



## Study of Sensory and Storage Quality of the RTS Juice Enriched with Papaya Leaf Flavonoid

Vignesh D., Tejas Muthal, Chidanand D. V. \* and Sunil C. K.

Indian Institute of Food Processing Technology (IIFPT),  
Ministry of Food Processing Industries (GOI), Thanjavur, Tamil Nadu, India-613005

\*Corresponding Author E-mail: [chidanand@iifpt.edu.in](mailto:chidanand@iifpt.edu.in)

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### ABSTRACT

Fruits and vegetables are known as protective foods as they are rich source of essential vitamins, minerals, fibers and phytochemicals. Therefore, the present study aimed to develop the ready to serve fruit juice product with different formulations of papaya, carrot, mango fruits to increase the nutritional value of final product. The product was added with the papaya leaf flavonoids extracted by using ultrasonication pre-treatment. The effect of addition of the papaya leaf flavonoids was studied and compared on the physicochemical properties and mineral content of the RTS juice. The addition of papaya leaf flavonoids found beneficial for extending the shelf life of RTS juice. The microbial growth was observed in the samples without addition of papaya leaf flavonoids after 30 days of storage; while samples with papaya leaf flavonoids did not show any microbial growth even after 45 days of storage. The sample with blending ratio 60:10:30 of papaya:carrot:mango fruits was accepted in the sensory evaluation. The physicochemical properties of the RTS juice like TSS, total sugars, titratable acidity, ascorbic acid and carotene content found decreasing with increase in the storage time. Finally, it was concluded that the addition of the papaya leaf flavonoids helps in the retention of the nutritional values of the final RTS fruit juice.

**Keywords:** Papaya leaf flavonoids, Ultrasonication, RTS juice, Physicochemical properties, Mineral content, Microbial growth

### INTRODUCTION

Fruits and vegetables are known as protective foods as they are rich source of essential vitamins, minerals, fibers and phytochemicals. They have strong antioxidant potential in scavenging free radicals (Kaur and Maini, 2001). Among them Papaya (*Caricaceae*) is

one of the major fruits grown in tropic regions of India (Vennila, 2007). The papaya fruit having rich source of  $\beta$ -carotene and mango is a good source of natural sugar, vitamins (c), fairly some minerals like calcium, phosphorus and ascorbic acid. (Wall, 2006).

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Papaya leaf extract has been traditionally used as medicine for various stomach disorders and was also found to exhibit antioxidant properties (Rahmat et al., 2004) and the papaya leaf extract has the potential against dengue fever (Ahmad et al., 2011). Papaya leaf are rich in flavonoids (Miean and Mohamed, 2001) and alkaloids (Khuzhaev & Aripova, 2000). The Flavonoid quercetin has the highest binding towards the protease complex and restricts the virus replication (Senthilvel et al., 2013) Both the leaves and the fruits of papaya have carotenoids such as  $\beta$ - carotene, lycopene (Anjum et al., 2013).

Carrot (*Daucus carota*) and mango (*Mangifera indica*) are rich source of carotenoids such as  $\beta$ -carotene and lycopene. These bio compounds are likely to be responsible for different aspects of immunity (Bub et al., 2003).  $\beta$ -carotene is an antioxidant compound which is the precursor of Vitamin A It is responsible for orange, yellow and red coloration in many vegetables and fruit. Specifically, several studies proposed that  $\beta$ -carotene (Carotenoid) will stimulate the immune system (Hughes, 1999). Lymphocytes proliferation in humans is stimulated by supplementation with pure  $\beta$ -carotene (Kramer and Burri, 1997). Therefore, supplementation of  $\beta$ -carotene in regular diet will considerably improve the immune functions in human. These nutritional qualities make carrot and mango efficient ingredients of the blend. De Carvalho et al. (2007) and Yu et al. (2012) improved the nutritional quality of the fruit juices; by following the blending of juices. This can help in enhancing the nutritional content of the fruit or vegetables based on the quality. Acidification could be achieved by blending with acidic fruit juices (Demir et al., 2004). Jan and Masih, 2012 developed blend of juices from pineapple

(*Ananas comosus*), carrot (*Daucus carota*), and orange (*Citrus sinensis*). Okoli and Ezenweke, 1990, have formulated papaya juice beverage to improve its quality and shelf-life of the product.

