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Evaluation of quality attributes and characterization of bio-active components in betel leaf blended fruit squashes using GC-MS

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Abstract
Betel leaf is a vine belongs to the family of *Piperaceae;* it is mostly consumed in Asia compared to other countries. Betel leaves possess immense therapeutic uses that effectively neutralizes pH imbalances in the stomach and intestine for improving the digestive health. These leaves can be value-added to include in diet. It can be consumed in the form of juices, powders, and dehydrated products to enhance the rate of metabolism. The objective of this study was to increase the consumption rate and to improve the value addition of betel leaves; thus, easily commercialized products have been prepared, that is, the squashes prepared from the betel leaf blended with three different fruits like pineapple, sweet lime and amla. Squash samples prepared with 30% amla juice, pineapple juice, and sweet lime juice with betel leaf juice was found to be more acceptable in terms of sensory and nutritional characteristics. The physicochemical analysis was done for both fresh and stored samples. The results reveal that betel leaf–blended fruit squash is highly acceptable for consumption for people of all age group.

Keywords: Betel leaf, sensory evaluation, squash, value addition

Introduction
Betel vine *(Piper betel Linn.)* belongs to the family of *Piperaceace*, which is one of the most important plants in south East Asia. It is commonly known as pan in India. The main cultivation of this vine is for its leaves as it is considered pivotal in every ceremony. Betel leaves were found in the spirit caves in Thailand in 5500-7000 BC, as stated by anthropologists. Its importance is explained in several ancient historic books known as "Mahavamsa" written in the Pali language (Rai *et al*., 2019) [7]. Majority of the people knew that these leaves are only used for chewing; therefore, these are commonly offered after lunch and dinner during social gatherings. These leaves are fully loaded with medicinal properties. In Ayurveda, *Piper* betel has its own importance for curing different kinds of health issues. Betel leaf extract is generally used for curing various diseases, and it is used as an adjuvant and mixed with various medicines for its superior benefits compared to individual usage. Thu these leaves are beneficial for voice laxative and appetizer (Bissa *et al* 2007) [2]. Due to the presence of the aromatic compounds, these leaves taste pungent (Pradhan *et al*., 2013) [6]. Nutrients and vitamins such as vitamin C, niacin, carotene, thiamine, riboflavin, carbohydrates, fiber, fat, protein, minerals and essential oils, starch, phenyl propane, cavibetol, cyneole, and calcium are present in these leaves, and they are also rich in antioxidants viz. flavonoids, terpenoids, tannins, alkaloids, and saponins. All these provide health benefits to the human body. It also inhibits various adverse effects by curing several diseases such as hypertension, diabetes, brain toxin, boils and abscesses, headache, leucorrhoea, cuts and injuries, ringworm infestation, swelling of gum, voice problems, rheumatism, wound healing, obesity, conjunctivitis, constipation, and abrasion. (Aishwarya *et al.*, 2016) [1]. Although it is having all these medicinal properties, due to its pungency, it is not used in value addition. Betel leaf blended with other fruits may raise the acceptable limit of consumption level along with nutritional benefits.

Materials and Methods
Piper betel leaves of Karpoora variety were procured from the local market in Madurai city, Tamil Nadu, India. Fruit like pineapple **(***Ananas comosus*), sweet lime (*Citrus limetta*), and amla (*Phyllanthus emblica*). Amla were purchased from the college campus (Agricultural College and Research Institute, Madurai, India) without any bruises and of equal size.

Non-perishable items such as sugar and citric acid were purchased in bulk from the local market. Glass bottles and PET bottles (750 ml) with caps were used for storing the prepared squash.

Methodology

The collected fruits were washed thoroughly with clean water. With the help of a sharp knife, pineapple eyes were removed and then cut into small thin pieces for juice extraction. The slices were added to a food blender and blended it for 5-10 minutes. With the help of a strainer, pineapple juice was extracted. Seed was removed from amla and then blended in the mixer for 5 min. With the help of muslin cloth, amla juice was extracted. After removing the outer layer and the fine inner skin of sweet lime, its juice was extracted by running it in a blender.

