Research Article

Utilization of *Carica papaya* Herbal Leaf Extract for Preparation of a Nutraceutical Functional Beverage

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Abstract

Present study aims to utilize the *Carica papaya* herbal leaf extract for the preparation of a functionally enriched beverage because of its reported immunity enhancing properties in various studies. The paper opted for extraction of papaya leaf extract by using cold extraction and hot extraction method with and without water. Further, to overcome the harsh taste of papaya leaf juice, blending with mango pulp was done to optimize the best blend in order to prepare nectar with 20% fruit juice/pulp, 15 TSS and 0.3% titratable acidity. Standardized functional nectar was further analysed for chemical and sensory analysis and stored under ambient and low temperature conditions. The hot juice extract method with 20% water was optimized on the basis of best quality attributes. For preparation of nectar, mango pulp and papaya juice in 70:30 ratio was standardized. Chemically, the prepared beverage had 133.06mg/mL of total sugars, 42µg/100mL of ascorbic acid, 1.07mg GAE/mL of total phenols with 23.39 % antioxidant activity which did not show prominent changes during 90 days of storage. Leaves of *C. papaya* have been reported quite effective in curing various diseases. However, no work has been done to prepare functional beverages of this extract due to its harsh taste. Thus, a blended beverage prepared from mixing mango pulp with papaya leaf extract will be acceptable among consumers due to its pleasant flavour.

Keywords: Papaya, herbal leaf extract, Mango-papaya leaf juice nectar, Antioxidant activity, Diseases

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Introduction

Papaya *(Carica papaya* L.) member of family Caricaceae, usually called versatile horticultural plant or wonder fruit of tropical and subtropical regions is a large quick growing soft stemmed plant with large leaves [1]. The different parts of papaya plant (the roots, stem, latex, leaves, fruits and seeds) are used for various medicinal purposes as claimed traditionally for the treatment of different aliments such as malaria, dengue, diabetes, etc. [2]. The herbal leaves of *Carica papaya* are known to increase the antioxidant activity in the blood. Papaya leaf extract are rich in tannins, flavonoids, glycosides, folic acid, vitamin B_{12}, Vitamin A and C, alkaloids, saponins, etc. and also possess anti-inflammatory, anticancer activity and protection against the oxidative damage [3]. The juice of papaya herbal leaf acts as a synergistic therapeutic dietary supplement in patients with oxidative stress related diseases [4]. Papaya leaf extract helps to increase platelet counts and shorten the hospitalization period during dengue fever [5]. The mechanism of action of *C. papaya* leaf extract has shown very good stabilizing properties to prevent lysis of platelets [6]. There are plenty of studies which reported that juice of papaya leaves increases the platelet production in the treated patient [7]. Thus, papaya leaf juice is considered to exert positive effect for the cure of many diseases [8]. In traditional medicines, the leaves are added into tea to cure malaria while dry leaves are used as cigar [9]. The papaya leaf drink encourages digestion and aid in treatment of ailment such as high blood pressure, chronic indigestion, obesity, overweight and weakening of heart [10]. In India, papaya leaves are being used specifically for treatment of fever, beriberi and asthma [11]. Though, the papaya leaves are store house of many pharmaceutical properties yet these have not been utilized on a commercial scale for preparation of functional food products. Development of products like functional immune boosting beverages from papaya leaf can be an alternative for efficient utilization of leaves. However, bitter taste of the leaf extract makes the product unacceptable by the masses. Preparation of leaf extract and blending of extract with fruit pulps for preparation of different beverages is an alternative for utilization of leaf extract with good functional properties. Keeping in view above points, present study was conducted to process and preserve mango pulp and papaya herbal leaf juice blended functional nectar.

Material and Methods

Selection of raw material

Fresh green papaya leaves of cv. *Arka Surya* were collected from local papaya orchard in district Hamirpur (HP), India (**Figure 1**). Leaves were washed in running tap water followed by shade drying to remove surface moisture. Fresh mango pulp was extracted from the mango fruit cv. *Amrapali* by passing through the pulper (**Figure 2**). The pulp was pasteurized, packed in glass bottles, heat processed and stored in cool dry place.