The ultrasonication treatment was used for the extraction of flavonoids from papaya leaves. In recent years ultrasound has been utilized as a food processing tool for modification of properties of food ingredients, as preservation tool, or for extraction of valuable compounds from vegetal cells (Khuzhaev & Aripova, 2000). Ultrasound can be defined as sound waves whose frequency is higher than 20 kHz. Application of ultrasound in food processing is based on the cavitation effect caused by high frequency ultrasonic waves when the amplitude is sufficiently high, and results in formation of cavitation bubbles in the liquid in which it propagates. These bubbles collapse explosively generating local fluctuations in pressures and high temperatures which are responsible for the mechanical and chemical effect to the nearby materials present (Miean, & Mohamed, 2001, Okoli & Ezenweke, 1990).

The present experiment was aimed to develop and study the physicochemical properties and mineral content of ready to serve fruit juice with incorporation of papaya leaf flavonoids during storage.

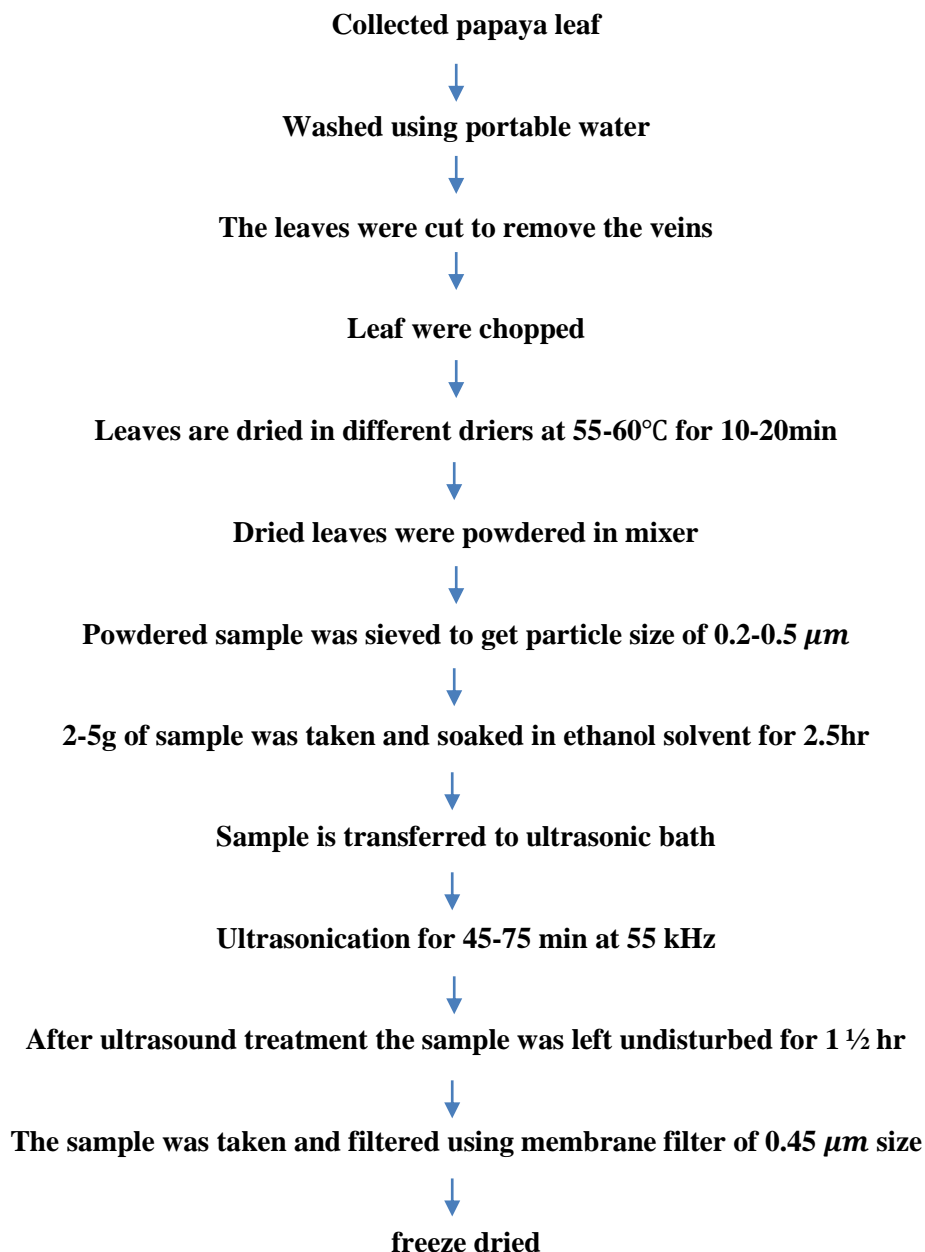
## Materials and methods

### 2.1 Procurement of raw materials

*Carica papaya* leaves were collected from the Nanjikottai, Thanjavur. Papaya, carrot and mango were collected from local cultivars and as well from local market Thanjavur.

### 2.2 Extraction of Papaya leaf flavonoids

Ultrasound assisted extraction method was used in extracting many bioactive compounds (Yang and Zhang, 2008)



### 2.3 Preparation of blended RTS

Fresh fruits of papaya and mango have been sorted based on their colour and healthiness of the fruit. Then it was washed with fresh water, then peeled with stainless peeler and the fruits were sliced and juices were extracted by using the juice blender. Fresh carrots were procured from the market and washed with fresh water, and it was given a treatment by soaking it in sodium hydroxide (40 g/l) at 95°C for 1 min which will make the peeling easy. Then following that blanching treatment, it was soaked with citric acid solution (60 g/l) at 95°C for 5mins this makes the endogenous

enzymes inactive and the tissues will get soften, at the end it was grounded with 1:1 ratio of water and it was filtered using muslin cloth then fresh juice is collected. Juices obtained from Papaya, carrot, and mango blended in different proportions i.e 100:0:0, 80:10:10, 60:10:30, 50:20:30 (Table 1) and with freeze dried powder of papaya leaf flavonoid was added to the blend at concentration ranging from 1-5g.