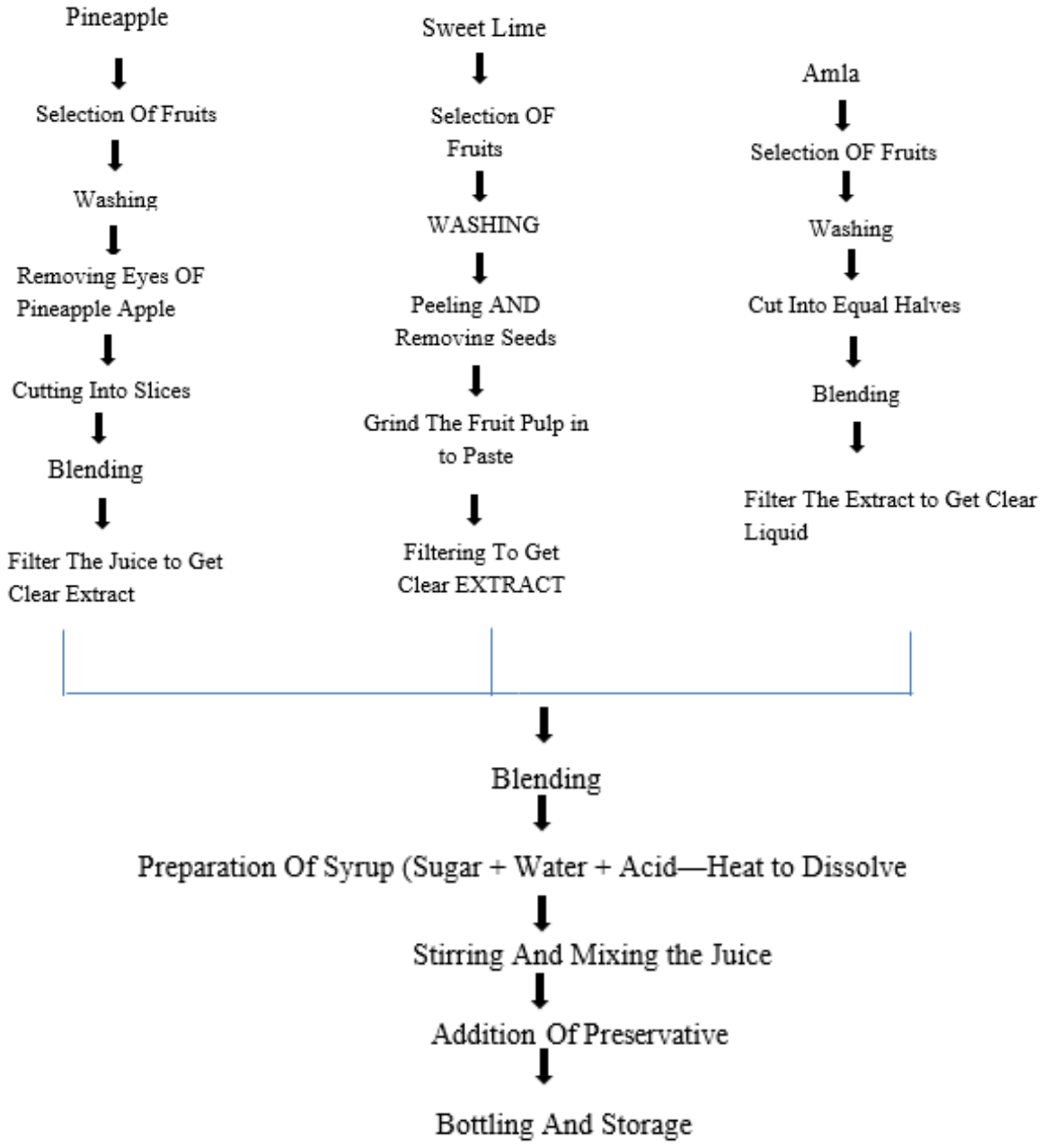

Fig 1: Flowchart for preparation of betel leaf–blended fruit squash

***Piper betel* leave extract**

For extraction, fresh betel leaves of desired variety were washed under running tap water, cut into small pieces with the help of scissors, and then grind it in a grinder for 2-3 min. The leave juice was filtered through folded muslin cloth, and extract was used in squash preparation.

Processing of mixed fruit squash

The betel leaf–blended fruit squash was prepared as per the FSSAI specifications (fruit pulp - 25 %, TSS - 45ºbrix, acidity - 1.0 %, and sodium benzoate - 600 ppm). The sugar syrup was prepared by heating the necessary amount of sugar and citric acid. The prepared syrup was filtered and allowed to cool. Then, required amount of fruit juice was added to the syrup to form desired combination. The required amount of preservative was added to the squash and mixed it well for one last time. The formulated squash was transferred to sterilized PET bottles (750 ml) leaving headspace 2 cm and airtight. Among all combinations, highly acceptable best-blended squash was selected and continued for further storage studies. Samples are stored under room temperature (32±2ºC) and refrigerant conditions (4±1ºC).