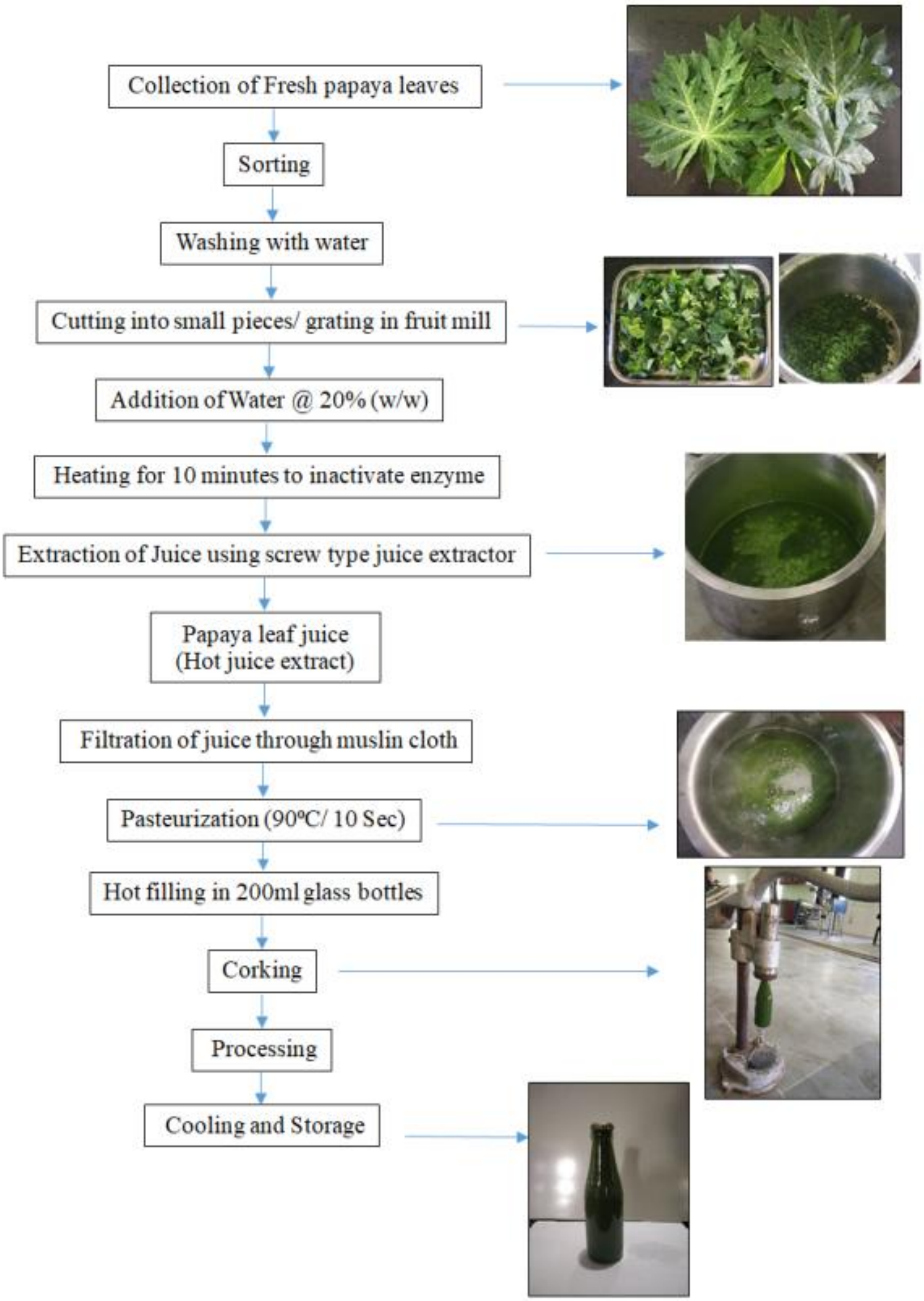

Figure 1 Optimized Flow chart showing processing of papaya leaf juice

Figure 2 (a) Peeled mango, (b) Mango pulp

Papaya herbal leaf juice extraction

Juice from papaya leaf herbs was extracted by using cold and hot pressing methods. In cold extraction (T_1) fresh leaves after washing were crushed in fruit mill and squeezed in screw type juice extractor to extract juice while in the hot pressing method (T_2), the crushed papaya leaves were heated for 10 minutes for inactivation of inherent-enzymes followed by passing through the screw type juice extractor to extract the juice. To standardize the optimum quantity of addition of water in the crushed leaves, the grated leaves were heated after adding 20% (T_3), 30% (T_4) and 40% (T_5) (w/v) water followed by juice extract though screw type juice extractor (M/S Bajaj Process Pack). The juices were pasteurized at 90°C for 10 seconds and packed in pre-sterilized glass bottles prior to their use for development of nectar (Figure 1).