### 2.4 Physicochemical properties:

#### 2.4.1 TSS

The total soluble solids content is determined by using lab level refractometer (Make:

ATAGO, RX-7000, Japan), as described by (Soltani et al., 2011). A scoop of banana pulp from the apical, middle and basal part of fruit placed on a muslin cloth separately, and a drop of it squeezed out onto the refractometer. In the case of unripe banana, the juice is extracted by squeezing pulp in a muslin cloth, three-four drops of squeezing banana set on the sensing screen of the refractometer. TSS value is expressed in terms of °Brix. The refractometer is based on the principle that as the density of a substance increases, its refractive index rises proportionately. Refractive index (nD) varies in the range

1.32422 to 1.70000. Brix varies in the range of 0.00 to 100.00% (Automatic Temperature Compensation).

#### 2.4.2 Total sugars

50 ml of clarified sugar solution was taken in 250 ml of volumetric flask and 1 mL of HCl was added then the solution was kept overnight for hydrolysis. 1-2 drops of phenolphthalein indicator was added on the next day and the acid was neutralized by adding standard NaOH till magenta colour develop. The volume was made up to 100 ml and titrated against sugar solution until the brick red precipitate was obtained.

$$\text{Total sugars (\%)} = \frac{\text{mg of invert sugar} \times \text{dilution factor}}{\text{titer value} \times \text{sample weight} \times 1000} \times 100$$

#### 2.4.3 Titratable acidity

The titratable acidity was determined by phenolphthalein indicator method as described by Ranganna (2007). Two grams were homogenized in 10 mL of distilled H<sub>2</sub>O, then

filtered through filter paper and made the final volume to 20mL using distilled H<sub>2</sub>O. then titrated against 0.1 NaOH. The formula used for calculating the titratable acidity

$$\text{Titervalue acidity (\%)} = \frac{\text{Titervalue} \times N \text{ of alkali} \times \text{volume made up} \times \text{Eq. wt of alkali}}{\text{volume of sample} \times \text{vol. of aliquot} \times 1000} \times 100$$

#### 2.4.4 Ascorbic acid

The ascorbic acid content of the sample was determined by following the standard method suggested by Ranganna (2007). 10g of sample was mixed with 3% of metaphoric acid and

volume was made to 100ml with metaphosphoric acid and filtered by filter paper. Then 5m of aliquot was taken and was titrated against std. as end point pink colour is observed.