GC-MS Analysis

GC-MS analysis of betel squash samples with hexane extract was done by using Shimadzu GC-MS QP- 2020 system comprising of auto sampler and gas chromatograph interfaced with mass Spectrometer (GC-MS) instrument with conditions like : Column Elite -1fused silica capillary column (30mm × 0.25 mm ×I.D × 1µM df , composed of 100 % Dimethyl poly siloxane),operating in electron impact mode at 70 eV ; helium (99.999%) was used as carrier gas at a constant flow of 1 ml / min and an injection volume of 1.0µl was employed (split less) injector temperature 250 °C; ion -source temperature 280 °C .The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/ min, to 200°C, then 5ºC/min to 280 °C, ending with 9 min isothermal at 280ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da.

Sample formulation

10 ml of squash sample was dissolved in HPLC grade hexane in 1:2 ratio followed by sonictaion Squash sample and HPLC grade hexane was used in 1:2 ratio to saturate the samples and then samples are placed is placed in sonicator for 15 minutes and continued it for another cycle and then the volume was made up using the same solvent. After completing the 2 cycles of sonication the solution was filtered using Whatman no.2 filter paper and concentrated on rotary evaporator at 40 °C and final the extract collected and filtered with anhydrous sodium sulphate. The sample for analysis was injected in vials using 2µm syringe filter.

Physico-chemical analysis

The physicochemical parameters were analyzed for the betel leaf–blended squash for every 30-day interval until 180 days. pH was measured according to AOAC 2007 method. Refractometer was used for measuring Total Soluble Solids of the prepared samples. Acidity in the samples were estimated by using titration method. Sugar content of the samples were analyzed using Nelson-Somogyi method (S. Sadasivam 2015). Tannins present in the sample was estimated using Folin-Denis method of spectrophotometer at 700 nm. Phenol was estimated using Folin-Ciocalteau reagent. Total flavonoid content was determined using the aluminum chloride colorimetric assay. Antioxidants were estimated by DPPH method.

Organoleptic score

The organoleptic characters like color, appearance, flavor, consistency, and overall acceptability of the squash samples were evaluated by 20 untrained panelists using nine-point hedonic scale.

Statistical analysis

The statistical analysis was performed for the impact of storage condition by completely randomized design (CRD) using AGRES software at 95% confidence level.

Results and discussion

Results of chemical changes during storage of sweet lime, amla and pineapple squash samples are presented in Fig 2, Fig 3 and Fig 4.

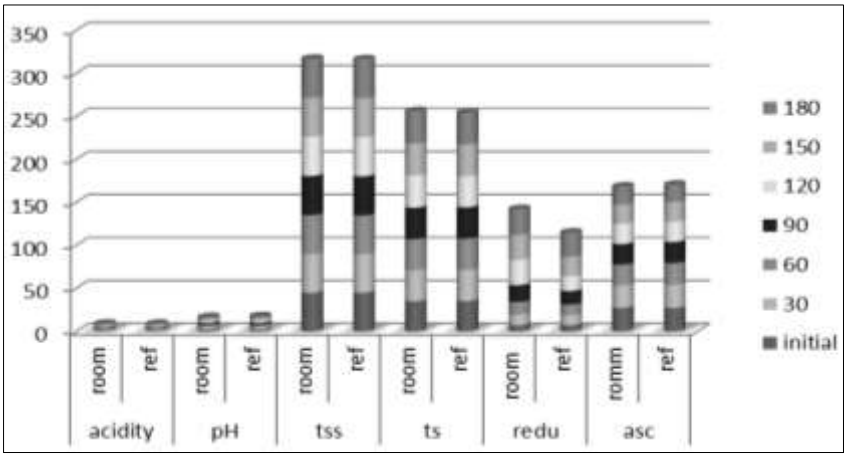

Fig 2: Graphical representation of chemical changes during storage of betel leaf blended sweet lime squash