Method for the preparation of papaya leaf herbal juice based mango nectar

For preparing papaya leaf herbal juice based mango nectar having minimum 15°B TSS, 0.3% titratable acidity and 20% fruit pulp/juice, eleven different combinations of papaya leaf juice and mango pulp (0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) were tried. The nectar after homogenization was strained and filled in hot pre-sterilized glass bottles (200 mL capacity) and sealed with crown corks. Sealed bottles were further processed at 85±5°C in hot water for 25 minutes. Bottles were then cooled in air, labelled and stored under ambient temperature (AT) and low temperature (LT) conditions.

Physico-chemical evaluation

Total soluble solids (TSS) was measured in degrees Brix using (Erma) hand refractometer (0-32°B). The pH of the samples was determined by pH meter (Deluxe pH meter Model-101). Buffer solution of pH 7 was used for periodical calibration of pH meter. Titratable acidity was determined by titrating 10 mL of the juice of appropriate dilution with standardized 0.1N NaOH and results were expressed as % citric acid [12]. The ascorbic acid content of the samples was estimated by titration method using freshly prepared 2, 6-dichlorophenolindophenol dye solution to a faint pink colour end point [13]. Reducing and total sugars of papaya leaf juice was estimated by using DNS (Dinitrosalicylic acid) method as an illustrated in Ranganna [12]. For determination of reducing and total sugars, absorbance was taken at 490 and 540 nm to calculate the concentration of reducing and total sugars from the test extract as glucose from the standard curve of glucose. Chlorophyll content in the extracted juice was also estimated [13]. The absorbance of the resultant solution was measured at 645nm, 663nm and 653nm against the solvent as blank and results were expressed as mg/mL. Total phenolic compounds (TPC) were determined in the methanolic extract using the Folin–Ciocalteu Colorimetric method [14]. Antioxidant activity in papaya leaf juice and prepared nectars was estimated according to Brand-Williams *et al*. [15] by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a source of free radical. To 0.1mL of sample extract in the glass cuvette 3.9 mL of 6x10 -5 mol\L DPPH in methanol was placed and the decrease in absorbance was measured at 515 nm for 30 minutes or until the absorbance become steady. Carotenoids were estimated as per the procedure given in Ranganna [12]. The absorbance to determine carotenoids was taken at 452 nm in a Ultra-Violet-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan).

Sensory/organoleptic evaluation

Papaya leaf herbal juice and nectar were evaluated for various sensory attributes such as colour, aroma, taste, body and overall acceptability according to the method described by Rangana [12] using 9-point hedonic scale (9 = like extremely and 1 = dislike extremely). The overall acceptability of the nectar was based on average scores for all sensory parameters. Sample with an average score of 6 and above were considered acceptable.

Statistical analysis of papaya leaf herbal juice extract and mango-papaya nectar

Data on physico-chemical attributes was analysed using completely randomized design (CRD) factorial. The critical difference at 5% level was used to compare different treatments during the storage period. Variance ratio test (f-test) and t-test was applied to study the significance of variation. Data pertaining to the papaya leaf herbal extract and sensory evaluation of nectar prepared from mango pulp and papaya leaf juice at different concentrations were evaluated by using randomized block design (RBD) according to Mahony [16].

Results and Discussion

Papaya leaf herbal juice extracted by using different methods showed significant variation in physical, nutritional and medicinal attributes. The yield of papaya leaf herb extract varied from 44.0 to 58.70% on fresh weight basis among different methods of extraction (**Table 1**). Dilution of crushed mass by adding water up to 10% or even sample heating without addition of water was not found appropriate in pre-liminary studies as it was difficult to heat and hence higher proportion of water i.e. 20, 30 and 40% was tried. Addition of water @ 20, 30 and 40% on the other hand caused increase in resultant juice from 44.00 to 58.70%. Thus, addition of water to grated mass prior to heating was considered appropriate, which helped in better heat transfer to inactivate the inherent enzymes.

