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Development and evaluation of vitamin C enriched low calorie *Aloe vera*- aonla blended functional squash using stevioside

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ABSTRACT

Experiment was conducted to develop vitamin C enriched, low calorie *Aloe vera* based functional squash by blending with aonla juice in different proportions and replacing sugar sweetness with stevioside. The products were also evaluated for their physico-chemical, nutritional and sensory quality attributes during storage. Among different combinations, the ratio of 65:35 (*Aloe vera*: aonla juice) with 30% juice part and 40° B TSS had recorded highest sensory score for taste and overall acceptability. The same treatment also had good amount of ascorbic acid (78.65 mg/100g) and phenolics (37.15mg/100g) compared to control sample (100% *A vera*). Further, the optimized formulation was used for the development of low/reduced calorie squash using stevioside. Sensory analysis of low calorie beverages indicated highest acceptability for the treatment LT_8 (30 sugar:70 non nutritive sweetener) prepared by 70% non-nutritive sweeteners (NS) sweetness level consisting of 90% stevioside and 10% sorbitol sweetness proportion. The developed product was found to have strong antimicrobial activity (28.50 mm inhibition zone) against *E. coli* as well as high antioxidant potential (66.90%). The calculated energy value of the developed product was recorded to be 53.65 Kcal/100g which was significantly low compared to the control sample (165 Kcal/100g). The developed beverages were successfully stored at ambient temperature for a period of 6 months without significant changes in chemical and sensorial quality profile. Overall, it was concluded that the developed products had better taste, palatability, nutritive value and storage stability beside reduced calorie value, hence can benefit the health conscious people.

Key words: Medicinal plant, ascorbic acid, functional beverages, non-nutritive sweeteners, sensory analysis.

INTRODUCTION

Aloe vera (L.) is one of the oldest known medicinal plants gifted by nature and is often called as *miracle plant* or *natural healer*. Its health benefits have been attributed to the polysaccharides contained in the mucilageous gel of leaves (Ahlawat and Khatkar, 1; Sharma *et al.,* 14). Various authors have suggested use of *A. vera* in food products such as beverages, energy drinks, jams, candies, wine and dairy products (Ahlawat and Khatkar, 1; Sharma *et al.,* 14; Boghani *et al*., 6). However, the bitter taste of *A. vera* juice has been reported to adversely affects the palatability of certain products especially beverages (Sharma *et al*., 14). To overcome this problem, blending seems to be one of the best alternatives as blending of two or more fruit juices/pulps to prepare most acceptable beverages with additional health benefits has also been reported earlier (Sasikumar *et al*., 12). Among many fruits, aonla is highly nutritious and important dietary source of vitamin C, minerals and amino acid and is well known for its nutraceutical and pharmacological properties (Jain and Khurdiya, 9). Thus, blending of *A. vera* juice with fruit juices like aonla juice could lead to the production of delicious beverage with improved organoleptic and nutritive value especially vitamin C content.

On the other hand, fruit based beverages (squash) contain large amount of sugars and provide excess amount of calories to the consumers which is partially responsible for various diseases like hypertension, cardiovascular diseases, increased incidence of diabetes mellitus and obesity (Barwal *et al*., 3). Therefore, the possibility of replacing such bulk calorie sweeteners with high intensity non-nutritive sweeteners could be explored for the development of reduced/ low calorie functional beverages. Stevioside is a natural high intensity sweetener obtained from stevia, a natural sweet herb and potentially used in various dietetic foods especially for diabetics (Kumar *et al*., 10; Sharma and Tandon, 13). Keeping these facts in view, the present investigation was undertaken to develop and evaluate *A vera*- aonla blended vitamin C enriched low calorie functional squash using stevioside for the benefit of consumers.

MATERIALS AND METHODS

Freshly harvested *A vera* leaves were washed thoroughly with water containing KMS @ 500 ppm and gel was extracted using cold extraction method. For