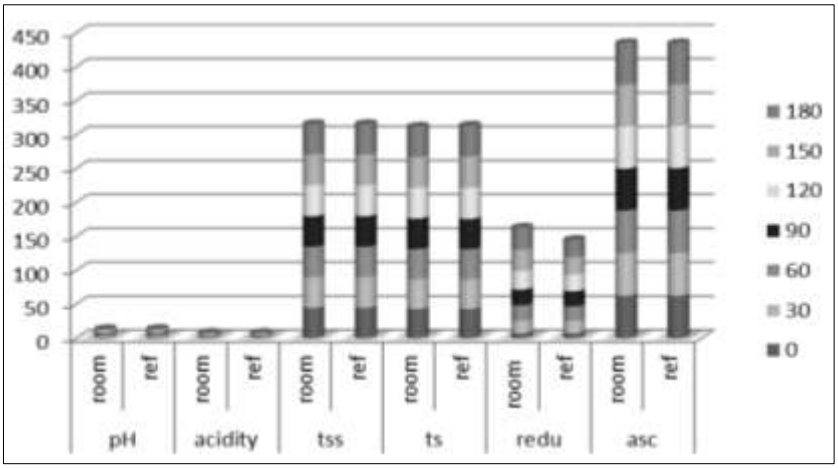

Fig 3: Graphical representation of Chemical changes during storage of betel leaf blended amla squash

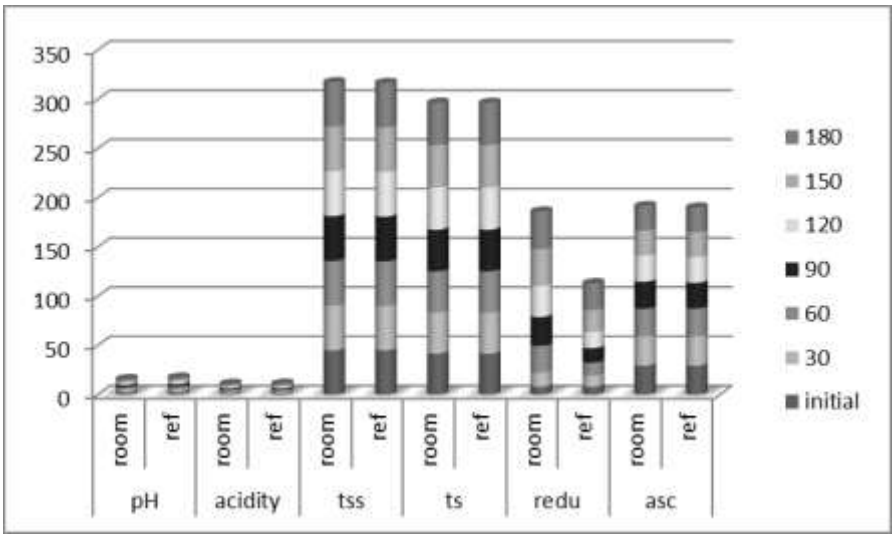

Fig 4: Graphical representation of chemical changes during storage of betel leaf blended pineapple squash

Proximate (control) values sweet lime, Pine apple, Amla squashes

Chemical parameters	Sweet lime	Pineapple	Amla
pH(°Bx)	1.5	3.7	2.7
Acidity (g)	0.4	1.2	1.0
TSS	45	45	45
Ascorbic acid(mg)	15.62	25.06	59.25
Total sugar(g)	32	41.07	43
Reducing sugar(g)	5.3	7.2	23.54

Chemical changes in chemical constituents of betel leaf blended squashes Changes in pH

The changes in pH during storage is presented in Table 1 and graphical depiction is given in Fig 1. The initial pH of the betel leaf–incorporated amla squash was 2.2, and final pH was noted as 1.91 and 1.89 were shown in table 5. From the data, it was observed that betel leaf–blended pineapple squash had pH 2.7 in initial stage and it slowly decreased to 1.99 and 2.0 at both room and refrigeration temperatures at their final stages of storage. For sweet lime, the initial pH was recorded as 2.6, whereas in final stages, it is decreased to 2.0 and 2.52 at room and refrigeration conditions. Selvi (2013) [3] observed gradual decrease in the pH in mixed fruit (i.e., guava, banana, and mango) squash with 4.15 as initial pH and 3.32 and 3.44 at room and refrigeration temperatures, respectively, at 180 days of storage and stated that due to increase in acidity, pH content of the squashes were decreased.