Table 1 Effect of different method of juice extraction on chemical characteristics of papaya leaf extract

Treatment		Juice yield (%)	TSS (°B)	pH	Titratable acidity, (%)	Total Phenol, mg GAE/mL extract	Chlorophyll, mg/ mL extract	Reducing sugar, mg/mL extract	Total sugar, mg/mL extract	Ascorbic acid, mg/ mL	Carotenoid (mg/ 100 mL)
T_1	Cold juice extract	45.0	5.10	7.20	0.05	2.31	0.214	3.02	3.04	0.86	2.84
T_2	Hot juice extract	44.0	5.43	6.84	0.06	2.47	0.157	4.90	4.97	0.62	1.80
T_3	Hot juice extract with 20% water	51.5	4.13	6.96	0.05	2.39	0.115	4.11	4.47	0.55	1.71
T_4	Hot juice extract with 30% water	53.6	3.13	7.48	0.03	2.30	0.087	3.29	3.95	0.52	1.50
T_5	Hot juice extract with 40% water	58.7	2.20	7.64	0.02	2.24	0.068	2.98	3.75	0.47	1.20
	$C.D_{0.05}$	1.210	0.261	0.102	0.003	0.100	0.013	0.260	0.460	0.029	0.040

Total soluble solids (TSS) of the papaya leaf extract in all five treatments ranged between 2.20-5.43°B. During analysis of papaya leaf extract, hot juice extract (T_2) was found to exhibit maximum TSS (5.43°B). Addition of water to the grated mass prior to juice extraction caused reduction in TSS. The juice extracted after addition of 20, 30 and 40% water showed TSS in the extract as 4.13, 3.13 and 2.20°B, respectively. Thus, boiling of grated leaves prior to juice extraction caused significant variation in TSS of the leaf juice extract.

Data in the same Table reveals that the pH of the papaya leaf extract in all treatments varied from 6.84-7.64. The juice obtained from T_5 exhibited highest pH (7.64) and minimum (6.84) in T_2. Titratable acidity of all the five treatment of papaya leaf extract ranged in between 0.02-0.06%. The maximum acidity (0.06% as citric acid) was found in T_2 while juice obtained from T_5 showed the minimum acidity (0.02%). Total phenolic content of papaya leaf extract ranged between 2.24-2.47 mg GAE/mL on fresh weight basis. The highest phenolic content was recorded in treatment T_2 as 2.47 mg GAE/mL followed by T_3 as 2.39 mg GAE/mL. Runnie *et al.* [17] earlier reported comparatively higher phenolic content of 4.24± 0.22 mg GAE/gm in dry leaves of papaya. Similarly, Paul and Ghosh [18] found that the heat treatment reduced values of TSS, titratable acidity and ascorbic acid of the pomegranate juice. It was observed during analysis that dilution of grated mass caused decrease in acidity and total phenolic content of the extracted herbal papaya juice. Maskat and Tan [19] during their studies reported that application of heating at different temperature for 5 to 15 minutes resulted into an increase in pH of mengkudu extract as compare to control.

The chlorophyll content in different treatment of papaya leaf herbal extract varied from 0.068-0.214 mg/mL. Maximum chlorophyll content was recorded in T_1 (0.214mg/mL) and minimum was observed in T_5 (0.068 mg/mL). All the treatments were found to be significant for chlorophyll. Further, addition of water along with heating resulted in decrease in chlorophyll content from 0.115 to 0.068 mg/mL as compared to 0.214 mg/mL in cold juice extraction. Shivputra *et al.* [20] in their studies have reported chlorophyll content in the range of 0.085-0.252 mg/gm on fresh weight basis in papaya leaves. Data revealed reducing sugar ranged from 2.98-4.90 mg/mL. The juice obtained from hot juice extract exhibited higher reducing sugar value (4.90 mg/mL) followed by juice in T_3 (hot juice extract with 20% water). Moreover, all the extraction techniques were found to be significant with each other for reducing sugar. Further, addition of water to the grated leaf mass resulted in decrease in reducing sugars from 4.11 to 2.98 mg/mL. Total sugars of papaya leaf extract in all treatments varied from 3.04-4.97 mg/mL. The maximum total sugar (4.97 mg/mL) was observed in T_2 followed by juice in T_3 as 4.47 and minimum as 3.04 mg/mL in T_1. In conformity with the trend of total phenols in herbal juice of different treatments, the total sugar content in the juice extract decreased with the increase in quantity of water to the grated mass prior to juice extraction. Data pertaining to the ascorbic acid content in papaya herbal leaf juice ranged from 0.47 to 0.86 mg/mL. Cold juice extract resulted in maximum ascorbic acid content (0.86 mg/mL) while hot juice extract with 40% water exhibited the minimum (0.47 mg/100mL). A slight decrease was observed in ascorbic acid content with the addition of water from 0.62-0.47 mg/mL. Our results for ascorbic acid are almost in line with the results recorded by Maisarah *et al.* [21] in papaya leaves. The carotenoid content in different treatment of papaya leaf extract varied from 1.20-2.84 mg/100 mL. Maximum carotenoid content was recorded in T_1 (2.84 mg/mL) under cold press condition. Addition of heat reduced carotenoids and all the treatments were found to be significant for carotenoids. Further, addition of water along with heating resulted in decrease in carotenoid content from 1.80 to 1.20 g/mL. Maisarah *et al.* [22] in their studies have reported carotenoid content of 3.86 mg/100 gm on fresh weight basis in papaya leaves which is slightly higher than results reported in this study. They further concluded that leaves of papaya exhibit ascorbic acid and carotenoid content even more than its ripe fruit.

