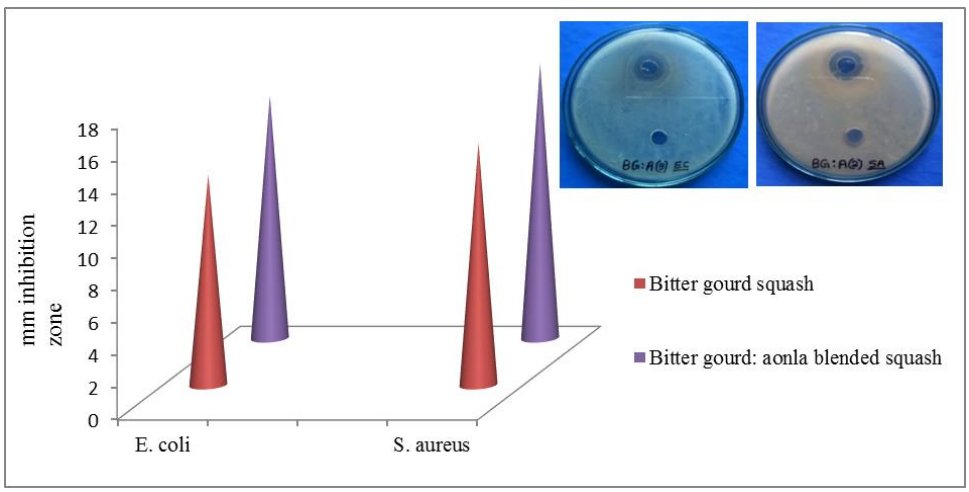

Fig 2: Antimicrobial activity of bitter gourd and bitter gourd: aonla blended squash

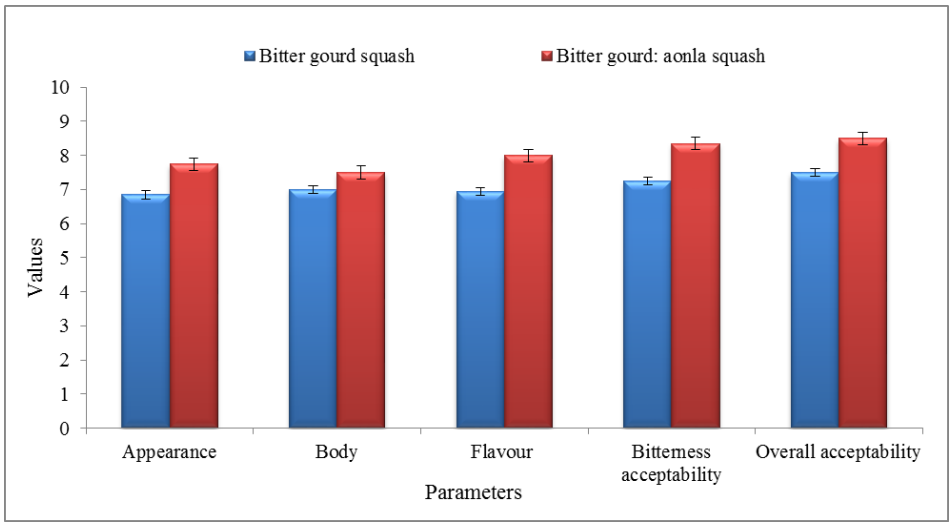

Fig 3: Sensory attributes of bitter gourd squash and bitter gourd: aonla blended squash

Cost of production of bitter gourd: aonla blended functional squash:

The cost of production of bitter gourd squash and bitter gourd: aonla 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. The cost of 100 percent bitter gourd squash was calculated to be Rs 68.21 per 750ml PET bottle. Whereas, it was Rs 66.57 per 750ml PET bottle for bitter gourd (70%): aonla (30%) 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.

