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# Development of Nutritionally Enriched Apple Based Ready-To-Serve (RTS) Beverage Infused with Papaya Leaf Extract

**Shivangi Bist[1], Gaurav Chandola[1*], Neha Singh[1], Yukta Gupta[1], Nishan C.K[1]**

[1]Department of Food Technology, School of Applied and Life Sciences, Uttaranchal University, Dehradun-248007, Uttarakhand, India

***Corresponding author:** Gaurav Chandola, Assistant Professor, Department of Food Technology, School of Applied and Life Sciences, Uttaranchal University, Dehradun-248007, Uttarakhand, India

**Abstract:** RTS (Ready-to-Serve) fruit beverages are prepackaged refreshing drinks designed for direct consumption. They contain edible parts of fruits, sugar, acid and water. These beverages offer convenience, diverse fruit flavors, and time-saving benefits, therefore, enjoyed by all age groups. However, they typically have low fruit content; high sugar levels and undergoes extensive processing, which can lead to the loss or reduction of certain nutrients such as vitamin C and volatile phytochemicals. Consequently, consumers are increasingly seeking healthier alternatives. To address these issues, development of a nutritionally enriched RTS beverage was attempted in the present study by infusing papaya leaf extract. The RTS beverage was formulated by blending 30% fruit content (apple pulp) with varying concentrations of papaya leaf extract ($T_1$: 2%, $T_2$: 3%, $T_3$: 4%, $T_4$: 5%) and compared against a control sample containing no papaya leaf extract. Sensory evaluation assessed the acceptability of the beverage among consumers. The control sample scored highest for taste (8.14±0.27) while, for color, odor, and overall acceptability, the $T_3$ formulation scored highest (8.54±0.14, 8.11±0.11, 8.18±0.45, respectively). Physicochemical analyses were conducted to ensure the stability and palatability of the beverage, reporting values of 10.00 °B for TSS, 3.595 for pH, and 0.256% for acidity. There was a significant increase in the total phenols (4.12 ± 1.55 mg/100g GAE), total flavonoids (150.23 ± 2.25 mg/100g CAE), ascorbic acid (3.35 ± 1.16 mg/100g) and antioxidant activity (65.23 ± 1.89 µg/ml) of the beverage infused with. The shelf life study, conducted under refrigeration conditions (4-7°C), indicated that the beverage remained stable and free from significant spoilage for 15 days. The results suggest that the infusion of papaya leaf extract significantly improved the nutritional profile of the RTS beverage, providing additional health benefits without compromising taste and quality.

**Keywords:** RTS beverage, papaya leaf extract, physiochemical properties, nutritional composition

## 1. Introduction

Fruits and herbal plants collectively represent the vibrant tapestry of nature's offerings, each contributing unique flavors, nutrients, and medicinal properties to our lives. The world leading producer of fruits are China, India, Brazil, USA, Turkey, Mexico, Indonesia, Span, Iran, Italy, in which India holds the second position in the world (Balali et al.2020). The contribution of India to the total worldwide in production of fruits stands at 11.38%. In the fiscal year 2017–18, India recorded a fruit production of 97.05 million metric tonnes, cultivated across 6.51 million hectares of land. India holds the top position globally in the production of banana, mango, papaya, guava, apple and pineapple (Sah et al.2022).

Fruits, with their complex sensory attributes, contain numerous bioactive constituents crucial for maintaining physiological health. From the anthocyanin found in berries to the bromelain present in pineapples, these diverse compounds offer significant nutraceutical benefits, supporting immune function and cardiovascular well-being.

The herbal medicinal plants are the nature's pharmacy, which offers a plethora of therapeutic compounds that have been traditionally harnessed for centuries to treat various ailments. The Botanical Survey of India (BSI) estimates India has over 8,000 medicinal plant species, with 2,500 species utilized in traditional medicines. Out of these, the majority of medicinal plants thrive within the 1800-meter elevation range, with Uttarakhand hosting the highest number of species, trailed by Sikkim and North Bengal. This unique concentration of medicinal flora sets India apart globally (Singh et al.2022). Some of the common medicinal plants are alovera, garlic, ginger, turmeric, peppermint, chamomile, lavender.

Among the widely used medicinal leaves are neem leaves (*Azadirachta indica*), basil leaves (*Ocimum basilicum*), tulsi leaves (*Ocimum tenuiflorum*), moringa leaves (*Moringa oleifera*), curry leaves (*Murraya koenigii*) and bay leaves (*Laurus nobilis*). Papaya leaves are also among such medicinal plants that have been known since ancient times for its therapeutic properties including immune system support, antioxidant properties, platelet boosting (in cases of dengue fever), digestive health improvement, anti-inflammatory effects, and possible anticancer properties.