Dilution of crushed mass by adding water up to 20% was considered optimum as it brought about reasonable increase in juice yield. Juice extract obtained from hot juice extract with 20% dilution also exhibited slight changes in chemical constituents thus was considered optimum. Moreover, addition of 20% water during hot juice extraction had positive effect which led to reduction in harshness of papaya extract. Conclusively, it was found that cold juice extraction (T_1) and hot juice extract (T_3) with 20% water dilution exhibited better juice yield, optimum values of TSS, chlorophyll, total phenols, ascorbic acid and thus can be used in papaya leaf juice extraction. Archana *et al.* [23] reported during their studies that the tamarind pulp extracted by hot enzymatic process contained higher amount of TSS, sugars, and lower amount of ascorbic acid in comparison to pulp extracted by cold extraction technique. Trappey *et al.* [24] did extraction mayhaw (*Crataegus opaca Hook*.) fruit pulp and concluded from their analysis that applied heat prior to extraction increases juice extraction. Gahlot *et al.* [25] extracted jamun leaf, curry leaf and litchi leaf extract by hot and cold extraction methods and found that yield of jamun, curry as well as litchi leaf extract was comparatively higher in hot juice extraction than the one obtained by cold extraction method.

Organoleptic evaluation of papaya leaf herbal extract

The organoleptic evaluation of papaya leaf herbal extract was carried out for different sensory attributes like colour, aroma, taste, consistency and overall acceptability on nine point hedonic scale (**Table 2** and **Figure 3**). Juice extraction method exerted a significant influence on the colour of the extracted leaf extract. Apparently cold juice extract was dark greenish in colour while boiling of leaf mass prior to juice extraction caused appreciable change in the appearance of the juice. The appearance of juice from hot extraction was brownish in colour which got diluted to acceptable appearance with the addition of water on dilution. The addition of water to the leaf mass prior of juice extraction caused improvement in colour acceptability of the juice extract with highest hedonic score of 8.50 in hot extract with 20% water addition. Use of water beyond 20% caused significant dilution in the colour of the leaf extract. Different juice extraction methods exerted a significant influence on the aroma and taste of the extracted leaf extract. Among various extraction techniques, the highest scores were obtained for hot juice papaya extract with 20% water. Further, with addition of water on dilution, the aroma goes on decreasing i.e. from 4.96 to 3.50. Taste score of cold juice extract was 2.66 which got increased to 4.83 in hot juice extract with 20% water and afterwards started decreasing again. The addition of water to leaf mass prior to juice extraction caused improvement in taste and aroma of the juice extract with highest hedonic scores in hot juice extracted with 20% water.