$$\text{Ascorbic acid \%} = \frac{\text{mg}}{100} = \frac{\text{titer value} \times \text{dye factor} \times \text{volume make}}{\text{wt of the sample taken} \times \text{vol of aliquot taken} \times 1000} \times 100$$

#### 2.4.5 β carotene

The total carotenoids content in the samples were determined by method suggested by Arun prabha, 2008, 1g of the samples was taken with petroleum ether (60-80°C), and acetone (3:2) the extract is extracted by grinding in silica dish with glass mortar. The extraction was continued till all fat soluble pigments are taken out and the extract is transferred into volumetric flask and volume is made up with acetone. Finally, the absorbance is measured at 45nm using petroleum ether as blank.

#### 2.4.6 Mineral content

Mineral content of the pre-treated green gram malt was estimated by ICPMS by using AOAC 2016 method.

#### Results and discussion

The ready to serve fruit juice with and without the addition of papaya leaf flavonoids was prepared successfully. The product found acceptable by the consumers. The ultrasonication extracted papaya leaf flavonoids observed with the positive results in the product.

The change in product composition was responsible for the changes in all properties of the product during storage of 45 days. Also, the addition of papaya leaf flavonoids changes the properties of RTS at very extent. The RTS 1 to 4 are prepared with different blending ratio as mentioned in Table 1. While, RTS 6 to 8 are the samples prepared with different blending ration and addition of papaya leaf extract. The results of each properties are described below.

### 3.1 TSS

The TSS values of the samples decreased as increased in the storage period. Samples with papaya leaf flavonoids showed higher TSS than untreated samples. Table 2 represents the TSS values of RTS juice. The samples without papaya leaf flavonoids such as RTS 5, RTS 6, RTS 7, RTS 8 showed the TSS of 10.45, 11.98, 13.27, 14.09 after 45 days of storage respectively. The rate of change of TSS was higher in the samples with papaya leaf flavonoids as compare to samples without papaya leaf flavonoids.

### 3.2 Total sugars

Total sugars content is the important parameter to be checked for RTS juice during storage. The level of total sugars was well maintained in the RTS juice with papaya leaf flavonoids samples as compare to the samples without papaya leaf flavonoids. The sugar levels decreased in samples with papaya leaf flavonoids during the storage period. It means that the addition of papaya leaf flavonoids helps to retain the sugar level in RTS juice during storage period. The change in the sugar level was noted from 14.45, 14.86, 15.28, 15.18 to the 13.11, 13.48, 14.63, 14.66 in the samples without papaya leaf flavonoids respectively. While, in samples with papaya leaf flavonoids the change was observed from 14.42, 14.84, 15.27, 15.14 to the 14.31, 14.74, 15.12, 15.08 respectively (Table 2).

### 3.3 Titratable acidity

The acidity of juice is responsible for defining the shelf life of the product. In case of RTS juice, the titratable acidity increased with the decreasing concentration of papaya fruit pulp for both with and without papaya leaf

flavonoids samples. The rate of change of titratable acidity was high in the samples without papaya leaf flavonoids and low in samples with papaya leaf flavonoids respectively (Table 2). The highest titratable acidity was found in sample with papaya leaf flavonoids RTS 7 after 45 days storage and lowest titratable acidity was found in sample without papaya leaf flavonoids RTS 1.

### 3.4 Ascorbic acid

The papaya leaf flavonoids showed great effluence on the decrease in ascorbic acid content. It helps in the prevention of the ascorbic acid loss during the storage of juice. The stability of the ascorbic acid was found more in RTS juice samples with papaya leaf flavonoids. The changes in the ascorbic acid values during the storage represented in the table 2.

### 3.5 $\beta$ carotene

The retention of the  $\beta$  carotene was more in the treated samples. Hence, it is considered that ultrasonication pre-treatments helps in the retention of the  $\beta$  carotene levels. The rate of decrease in the values were found higher in the untreated samples as compare to the treated samples. The table 5 represents the change in the values of the  $\beta$  carotene content in the RTS juice during storage period. The  $\beta$  carotene was highest in RTS 7 (0.32) and RTS 8 (0.34) respectively (Table 2).

### 3.6 Calcium

The calcium content in samples with papaya leaf flavonoids found stable during 45 days storage. But in samples without papaya leaf flavonoids the calcium content decreased gradually. Addition of papaya leaf flavonoids was not suitable for maintaining the calcium content in ready to serve fruit juice during storage. The change in calcium content of RTS juice samples was shown in Table 3.