Table 1: Changes in pH during storage of betel leaf-blended pine apple, amla and sweet squash samples

Storage days	Pine apple		Sweet lime		Amla	
	T1	T2	T1	T2	T1	T2
0 days	2.7±0.018	2.7±0.025	2.6±0.016	2.6±0.041	2.2±0.005	2.2±0.059
30	2.5±0.008	2.69±0.023	2.6±0.005	2.59±0.038	2±0.016	2.19±0.052
60	2.4±0.416	2.68±0.006	2.5±0.022	2.58±0.0473	1.97±0.012	2.18±0.056
90	2.3±0.419	2.59±0.007	2.5±0.040	2.55±0.069	1.95±0.031	2±0.0598
120	2.0±0.810	2.55±0.0518	2.4±0.076	2.54±0.019	1.94±0.017	1.9±0.056
150	2.1±0.038	2.19±0.016	2.3±0.050	2.53±0.053	1.92±0.007	1.97±0.057
180	1.99±0.040	2.0±0.020	2±0.029	2.52±0.039	1.91±0.062	1.89±0.047

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.02367	0.04779**	0.02215	0.04471**	0.02266	0.04574**
P	0.01265	0.02554*	0.01184	0.02390**	0.01211	0.02445 NS
SP	0.03348	0.06758**	0.03132	0.06322**	0.03205	0.06469**

*SED – Standard Error Difference, CD-Critical Difference

Changes in acidity

The changes in acidity during storage are presented in Table 2 and graphical depiction is given in Fig 2. An increasing trend was observed in the acid content of the betel leaf–incorporated fruit squashes. The initial acid content of betel leaf blended with amla is 1g, and it was raised to 1.3 and 1.27 at room and refrigeration temperatures, respectively, at 180 days of storage. The data for betel leaf–blended pineapple was showing initial as 1.56 percent, and in its final days of storage, it was recorded with 1.62 and 1.66 percent at both room and refrigeration temperatures, respectively. The initial and final values for betel leaf blended with sweet lime are noted with 1.26 in initial stage and 1.37 and 1.36 percent at final stages at room and refrigeration temperatures, respectively, after 180 days of storage. Thirukkumar (2018) [8] in his research on noni fruit juice blended with amla squash stated that the increasing trend in the acidity in the squash during storage was mainly due to methyl groups of pectins to liberate free –COOH groups the Kerb's cycle will contribute to acidity and interaction of citric acid in the beverages

Table 2: Changes in acidity during storage of betel leaf-blended pine apple, amla and sweet squash samples.

Storage days	Pine apple (g/100 ml)		Sweet lime (g/100 ml)		Amla (g/100 ml)	
	T1	T2	T1	T2	T1	T2
0 days	1.56±0.025	1.56±0.003	1.26±0.005	1.26±0.014	1±0.017	1±0.018
30	1.57±0.049	1.58±0.065	1.28±0.029	1.27±0.042	1.1±0.035	1.12±0.018
60	1.57±0.058	1.59±0.074	1.29±0.038	1.28±0.019	1.13±0.014	1.17±0.032
90	1.58±0.009	1.62±0.044	1.34±0.040	1.31±0.015	1.21±0.036	1.2±0.02
120	1.59±0.050	1.62±0.03	1.35±0.022	1.32±0.007	1.24±0.010	1.22±0.033
150	1.61±0.002	1.64±0.058	1.35±0.0358	1.36±0.012	1.28±0.003	1.24±0.031
180	1.62±0.066	1.66±0.027	1.37±0.0177	1.36±0.020	1.3±0.0256	1.27±0.023

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.01274	0.02571**	0.01274	0.02571**	0.01274	0.02571**
P	0.00681	0.01374NS	0.00681	0.01374 NS	0.00681	0.01374 NS
SP	0.01801	0.03636**	0.01801	0.03636**	0.01801	0.03636**

*SED – Standard Error Difference, CD-Critical Difference

Changes in TSS

The changes in TSS during storage is presented in Table 3 and graphical depiction is given in Fig 3. At the initial stages, squash had 45º brix of total soluble solids, and during the storage periods, it was observed that there is a slight decrease in both room and refrigeration temperatures. The statistical analysis showed 45.4 and 45.35˚brix at 180 days of storage at both room and refrigeration conditions. For betel leaf blended with pineapple, it was 45.21 (table 1) and 45.4˚brix in betel leaf–blended sweet lime (table 3), it was recorded as 45.21 at both room and refrigeration conditions. Similar trend was reported in the study by Arun (2019) on papaya and mango squash with maximum TSS 45.5˚ brix and minimum value of 43.9º brix in seedless papaya.