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extraction of juice, gel was passed through grinder, homogenized and treated with 0.5% pectinase at 40-50ºC for 30 min. followed by filtration and pH adjustment (3.5) by adding citric acid (Sharma *et al.*, 14). The juice was then pasteurized and stored for further studies. The aonla juice was extracted through hydraulic press after grating the de-stoned fruits. The juice was preserved by heat processing in glass bottle and kept under refrigerated conditions for further studies. Different combinations of *A. vera* juice (AVJ) and aonla juice (AJ) were tried for optimization of a suitable combination for the preparation of palatable A*loe-aonla* squash as per following treatments: T_1 (Al_{100}:An_0) = AVJ 100% ; T_2 (Al_{90}:An_{10}) = AVJ 90% + AJ 10%; T_3 (Al_{85}:An_{15})= AVJ 85% + AJ 15%; T_4 (Al_{80}:An_{20})= AVJ 80% + AJ 20%; T_5 (Al_{75}:An_{25})= AVJ 75% + AJ 25%; T_6 (Al_{70}:An_{30}) = AVJ 70% + AJ 30%; T_7 (Al_{65}:An_{35})= AVJ 65% + AJ 35%; T_8 (Al_{60}:An_{40}) = AVJ 60% + AJ 40%;T_9 (Al_{55}:An_{45})= AVJ 55% + AJ 45%;T_{10} (Al_{50}:An_{50})= AVJ 50% + AJ 50%. The beverages were prepared as per standard method and specifications of FSSA-2006 using 30% fruit part (blended), maintaining TSS and acidity between 40-41ºB and 1.2-1.3 per cent, respectively in all the treatments. Best combination/ blend was selected on the basis of sensory evaluation.

Further, the low calorie *Aloe vera*- aonla squash was prepared by replacing sugar sweetness (S) with equi-sweetness of stevioside (St) and sorbitol (So) at different proportions as per the method given by Sharma and Tandon (13). Various treatments were, LT_1= 100%S; LT_2= 90%S+10%NS (90st:10So); LT_3= 80%S+20%NS (90st:10So); LT_4= 70%S+30%NS (90st:10So); LT_5= 60%S+40%NS (90st:10So); LT_6= 50%S+50%NS (90st:10So); LT_7= 40%S+60%NS (90st:10So); LT_8= 30%S+70%NS (90st:10So); LT_9= 20%S+80%NS (90st:10So); LT_{10}= 100% St.

All the beverages were evaluated for their physico-chemical characteristics viz. TSS, titratable acidity, pH, sugars, total phenols and ascorbic acid as per standard methods for a period of 6 months at different intervals of 0, 3 and 6 months (Ranganna, 11). Energy value was calculated by taking into account the amount of sugars, proteins and fats content present in the squash (Sharma and Tandon, 13). 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, taste and overall acceptability (Ranganna, 11). The antimicrobial activity of the developed beverages against *E. coli* was measured by well diffusion method (Aneja, 2) and 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*. (7), where DPPH (2, 2 diphenyl-1-picrylhydrazyl) was used as a source of free radical.

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 (Cochran and Cox, 8).

RESULTS AND DISCUSSION

In the present investigation, while optimizing suitable blend of *Aloe vera* and aonla juice, the colour score of the beverages was found to range between 7.2 to 7.5, which increased gradually with increase in aonla juice incorporation (Fig. 1). It was observed that the beverages having higher levels (up to 85%) of *A vera* juice obtained higher body scores (7.5), while further increase in *A vera* juice content reduced the body score gradually to minimum (7.0). The taste profile among different beverages ranged between 6.75 and 8.0 with maximum score recorded in treatment T_7 (AVJ 65% + AJ 35%) whereas minimum in control sample (100% AVJ). The beverages having higher *A vera* juice concentration resulted in drastic reduction of taste score which might be due to bitter taste of *Aloe vera* juice. The highest overall acceptability score (7.75) was recorded in T_7 followed by T_8 which remained statically significant ($p<0.05$) with rest of the samples (Fig. 1). The possible reason for this may be improvement in mouth feel of product by blending with *aonla* juice. Various authors have reported improvement in sensory quality of beverages prepared by blending different fruit juices (Sasikumar *et al*., 12; Sharma and Tandon, 13; Bhardwaj and Pandey, 5). Therefore, based on sensory evaluation, the squash (T_7) prepared by using 65% *Aloe vera* juice + 35% aonla juice with 30% blended juice and 40º B TSS, was adjudged the best by the panelists. Further,

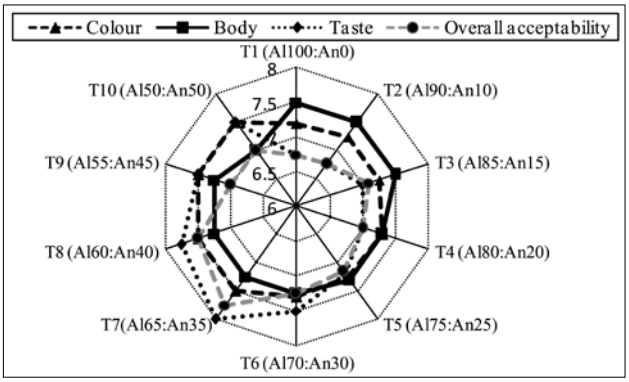

Fig. 1. Sensory evaluation of different *A. vera-aonla* blended functional squash.