### 3.7 Phosphorous

The phosphorous present the juice sample decreased as increased in the storage time. The rate of change of phosphorous in both samples with and without papaya leaf flavonoid was same. The values drop from 1.82, 1.84, 1.88, 1.89, 1.80, 1.83, 1.86, 1.87 to the 1.80, 1.82, 1.85, 1.87, 1.79, 1.81, 1.84, 1.85 in the

samples RTS 1 to RTS 8 respectively. The values are mentioned in the Table 3.

### 3.8 Potassium

The potassium showed the case similar to phosphorous content. Table 3 represents the data for potassium content of all RTS juice samples. The values decreased with increased in the storage time. The highest potassium content was found in RTS 4 (12.78) and RTS 8 (12.79) after 45 days storage period. While, lowest potassium content was observed in RTS 1 (9.37) and RTS 5 (9.33) after 45 days storage time

### 3.9 Iron

Iron content of the juice increased with decreased in the concentration of papaya fruit pulp according to the blending ratio. The values of iron found stable throughout the storage time of 45 days. Initially, the highest values was observed in the RTS with lowest papaya fruit pulp that is RTS 4 (0.12) and RTS 8 (0.11) respectively. The observed values was shown in the Table 3.

### 3.10 Microbial analysis

The overall microbial analysis of all the 8 samples was carried out in order to check the shelf life of the product and to study the effect

of the papaya leaf flavonoids on the storage capacity of the ready to serve juice. The samples with addition of papaya leaf flavonoid did not show any presence of the microbial load during the 45 days storage time. But the samples without papaya leaf flavonoids observed with the microbial growth after 30 days of storage. Hence, it was suggested that the addition of papaya leaf flavonoids would help for extending the shelf life of the ready to serve fruit juice. The values of microbial growth observed after 45 days were 4.08, 4.07, 4.02, 3.03 in the sample RTS 1 to RTS 4 respectively. The results are shown in Table 4.

### 3.11 Sensory evaluation

Sensory evaluation is an important parameter to test the acceptability of the product by consumer. So, the sensory evaluation of the ready to serve fruit juice samples with addition of papaya leaf flavonoids was carried out with the help of 10 pannelist. The RTS 7 sample was found highly accepted before (8.00) and after (7.18) 45 days storage time. The RTS with high concentration of papaya fruit pulp blending ratio (6.01) was not much accepted by the pannelist. Table 5 represents the sensory evaluation data of RTS juice samples.

**Table 1. Blending ratio of ready to serve fruit juice**

Sr. no.	Juice	Blending ratio
1	Papaya: carrot: mango	100:0:0
2	Papaya: carrot: mango	80:10:10
3	Papaya: carrot: mango	60:10:30
4	Papaya: carrot: mango	50:20:30

**Table 2. Change in physicochemical properties of RTS fruit juice**

Storage time (Days)	Samples							
	RTS 1	RTS 2	RTS 3	RTS 4	RTS 5	RTS 6	RTS 7	RTS 8
<b>TSS</b>								
0	11.09	11.07	11.26	14.12	10.53	12.05	13.33	14.12
15	11.09	11.05	11.24	14.10	10.51	12.03	13.31	14.11
30	11.07	11.02	11.21	14.07	10.49	12.01	13.29	14.10
45	11.05	11.00	11.19	14.05	10.45	11.98	13.27	14.09
<b>Total sugars</b>								
0	14.45	14.86	15.28	15.18	14.42	14.84	15.27	15.14
15	14.02	14.69	15.04	15.01	14.36	14.81	15.26	14.11
30	13.67	13.57	14.80	14.82	14.32	14.77	15.23	15.09
45	13.11	13.48	14.63	14.66	14.31	14.74	15.12	15.08