Table 3: Changes in TSS (º Bx) during storage of betel leaf-blended pine apple, amla and sweet squash samples.

Storage days	Pine apple		Sweet lime		Amla	
	T1	T2	T1	T2	T1	T2
0 days	45±1.272	45±0.742	45±1.03	45±0.29	45±0.477	45±0.212
30	45.46±0.433	45±1.347	45±0.918	45±0.428	45±1.071	45±1.102
60	45.46±0.77	45.41±0.94	45.5±0.092	45±1.439	45±1.255	45±0.401
90	45.46±0.711	45.39±1.28	45.44±0.92	45.44±0.92	45±0.061	45±1.05
120	45.44±0.98	45.36±0.80	45.43±0.64	45.42±0.370	45.48±1.206	45.45±0.987
150	45.32±0.585	45.32±1.42	45.43±1.23	45.4±0.463	45.4±0.154	45.44±0.092
180	45.21±0.30	45.21±1.29	45.41±0.95	45.4±0.926	45.4±0.864	45.35±0.276

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.45656	0.92159NS	0.43189	0.87180 NS	0.46799	0.94467 NS
P	0.24404	0.49261 NS	0.23086	0.46600 NS	0.25015	0.50495 NS
SP	0.64567	1.30332 NS	0.61079	1.23291 NS	0.66184	1.33596 NS

*SED – Standard Error Difference, CD-Critical Difference

Changes in total sugar

The changes in total sugar during storage is presented in Table 4 and graphical depiction is given in Fig 4. The initial total sugar content of betel leaf–incorporated amla squash is 45.24 and it was reduced in the final stages (table 6). In the last 180 days of storage, the data show that the squash having 43.44 and 42.15 g/100 ml in room and refrigeration storages. Similarly, in the betel leaf blended with pineapple squash, initially, it was 43.4, and then, it showed decrease in final stages which was recorded as 41.7 and 39.5 g/100 ml in room and refrigeration stages of storage. For betel leaf–blended sweet lime, it was 37.54 at initial and 35.4 and 33.42 g/100 ml at final storage time at room and refrigeration temperatures. This might be due to polymerization of sugars in the presence of acids. Papaya-based mixed fruit squash of Vennila *et al.* (2015) [9] stated that six months of study at room temperature had showed change in the total sugar content from 36.94 to 32.92 %.

Table 4: Changes in Total sugar during storage of betel leaf-blended pine apple, amla and sweet squash samples.

Storage days	Pine apple (g/100 ml)		Sweet lime (g/100 ml)		Amla (g/100 ml)	
	T1	T2	T1	T2	T1	T2
0 days	43.40±0.1	43.40±0.7	37.54±0.5	37.54±0.6	45.24±0.45	45.24±0.39
30	43.38±0.7	43.24±1.4	37.25±0.4	37.19±0.7	45.16±1.07	45.20±0.30
60	43.24±0.1	43.16±1.2	36.55±0.8	37.02±1.1	45.05±0.42	44.56±0.57

90	42.85±1.2	42.29±0.3	36.48±0.7	36.39±0.17	44.29±1.08	44.25±0.33
120	42.56±0.8	41.28±1.0	36.29±0.4	35.14±0	44.24±0.09	44.03±0
150	41.25±1.3	40.52±0.2	36.16±0.8	34.02±0.1	43.58±1.09	43.25±1.08
180	41.07±0.2	39.05±0.1	35.4±0.6	33.42±0.7	43.44±0.26	42.15±0.65

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.45049	0.90934 ^{NS}	0.39955	0.80652*	0.38119	0.76946*
P	0.24080	0.48606 ^{NS}	0.21357	0.43110 ^{NS}	0.20376	0.41129 ^{NS}
SP	0.63709	1.28600 ^{NS}	0.56505	1.14059 ^{NS}	0.53909	1.08818 ^{NS}