Table 3: Cost of production of bitter gourd: aonla blended squash

Particular	Rate (Rs)	Bitter gourd squash		Bitter gourd: aonla blended squash	
		Quantity	Amount (Rs)	Quantity	Amount (Rs)
Bitter gourd juice	65/L	3000 mL	195.00	2100 mL	136.50
Aonla juice	50/L	-	-	900 mL	45.00
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	-	569.00
Processing cost @ 20%	-	-	116.60	-	113.80
Depreciation on machinery @ 10 %	-	-	58.30	-	56.90
Total preparation cost	-	-	757.90	-	739.70
Profit @ 20%	-	-	151.58	-	147.94
Total cost of production (10 L)	-	-	909.48	-	887.64
Sale price/bottle (750 ml)	-	-	68.21	-	66.57

Discussion

Proximate analysis shows that bitter gourd and aonla fruits are good source of ascorbic acid, phenolics and antioxidants and can be successfully utilized for the development of functional beverages. Different authors reported that bitter gourd juice contained 3.20ºB TSS, 0.04 percent titratable acidity, 84.99 mg/100g ascorbic acid, 0.61 percent reducing sugars and 1.93 percent total sugars, respectively (Satkar *et al*., 2013; Kaur and Aggarwal, 2014) [9-10]. Whereas, aonla juice contained higher amount of ascorbic acid, phenolics and have higher antioxidant activity (Sasikumar, 2013; Sasikumar *et al*., 2013; Kaur and Kapoor, 2005; Reddy and Chikkasubbanna, 2009) [11-14]. Sensory analysis test are essential for the development of a product that gives the acceptability and meet the needs of consumer. The colour (appearance) score decreased when the proportion of aonla juice was increased beyond 30 percent. The colour score decreased significantly and this was in conformity with the findings of Sharma *et al*., 2002 [15] and Barwal *et al*., 2006 [16] in spiced plum squash and bitter gourd RTS drink. It was found that with the increase in proportions of fruit pulp above 30 percent, the squash gave the cloudy appearance, hence decrease in body score was noticed (Bhardwaj and Mukherjee, 2017; Kausar *et al*., 2012) [17-18]. Further, blending of bitter gourd juice with appropriate proportion of aonla juice might have enhanced the flavour profile of the product due to balanced sugar: acid blend, desirable aroma and taste (Sharma *et al*., 2002; Barwal *et al*., 2006; Bhardwaj and Mukherjee, 2017) [15-17]. It was observed that addition of aonla juice beyond 20 percent had successfully reduced the bitter taste of the product. Whereas, it increased when aonla juice was used at higher concentration of 50 percent, which was not liked by the panelists. It might be due to the fact that at higher levels, aonla juice gave its own astringent flavour which failed to mask the bitter taste of bitter gourd juice. In case of overall acceptability it was observed that with the addition of aonla juice beyond 20

percent, the score for flavour as well as bitterness acceptability increased which has resulted in higher overall acceptability score of the products. The blended squash was found to have higher ascorbic acid content, phenolic content, higher antioxidant potential and strong antimicrobial activity. Whereas, blending has also exerted positive effect on sensory attributes of the beverage as the blended squash had recorded higher score for flavour, bitterness acceptability and overall acceptability compared to standard bitter gourd squash. Our results are in conformity with earlier studies conducted by other authors, who 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 Sruthi, 2014; Sharma *et al*., 2016; Selvamuthukumaran and Chowdary, 2017; Sharma and Thakur, 2017) [19-24].

Conclusion

From the present study, it emerged that blending of bitter gourd juice with aonla juice resulted in the improvement of both sensory and nutritional characteristics of the developed beverage. The beverage prepared by blended juice (70% BGJ + 30%AJ) with 45° B TSS had recorded highest sensory scores for taste, bitterness acceptability and overall acceptability. The blended squash contained similar amount of acidity and sugar but significantly higher amounts of ascorbic acid and total phenolics. The developed product had also shown higher antioxidant as well as antimicrobial activity compared to the control sample (bitter gourd squash). Besides improving the taste profile as well as nutritional value of the blended beverages, blending of bitter gourd juice with aonla juice was economical and cost effective. Hence, development of such beverages at commercial scale seems to be a profitable venture and their availability in the market will definitely benefit the health conscious people.

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