Table 2 Effect of different methods of juice extraction on the sensory quality (nine point hedonic score) of papaya leaf juice extract

Treatment/ Method	Colour	Aroma	Taste	Consistency/ Body	Overall acceptability

(T_1) Cold juice extract without water	7.60	4.00	2.66	5.33	4.83
(T_2) Hot juice extract without water	7.50	4.05	3.00	4.33	3.33
(T_3) Hot juice extract with 20% water	8.50	4.96	4.83	6.50	5.16
(T_4) Hot juice extract with 30% water	7.70	3.90	3.83	5.29	4.33
(T_5) Hot juice extract with 40% water	6.50	3.50	2.33	3.66	4.16
$C.D_{0.05}$	0.71	0.85	0.89	0.95	0.75

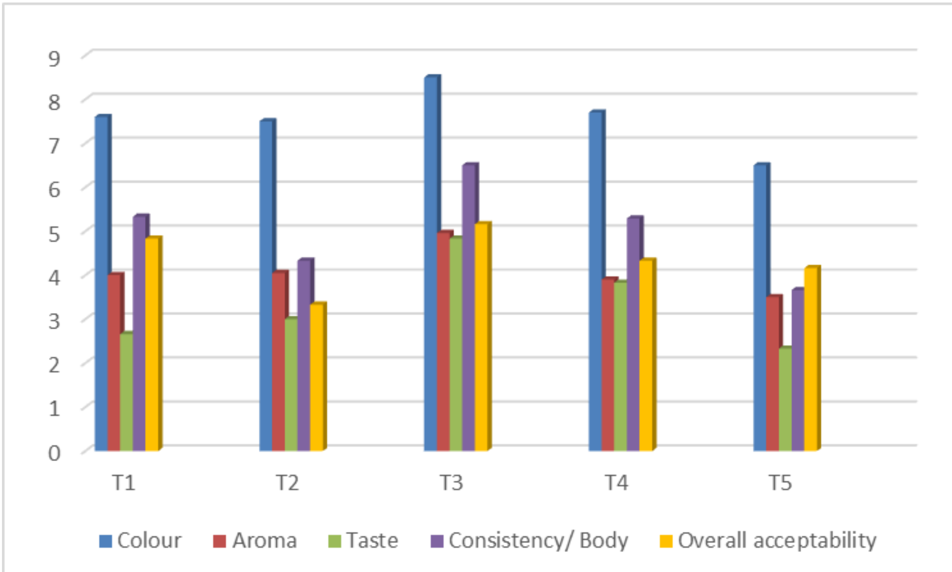

Figure 3 Sensory evaluation of herbal papaya leaf juice extract on 9 point hedonic scale

The consistency/body of papaya leaf herbal extract of all the five treatments ranged in between 3.66-6.50. Juice extraction method exerted a significant influence on the consistency of the extracted leaf juice. Among the various treatments, maximum hedonic score for consistency (6.50) was recorded in hot juice extract with 20% water which was significantly higher in comparison to the remaining treatments. Consistency score decreased in all other treatments with the addition of water to the grated mass for dilution. Among all the treatments under study, treatment (T_3) hot juice extract with 20% water gave the best overall acceptability (5.13) and adjudged appropriate with respect to physico-chemical and sensory characteristics and hence optimized for further experimentation for the development of blended functional nectar.

Among the various extraction techniques, the highest sensorial scores were observed in hot juice herbal extract with 20% water, but taste and aroma scores even with this treatment were not within the acceptable range. Thus, papaya herbal leaf juice extract could not be used as such for consumption due to very harsh and typically unacceptable flavour. Therefore, blending of papaya leaf juice with mango pulp was done to develop a tasty functional drink.

Organoleptic evaluation (9 point hedonic scale) of mango-papaya herbal leaf juice nectars

Sensory attributes of mango-papaya herbal leaf juice nectar is presented in **Table 3**. Various combination of mango-papaya herbal leaf juice nectar prepared by using different proportions of mango pulp and papaya leaf juice responded to the sensory attributes differently. The nectar prepared by using 70% mango pulp and 30% papaya leaf herbal extract was considered acceptable in terms of sensory attributes with colour, aroma, taste, body and overall acceptability scores of 8.13, 8.00, 7.70, 7.90, 8.10, representing very good scores on the 9 point hedonic scale. Further, increase of mango pulp didn't exhibit significant changes in sensorial attributes. Hence, on the basis of sensory attributes of papaya leaf juice based mango nectar, the product prepared by using mango pulp (70%) and papaya leaf juice (30%) was adjudged the best and hence this treatment was used for the preparation of final product (**Figure 4**). Similar trend of results were reported during preparation of papaya and mango blended beverage [24]. They found 70 percent of papaya pulp with 30 per cent mango pulp as highly acceptable for the preparation of blended beverage. Bohra *et al.* [26] recommended a ratio of 65:30:05 of pumelo juice blended with mango ginger and kokum juice on the basis of sensory evaluation. Punam *et al.* [27] prepared blended squash of bael and mango pulp and tried different proportion juice and pulp. They optimized proportion of juice of mango and bael as 75:25. Selvamuthukumaran *et al.* [28] prepared spiced squash of seabuckthorn and pineapple juice with different blended ratios of their juices. They also reported that the beverage prepared from 70 percent sea buckthorn and 30 percent pineapple blend as best blend on the basis of organoleptic scores. Om *et al.* [29] optimized 25 percent aonla juice and 75 percent mango pulp for the preparation of blended squash. Priyanka *et al.* [30] prepared blended squash of jamun and grape juice, the maximum overall acceptability was obtained in the squash prepared from 75 per cent jamun juice and 25 percent juice of grapes. Megha and Alka [31] also prepared value added beverages from papaya leaf juice extract blended with banana shake, mosamobi, pineapple and pomegranate juice and found prepared products acceptable on the basis of sensory evaluation.