*SED – Standard Error Difference, CD-Critical Difference

Changes in reducing sugar

The changes in reducing sugar during storage is presented in Table 5 and graphical depiction is given in Fig 5. Reducing sugars showed increasing trends along with increase in the storage periods. The initial reducing sugar in betel leaf–blended amla squash is 7.72 g/100 ml, and it gradually increased to 32.91 and 26.14 g/100 ml in room and refrigeration period after 180 days of storage period (table 6). In betel leaf–blended pineapple squash, the initial value was recorded as 8.12, but after reaching 180 days of storage, it reached to 38.43 and 27.43 g/100 ml in room and refrigeration time (table 2). In the case of betel leaf blended with sweet lime squash, initial value was noted as 7.49, whereas at 180 days of storage, it was 30.43 and 28.36 at both room and refrigeration storage conditions. Ibrahim *et al*. (2017) [4] study of tamarind plum squash showed that the reducing sugars of tamarind plum was 17.10 at initial day and reached to 28.10 to 33.23 in different temperature conditions. The study also stated that it was due to the reduction in the sucrose and concluded it by stating that irrespective of time periods reducing sugars increased.

Table 5: Changes in reducing sugar during storage of betel leaf-blended pine apple, amla and sweet squash samples

Storage days	Pine apple (g/100 ml)		Sweet lime (g/100 ml)		Amla (g/100 ml)	
	T1	T2	T1	T2	T1	T2
0 days	8.12±0.786	8.12±0.639	7.49±0.008	7.49±0.136	7.72±0.18	7.72±0.81
30	14.19±0.397	11.19±0.501	12.1±0.304	11.53±0.039	18.14±0.024	18.01±1.179
60	27.21±1.082	13.22±0.333	14.91±0.44	12.43±0.262	22.71±0.030	21.31±0.121
90	29.43±0.201	14.73±1.309	19.51±0.398	15.64±0.127	23.43±0.33	22.43±0.883
120	31.41±0.319	16.42±0.279	29.13±0.376	17.26±0.540	27.18±0.110	24.14±0.372
150	37.41±1.315	22.19±0.49	29.01±0.256	22.48±0.703	31.41±0.72	25.73±0.780
180	38.43±1.151	27.43±0.094	30.43±0.242	28.36±0.424	32.91±1.07	26.14±0.473

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.38532	0.77778**	0.17612	0.35551**	0.32483	0.65568**
P	0.20596	0.41574 ^{NS}	0.09414	0.19003**	0.17363	0.35048**
SP	0.54492	1.09995 ^{NS}	0.24908	0.50277 **	0.45938	0.92728**

*SED – Standard Error Difference, CD-Critical Difference

Changes in ascorbic acid

The changes in ascorbic acid during storage are presented in Table 6 and graphical depiction is given in Fig 6. Deduction in the content of ascorbic acid was observed throughout the 180 days of storage period. Initially, the ascorbic content was 29.52 and finally 24.9 and 25.13 at room and refrigeration temperatures in betel leaf–incorporated pineapple squash. As in the case of betel leaf– incorporated sweet lime, the initial value of ascorbic acid was 27.65, and in final stages, it was 21.09 and 20.19 at both types of storage conditions.

Table 6: Changes in ascorbic acid during storage of betel leaf-blended pine apple, amla and sweet squash samples.

Storage days	Pine apple Mg/100 ml		Sweet lime Mg/100 ml		Amla Mg/100 ml	
	T1	T2	T1	T2	T1	T2
0 days	29.52±0.15	29.52±0.59	27.65±0.570	27.65±0.325	62.84±1.3	62.84±0.8
30	29.41±0.36	29.5±0.90	26.13±0.124	26.63±0.018	62.84±1.4	62.8±0.1
60	28.32±0.50	28.42±0.61	24.12±0.672	25.39±0.691	62.82±1.8	62.79±0.08
90	27.46±0.85	26.12±0.17	23.71±0.193	24.52±0.133	61.8±1.6	62.64±1.8
120	26.53±0.23	26.08±0.72	23.61±0.433	23.42±0.063	61.79±0.7	61.59±0.3
150	25.47±0.39	25.42±0.50	22.42±0.335	23.12±0.550	61.77±1.8	61.42±0.6
180	24.9±0.81	25.13±0.58	21.09±0.545	20.19±0.549	61.76±0.7	61.4±0.1

*Values are represented as mean ±standard deviation of triplicates
T1: Room temperature T2: Refrigeration

Treatments	Pine apple		Sweet lime		Amla	
	SED	CD (0.05)	SED	CD (0.05)	SED	CD (0.05)
S	0.29408	0.59362**	0.22296	0.45006**	0.64804	1.30811**
P	0.15719	0.31730 NS	0.11918	0.24057 NS	0.34639	0.69921**
SP	0.41589	0.83950**	0.31531	0.63648**	0.91647	1.84994**

*SED – Standard Error Difference, CD-Critical Difference

The graphical depiction of changes in the chemical properties of the developed squashes is given in Fig 2, Fig 3 and Fig 4.