a comparison of data presented in Table 1 revealed that addition of aonla juice had also improved the nutritional quality of the squash as evident from its higher ascorbic acid (78.65 mg/100g) and total phenolic contents (37.15 mg/100g) compared to standard *Aloe vera* squash (32.25 mg/100g and 15.80 mg/100g), respectively. Similarly, Jain and Khurdiya (9) reported vitamin C enrichment of fruit juice based RTS beverage through blending with Indian gooseberry juice. The blended squash was also found to have higher antioxidant potential (66.90 % free radical scavenging activity) and strong antimicrobial activity against human pathogen *E coli* (28.50 mm inhibition zone). Improvement in nutritional quality attributes of beverages prepared by blending different fruit juices/pupls has been reported earlier (Bhardwaj and Pandey, 5; Sharma and Thakur, 15). Hence, the treatment T_7 was selected for further preparation of low calorie *A vera* - aonla blended beverage by replacing sucrose sweetness with the sweetness of stevioside.

The chemical changes in physico-chemical characteristics of low calorie *Aloe vera*- aonla squash during storage are presented in Table 2. Perusal of data shows that total soluble solids (TSS) among different treatments decreased continuously as the per cent share of stevioside sweetness increased. It might be due to the reason that stevioside do not add to the TSS (Sharma and Tandon, 13). On the preparation day, maximum TSS (40.05ºB) was recorded in LT_2 (90%S+10%NS) and minimum (7.50ºB) in LT_{10} (100% St) which increased to 41.15ºB and 9.00ºB, respectively after 6 months of storage

Table 1. Physico-chemical, nutritional and sensory characteristics of standard *Aloe vera* and *Aloe vera*-aonla blended squash.

Parameters	*Aloe vera* (100%) sqaush*	*Aloe vera* – aonla (65:35) blended squash*
TSS (°B)	40.0 ± 0.20	40.0 ± 0.50
Acidity (%, CA)	1.28 ± 0.05	1.20 ± 0.07
pH	2.30 ± 0.04	2.50 ± 0.02
Reducing sugars	20.50 ± 0.90	25.85 ± 1.02
Total sugars	36.12 ± 1.35	38.75 ± 1.15
Ascorbic acid	32.25 ± 2.63	78.65 ± 2.85
Total phenols	15.80 ± 2.08	37.15 ± 1.75
Antioxidant activity (%)	38.80 ± 0.95	66.90 ± 0.88
Antimicrobial activity (zone of inhibition, mm)	26.0 ± 1.05	28.50 ± 1.12

* Mean ± SD with each value a average of 3 determinations; SD = Standard Deviation

Table 2. Changes in TSS, titratable acidity, ascorbic acid, reducing sugars, and total phenols of *Aloe vera*: aonla blended low calorie squash during storage.