<b>Titratable acidity</b>								
0	0.19	0.24	0.38	0.36	0.17	0.23	0.36	0.34
15	0.16	0.21	0.34	0.33	0.16	0.22	0.35	0.33
30	0.12	0.18	0.32	0.31	0.13	0.18	0.33	0.31
45	0.10	0.16	0.30	0.29	0.11	0.17	0.31	0.27
<b>Ascorbic acid</b>								
0	3.76	5.33	6.74	6.56	3.52	5.21	6.62	6.49
15	3.36	5.28	6.68	6.51	3.48	5.19	6.61	6.46
30	3.12	5.21	6.63	6.45	3.43	5.18	6.58	6.45
45	2.88	5.17	6.58	6.34	3.41	5.17	6.55	6.42
<b>β carotene</b>								
0	0.26	0.31	0.36	0.38	0.24	0.29	0.35	0.36
15	0.23	0.28	0.34	0.35	0.23	0.28	0.34	0.36
30	0.20	0.25	0.31	0.32	0.21	0.27	0.33	0.34
45	0.15	0.22	0.29	0.30	0.20	0.25	0.32	0.34

Table 3. Change in mineral content of RTS fruit juice

Storage time (Days)	Samples							
	RTS 1	RTS 2	RTS 3	RTS 4	RTS 5	RTS 6	RTS 7	RTS 8
<b>Calcium</b>								
0	1.68	1.62	1.56	1.50	1.58	1.53	1.49	1.44
15	1.68	1.62	1.56	1.50	1.58	1.52	1.47	1.42
30	1.67	1.61	1.55	1.49	1.57	1.51	1.45	1.39
45	1.65	1.61	1.55	1.49	1.56	1.50	1.44	1.37
<b>Phosphorous</b>								
0	1.82	1.84	1.88	1.89	1.80	1.83	1.86	1.87
15	1.82	1.84	1.87	1.88	1.80	1.82	1.85	1.86
30	1.80	1.83	1.86	1.88	1.80	1.81	1.84	1.85
45	1.80	1.82	1.85	1.87	1.79	1.81	1.84	1.85
<b>Potassium</b>								
0	9.40	10.56	12.20	12.82	9.35	10.52	12.18	12.80
15	9.40	10.55	12.19	12.80	9.35	10.51	12.17	12.79
30	9.38	10.53	12.19	12.80	9.34	10.51	12.17	12.78
45	9.37	10.51	12.17	12.78	9.33	10.50	12.17	12.76
<b>Iron</b>								
0	0.07	0.09	0.11	0.12	0.06	0.08	0.10	0.11
15	0.07	0.09	0.11	0.12	0.06	0.08	0.10	0.11
30	0.07	0.08	0.10	0.12	0.05	0.07	0.09	0.11
45	0.06	0.08	0.10	0.11	0.04	0.06	0.09	0.10

Table 4. Change in microbial analysis of RTS fruit juice

Storage time (Days)	Samples							
	RTS 1	RTS 2	RTS 3	RTS 4	RTS 5	RTS 6	RTS 7	RTS 8
<b>Microbial growth</b>								
0	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
30	4.05	4.03	4.00	3.02	-	-	-	-
45	4.08	4.07	4.02	3.03	-	-	-	-

Table 5. Sensory evaluation of RTS fruit juice

Storage time (Days)	Samples			
	RTS 5	RTS 6	RTS 7	RTS 8
<b>Sensory evaluation</b>				
0	6.34	6.64	8.00	7.55
15	6.31	6.45	7.88	7.51
30	6.20	6.23	7.55	7.44
45	6.01	6.12	7.18	6.77

## CONCLUSION

The ready to serve fruit juice from the blending of papaya, mango and carrot fruits was successfully prepared and accepted by the consumers. The ultrasonication treatment found effective for the extraction of flavonoids from the papaya leaves. The addition of the papaya leaf flavonoids found beneficial for extending the shelf life of the product as well as to maintain the nutritional values of the final RTS product. The storage studies of the blended fruit juice were done with the evaluation of the properties like TSS, total sugars, titratable acidity, ascorbic acid, carotene content, minerals content, microbial analysis and sensory evaluation respectively. It was observed that the addition of the papaya leaf flavonoids helps in the maintaining the level of minerals in the final products. The microorganisms observed deactivated or suppressed in the samples with addition of the papaya leaf flavonoids. The physicochemical properties such as TSS, total sugars, titratable acidity, ascorbic acid and carotene content of the ready to serve fruit juice was found decreasing with the increase in storage time. The present formulated product with blending ratio 60:10:30 of papaya:carrot:mango which was accepted in the sensory evaluation will help in supplementing the nutrition to the consumers.

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