Organoleptic characteristics of betel leaf–blended squash

The betel leaf–blended fruit squashes scored highly acceptable values during the period of storage. Among the three varieties of fruits blended with betel leaves, the mean score of overall acceptability for betel leaf–blended amla was 8.2 and overall acceptability for pineapple was 8.26, whereas for betel leaf–blended sweet lime, it was 8.22 at the end of 180 days. Manimegalai (2003) stated that increased storage period will decrease the overall acceptability trend.

Table 7: Treatment combinations and their sensory attributes of prepared squashes

Squashes	Combinations	Sensory attributes				
Control	Fruit juice	Color	Flavor	Consistency	Taste	Overall acceptability
Betel leaf + amla	30% Betel juice 70 % Amla juice	7.8±0.7	8.1±0.8	7.7±0.8	7.7±0.7	8.2±0.6
Betel leaf + pine apple	30% Betel juice 70% Pineapple juice	8.24±0.4	8.27±0.6	8.09±0.6	8.49±	8.26±0.4
Betel leaf + sweet lime	30% Betel juice 70% sweet lime	8.04±0.6	7.77±0.7	8.22±0.6	8.04±0.7	8.22±0.6

*Values are represented as mean ±standard deviation of triplicates

GC-MS analysis for presence of bio-active components

The analyses of the betel leaf incorporated squashes using GC-MS have revealed the presence of more than 25 bioactive components in all the squash samples. The bio-active components with their biological activity are presented in table 8.

Table 8: GC-MS analyzed compounds of the betel-leaf blended squash samples

S. No	Compounds (amla)	Retention Time	Area %	Biological activity	References
1	Alpha.-Terpineol	12.376	5.36	Antioxidant , Anticancer , Antiulcer, Anti-hypertensive &Antitumor.	Christina khaleel *et al.*, (2018) Saadia Bashir Hassan *et al.*, (2010).
2	Hexadecane	21.436	0.92	Antibacterial and antioxidant activities	Kalpana Devi *et al* ., (2012)
4	n-Nonadecane-1	23.101	2.26	Antimicrobial and cytotoxic properties	Kuppuswamy *et al*.,(2013)
5	Heneicosane	25.495	5.65	Anti asthamatic activity& Anti-microbial	Usha *et al* .,(2019)
6	Eicosane	26.502	1.98	Antibacterial activity , Anti-oxidant Anti-fungal	Ammar *et al*.,(2007)
7	1,4-Dioxane-2,5-diol, 2TBDMS derivative	42.987	3.20	Antibacterial activity	Rajesh 2011
8	Heptadecane	20.268	1.60	Alkane	
9	Nonadecane	23.099	2.80	Antioxidant, Antibacterial 45, Antimicrobial	Mahmoodreza., 2010 Javidnia.,2008
10	Tricosyl acetate	36.860	3.91	Anti-inflammatory	Anjum mobeen 2020
11	Octadecanoic acid, 3-oxo-, ethyl ester	42.640	2.03	Anticancer ,anti -microbial	Sergio rosella *et al.*,2007
12	n-Hexadecanoic acid,	43.458	7.00	Antioxidant, Hypocholesterolemic.	Tulika tyagi *et al.*, (2016)
13	Nonacosane		2.34	antibacterial activity	Mihailovi´c, V 2011
14	Octadecanoic acid	43.059	2.52	Antioxidant,anti-inflammatory	Dr. Dukes,1992-2016
15	Nonadecyl pentafluoropropionate	43.460	0.1	Anti-oxidant	Renukadevi *et al.*, 2011

Conclusion

The study showed that the betel leaves are highly acceptable when they are combined with other raw materials such as fruits, and also these leaves increase the nutrient contents in the squash. Among the three fruits that were blended with betel leaf, pineapple was little higher in overall acceptability compared to betel leaf blended with amla and sweet lime. This can be stored for longer shelf-life at both room and refrigeration temperatures. The appearance of bioactive compounds in the formulated squash will convey that squash will be highly recommended for human consumption

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