Storage period (months)/ treatments	TSS (ºB)				Titratable acidity (%)				Ascorbic acid (mg/100 g)				Reducing sugars (%)				Total phenols (mg/100 g)			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
LT_1	40.00	41.00	42.00	41.00	1.20	1.20	1.22	1.21	78.65	75.51	72.84	75.67	25.85	26.91	27.25	26.67	34.00	30.80	26.15	30.31
LT_2	40.05	40.66	41.15	40.62	1.20	1.20	1.22	1.21	80.00	76.82	73.08	76.63	24.75	25.35	26.08	25.39	34.15	31.50	26.25	30.63
LT_3	39.35	40.50	41.10	40.31	1.22	1.23	1.24	1.23	85.20	82.50	78.32	82.00	23.25	24.08	24.85	24.06	35.50	31.85	26.60	31.31
LT_4	35.15	36.00	36.60	35.91	1.20	1.21	1.24	1.22	76.56	74.05	70.85	73.82	20.35	21.16	21.80	21.10	36.00	32.75	27.85	32.20
LT_5	33.02	33.80	34.20	33.67	1.22	1.22	1.24	1.22	81.50	79.10	76.86	79.15	15.20	16.32	17.50	16.34	38.30	34.25	28.50	33.68
LT_6	28.65	29.15	29.80	29.20	1.21	1.22	1.23	1.22	78.60	76.41	72.15	75.72	12.00	13.65	14.36	13.34	38.85	34.00	28.50	33.78
LT_7	24.25	25.05	25.70	25.00	1.20	1.20	1.22	1.21	80.50	77.25	72.00	76.58	10.75	12.18	13.02	11.98	39.62	34.00	28.50	34.04
LT_8	19.00	20.50	21.00	20.16	1.22	1.22	1.24	1.22	80.65	76.76	70.50	75.97	7.10	7.86	8.94	7.97	39.75	34.36	30.00	34.70
LT_9	13.40	14.25	15.05	14.23	1.22	1.23	1.25	1.23	80.50	78.50	71.25	76.75	5.28	6.00	6.78	6.02	40.05	35.05	30.10	35.06
LT_{10}	7.50	8.26	9.00	8.25	1.20	1.22	1.25	1.22	80.50	76.50	70.05	75.68	3.10	4.02	4.85	3.99	41.00	35.50	32.30	36.26
Mean	28.03	28.91	29.56	28.83	1.20	1.21	1.23	1.22	80.26	77.34	72.79	76.79	14.76	15.75	16.54	15.68	37.72	33.40	28.47	33.20
	$CD_{0.05}$ Treatment (T) : 0.25 Storage (S) : NS T × S : NS				$CD_{0.05}$ Treatment (T) : NS Storage (S) : NS T × S : NS				$CD_{0.05}$ Treatment (T) : NS Storage (S) : 0.62 T × S : NS				$CD_{0.05}$ Treatment (T) : 1.62 Storage (S) : NS T × S : NS				$CD_{0.05}$ Treatment (T) : NS Storage (S) : 0.12 T × S : NS			

period. The increase in TSS during storage was found to be non-significant however; slight increase might be due to solubilization of pulp constituents into simple sugars (Boghani *et al.,* 6; Barwal *et al*., 3). A slight increase in acidity was observed during storage which might be due to release of acids from juice by autolysis (Sasikumar *et al*., 12). The mean value of ascorbic acid content among different treatments decreased from an initial value of 80.26 mg/100g to 72.79 mg/100 g after 3 and 6 months of storage, respectively. Significant decrease in ascorbic acid during storage may be attributed to its degradation into dehydro-ascorbic acid, furfural and hydroxy furfural at ambient conditions (Barwal *et al*., 4). Perusal of data presented in Table 2 revealed that with the increase in proportion of stevioside, corresponding decrease in sugar contents was registered. It might be attributed to the fact that stevioside is carbohydrate free, so does not contribute to reducing sugars during analysis (Kumar *et al*., 10). On the preparation day, highest reducing sugars (25.85%) were recorded in LT_1 and lowest (3.10%) in LT_{10}. The increase in reducing sugars during storage might be due to hydrolysis of polysaccharides to reducing sugars in the presence of citric acid (Sharma and Thakur, 15). The phenolic contents in different beverages varied insignificantly between 30.31 mg/100g to 36.26 mg/100g (Table 2). Whereas, during storage it decreased from an initial mean value of 37.72 mg/100g to 28.47 mg/100g after 6 months. The decrease in total phenolic contents during storage might be due to their involvement in the formation of polymeric compounds by complexing with protein and their subsequent precipitations (Sharma and Thakur, 15; Barwal *et al*., 4).

Among different treatments, the sensory appearance score ranged between 7.20 to 7.50 (Table 3). It was observed that at higher concentrations of stevioside (>70%), the appearance score decreased. However, addition of sorbitol @10% has improved the overall appearance/ body of the beverages as it is an excellent bulking or bodying agent and has good heat stability (Barwal *et al*., 3). Significantly higher mean scores for taste (7.93) and overall acceptability (7.60) were recorded in treatment LT_8 (30% sucrose+70% non-nutritive sweetener), whereas minimum was recorded in LT_{10} (100% stevioside). It might be attributed to the bitter after taste of stevioside which becomes more evident at higher concentrations. Our findings are in line with the observations of Sharma and Tandon (13) in low calorie bitter gourd spiced squash. Slight decrease in taste and overall acceptability scores during storage could be possibly due to the loss of volatile aromatic substance in storage at ambient conditions (Sharma and Thakur, 15). However, interaction between treatment and storage intervals was found non-significant. The results pertaining to energy value of different low calorie *A vera*- aonla blended beverage are presented in Fig. 2. The

Table 3. Changes in sensory appearance, taste and overall acceptability of *Aloe vera*: aonla blended low calorie squash during storage.