Table 3 Sensory evaluation (9-point hedonic scale) of papaya leaf juice based mango nectar

Attributes Mango-PLJ Nectar (Ratio)	**Colour**	**Aroma**	**Taste**	**Consistency/ Body**	**Overall acceptability**
T_1 (0:100)	4.63	3.90	2.93	4.26	4.46
T_2 (10:90)	5.10	4.70	3.90	5.00	5.23
T_3 (20:80)	5.86	5.36	5.10	5.36	5.90
T_4 (30:70)	6.06	5.96	5.90	5.93	6.46
T_5 (40:60)	6.83	6.20	6.06	5.93	7.13
T_6 (50:50)	7.53	7.10	7.06	7.13	7.63
T_7 (60:40)	7.90	7.70	7.50	7.70	7.90
T_8 (70:30)	8.13	7.90	7.70	7.90	8.10
T_9 (80:20)	8.20	7.95	7.80	8.00	8.15
T_{10} (90:10)	8.30	8.05	7.89	8.13	8.25
T_{11} (100:0)	8.45	8.15	8.00	8.25	8.40
$C.D_{0.05}$	0.17	0.23	0.21	0.20	0.30

*On 9-point Hedonic scale (Appendices-I)
PLJ= Papaya leaf juice

Figure 4 Processed blended mango papaya nectar, mango nectar and papaya leaf juice nectar.
(a) Mango- papaya leaf juice nectar, (b) Mango nectar, (c) Papaya leaf juice nectar

Changes in total soluble solids, reducing sugars, total sugars, pH and titratable acidity of optimized blended mango-papaya herbal leaf juice nectar during storage

Changes in total soluble solids (TSS), reducing sugars, total sugars, pH and titratable acidity of mango-papaya herbal leaf juice nectar (70:30) during storage is represented in Table 4. Increase in TSS were recorded from initial value of 15°B to final values of 15.77°B under ambient temperature (AT) and 15.67°B under low temperature (LT) conditions, respectively. There is also an increase in reducing sugars from 49.46 mg/mL to 68.30 mg/mL and 61.30 under AT and LT, respectively. Total sugars got increased from 133.06 mg/mL to 150.74 and 147.84 mg/mL.

Increase in TSS and sugars of nectar during storage were probably due to conversion of left over poly saccharides like starch into soluble sugar. Similar trends of increase in TSS have been reported by Sharma and Singh [32] in lime juice, Patel *et al.* [33] in mango nectar and Datey and Raut [34] in mango nectar during storage. In case of sugars, similar trend of results were reported by Jain *et al.* [35] who observed an increase in sugars in nectar with the advancement of the storage period.

During storage, the pH of nectar decreased from 4.31 to 3.86 under AT and 3.91 under LT conditions, respectively. The reduction in pH could be attributed to simultaneous slight change in acidity of nectar. Byanna and Gowda [36] and Ravani and Joshi [37] have reported decrease in the pH of the RTS beverages during 90 days of storage. Also, Nilugin and Mahendran [38] and Rustagi and Kumar [39] reported that the pH of nectar decreased as the storage period increased. Change in titratable acidity from 0.30% to 0.38% and 0.36% under AT and LT conditions were observed. Kumari and Sandal [40] also reported that there was gradual increase in acidity value with an increase in storage period in mango RTS.

Changes in ascorbic acid, total phenols, carotenoids and antioxidant activity of optimized blended mango-papaya herbal leaf juice nectar during storage

The effect of storage on ascorbic acid, phenols, carotenoid content and antioxidant activity of the prepared nectar is presented in **Table 4**. During 90 days of storage, a significant decrease in ascorbic acid content from 42 µg/100mL to 33 µg/100mL and 35 µg/100mL under AT and LT conditions have been observed, respectively. This reduction was attributed to the oxidation or irreversible conversion of L-ascorbic acid into de hydro ascorbic acid in the presence of enzyme ascorbic acid oxidase caused by presence of entrapped or residual oxygen in the glass bottles. Das [41] also reported decrease in ascorbic acid content in fruit nectar with the advancement in storage period.

The total phenol content in the prepared nectar ranged between 1.07- 1.20 mg GAE/mL during 90 days of storage. Nectar exhibited an increase in total phenol content from 1.07 to 1.17 and 1.20 mg GAE/mL after 90 days at low and ambient temperature conditions, respectively. Similar results have been reported by Beh *et al.* [42] who reported increase in phenolic content with advancement in the storage periods. However, the variation of phenolic content depends upon many factors such as cultivar, harvest date, ripeness, processing technique and location [43].

Table 4 Changes in chemical characteristics of mango- papaya leaf juice nectar during storage at ambient (30-35°C) and low temperature (5±1°C)

Attributes	Initial	30 days		60 days		90 days		F	t Statistic	Probability*
	Zero Day	AT	LT	AT	LT	AT	LT	Statistic		
TSS (^{0}B)	15.00	15.36	15.23	15.56	15.37	15.77	15.67	1.01	2.12	0.10
Reducing sugar (mg/mL)	49.46	56.93	52.88	63.32	58.99	68.30	61.30	1.14	18.73	0.13
Total sugar (mg/mL)	133.06	138.46	136.09	142.80	140.12	150.74	147.84	1.66	2.01	0.11
pH	4.31	4.23	4.25	4.09	4.13	3.86	3.91	1.05	1.73	0.15
Titratable Acidity (%)	0.30	0.32	0.31	0.35	0.33	0.38	0.36	1.02	0.90	0.41
Ascorbic Acid (µg /100mL)	42.0	39.0	40.0	36.0	38.0	33.0	35.0	1.73	2.45	0.07
Total phenol (mg GAE/100 mL)	1.07	1.09	1.12	1.14	1.18	1.17	1.20	1.03	3.67	0.02
Carotenoids (µg/100mL)	22.00	21.55	21.80	21.10	21.68	20.79	21.50	1.09	9.40	0.04
Antioxidant activity (%)	23.39	20.39	21.78	18.41	19.73	16.89	17.42	1.04	10.41	0.03

AT- Ambient temperature (30-35°C), LT- Low temperature (5±1°C)

Carotenoids got decreased from initial value of 22 to 20.79 and 21.50 µg/100mL under AT and LT conditions, respectively. This decrease in carotenoid content might be due to photooxidation of carotenoids which resulted in isomerization of this pigment. The mango-papaya leaf juice (70:30) nectar exhibited decrease of antioxidant activity from 23.39% upto 16.89% and 17.42% during 90 days of storage under AT and LT conditions, respectively. Reduction in antioxidant activity might be due to decrease of ascorbic acid and carotenoids. In fact, antioxidant activity has been correlated with ascorbic acid and carotenoids of the prepared nectar Similarly, Jingfei and Vasantha Rupasinghe [44] reported a decrease in carotenoids and antioxidant activity in apple-carrot juice blends during storage.

Conclusion

On the basis of chemical and organoleptic characteristics of papaya leaf extract, it is concluded that extraction of juice after heating the crushed papaya leaf mass along with 20% water (T_3) was most appropriate for juice extraction. Blending of mango pulp and papaya leaf juice in 70:30 proportions was found optimum for the preparation of tasty functional nectar in order to remove bitterness and harsh flavor of papaya leaf extract in the end product. The beverage prepared by using 20% fruit pulp/ juice consisting of 70% mango pulp and 30% proportion of papaya leaf juice by maintaining 15°B TSS and 0.3% acidity followed by heat processing was adjudged the best with respect to chemical and sensory attributes. The product can successfully be stored up to 90 days at both low (5±1°C) and ambient (27-35°C) temperature conditions. Thus, papaya leaf juice can be utilized for preparation of papaya juice based mango nectar for its regular consumption as an immunity boosting beverage.

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