Storage period (months)/ treatments	Appearance				Taste				Overall Acceptability			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
LT_1	7.25	7.25	7.20	7.23	7.75	7.68	7.50	7.64	7.50	7.25	7.10	7.28
LT_2	7.20	7.20	7.10	7.17	7.25	7.00	6.90	7.05	7.20	7.00	6.80	7.00
LT_3	7.22	7.20	7.00	7.14	7.50	7.30	7.20	7.33	7.50	7.30	7.00	7.27
LT_4	7.35	7.30	7.15	7.27	7.75	7.65	7.50	7.63	7.60	7.50	7.10	7.40
LT_5	7.40	7.30	7.20	7.30	7.65	7.60	7.50	7.58	7.70	7.50	7.20	7.47
LT_6	7.45	7.45	7.20	7.37	7.85	7.75	7.60	7.73	7.80	7.50	7.20	7.50
LT_7	7.50	7.50	7.30	7.43	8.00	7.80	7.60	7.80	7.75	7.50	7.20	7.48
LT_8	7.50	7.45	7.30	7.42	8.20	8.00	7.60	7.93	8.00	7.60	7.20	7.60
LT_9	7.30	7.30	7.10	7.23	7.00	6.80	6.50	6.77	7.00	6.60	6.20	6.60
LT_{10}	7.20	7.00	7.00	7.07	6.50	6.30	6.05	6.28	6.50	6.20	6.00	6.23
Mean	7.34	7.30	7.16	7.26	7.55	6.99	7.20	7.38	7.46	7.20	6.90	7.18
	$CD_{0.05}$ Treatment (T) : 0.04 Storage (S) : NS T × S : NS				$CD_{0.05}$ Treatment (T) : 0.08 Storage (S) : NS T × S : NS				$CD_{0.05}$ Treatment (T) : 0.11 Storage (S) : 0.04 T × S : NS			

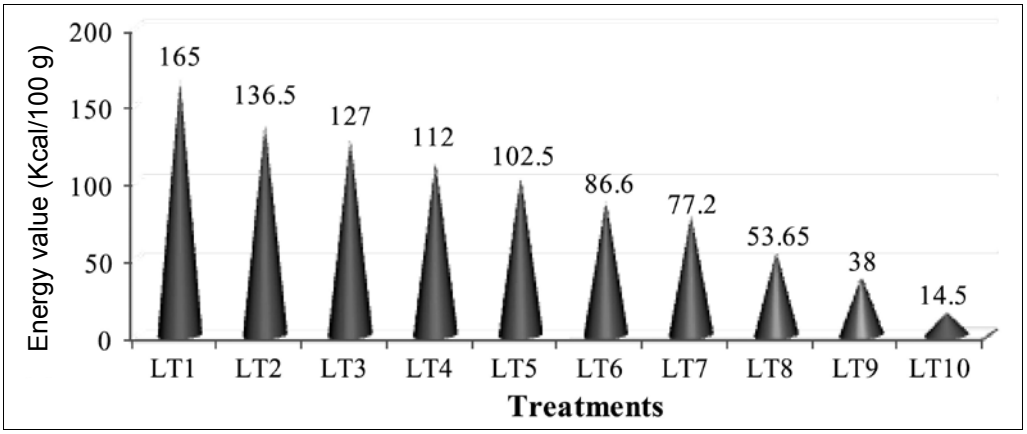

Fig. 2. Calculated energy value of different *A vera*- aonla blended low calorie functional squash.

highest calculated energy value (165.0 Kcal/100g) was obtained by the control treatment LT_1 (100% sucrose), whereas the lowest was recorded in LT_{10} (100% stevioside sweetened). It can be seen that with the increase in per cent share of stevioside, the calorie/energy value of beverages decreased which might be due to the fact that stevioside is zero energy high intensity sweetener (Kumar *et al*., 10). The best rated treatment (LT_8= 30% sucrose : 70% NS) had 53.65 Kcal/100g energy value and the developmental effort has successfully reduced the energy value up to 56% per serving when 70% sugar was substituted with the sweetness of stevioside and sorbitol. Sharma and Tandon (13) had also attained 60% reduction in calorie value per serving at 75% sweetness level of stevioside without compromising sensory quality in bitter gourd spiced squash.

Conclusively, the results of present investigation provide an effective way of delivering health benefits of *Aloe vera* and aonla to the consumers in the form of a beverage having good palatability, nutritive value besides reduced calorie value.

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