Presently, herbal plants are available in the market in the form of teas, oils, tinctures, syrups, salts and seasoning blends. Majority of the herbal plant based products significantly lacks taste and feels more of a medicine. This creates a need for development of food products that not only harness the nutritional richness of herbal plants, but can also be enjoyed by all the age groups with convenience.

RTS (Ready-to-Serve) fruit beverages are prepackaged drinks that are made from the blend of fruits, sugar, acid and water for direct consumption. They offer convenience, diverse flavors of fruits, and time-saving benefits. Moreover, RTS fruit beverages are enjoyed by all age groups. However, low concentration of fruit part, high content of sugar and greater extent of processing raises concerns for nutritional losses as well as health issues associated with obesity and diabetes. Therefore, herbal extract infused RTS beverages can not

only enhance palatability of medicinal plants but can also significantly improve the nutritional properties of traditional RTS beverages. This type of infusion can resolve various health problems related to platelet count, immune system, digestion, liver disorders and inflammatory diseases due to the intermingle beneficial properties of both ingredients. The present study was thus, aimed at developing an apple based ready to serve beverage by infusing papaya leaf extract for its nutritional enrichment.

## 2. Material and Methods

Fresh papaya leaves were gathered from the nearby area of Dehradun. Leaves were thoroughly washed before being sun-dried for eight hours. Once dried, they were hand-crushed and finely ground into powder. The resulting powder was carefully stored in an airtight container at room temperature until it was ready to be used. The fresh apple, sugar, acid (food grade citric acid) were collected from nearby market of Dehradun. Glassware and instruments during the study *viz.*, rotary evaporator, grinder, beaker, conical flask, filter paper, pan, ethanol, air tight container, funnel, aluminium foil were used from Advance Food Technology Lab of Uttaranchal University.

### 2.1 Preparation of Papaya Leaf Extract

The papaya leaf extract was prepared using cold extraction maceration method (Suriaman, 2017). Approximately 100 g of finely ground papaya leaf powder was dissolved in 500 ml 96 % ethanol solution (extract material to solvent ratio of 1:5). The solution was then kept for maceration at room temperature (30 ± 2° C) for 72 hours. Upon completion of maceration, solution was filtered through a filter paper (WM No. 1). Subsequently, the filtrate underwent evaporation in a rotary evaporator at 60°C and 40 rpm for about an hour to yield a concentrated papaya leaf extract. The extract was transferred into a PET bottle, and stored in a refrigerator at 4°C to prevent any potential damage (Fig 2.2).

**Fig 2.1: Papaya Leaf Powder**

**Fig 2.2: Papaya Leaf Extract**

### 2.2 Extraction of Apple Pulp

The apple fruit was washed thoroughly and the skin was peeled off. After peeling it was cut into 4 equal parts and was heated with about 10% water until they became soft. After softening the entire mass was grinded in the grinder for getting a homogenized pulp.

**Fig 2.3: Fresh Apple Fruit**

**Fig 2.4: Apple Pulp**

### 2.3 Preparation of Apple Based RTS Beverages

According to FSSR (2011), RTS fruit beverages should have minimum 10% fruit part, 10% TSS and not more than 1.5% acidity. In the present study, 30% fruit part in the beverage was fixed, considering the mouthfeel of beverage and maximum utilization of fruit. Therefore, approximately 100 g of apple pulp corresponding to 30 % fruit part was taken for the preparation of RTS beverage and the expected quantity of RTS beverage to be prepared from 100 g pulp was estimated as approximately 333 g (since 100 g corresponds to 30 % of the beverage). Calculations on sugar and acid were done based on the actual TSS and acidity of the apple pulp as follows:

TSS Contribution from apple pulp = 10%

Therefore, Sugar already present in 100 g pulp **(A)** =10g

Required TSS of Final RTS beverage = 10%

Therefore, sugar that should be present in 333 g RTS beverage **(B)** = 33.3g

Therefore, sugar to be added = **B-A =** 33.3- 10 = 23.3g

Acidity contribution from pulp = 0.12%

Therefore, acid already present in 100 g pulp **(C)** = 0.12g

Required acidity of final RTS beverage = 0.2%

Therefore, acid that should be present in 333 g RTS beverage **(D)** = 0.66g

Therefore, acid (citric acid) to be added = **D-C =** 0.66 - 0.12 = 0.54g

Amount of water to be added was calculated using the following equation:

$$Amount\ of\ water\ to\ be\ added\ (g) = Expected\ quantity\ of\ RTS\ beverage\ to\ be\ prepared\ (g) - \{Amount\ of\ pulp\ (g) + sugar\ to\ be\ added\ (g) + acid\ to\ be\ added\ (g)\}$$

$$= 333 - (100 + 23.3 + 0.54)$$

$$= 209.16\ g$$

As a next step, calculated amount of sugar and acid were added to weighed amount of water and heated to boiling, followed by filtration through muslin cloth.

Subsequently, the apple pulp was mixed with the filtered syrup and hated to 85 ± 2 °C. The prepared RTS beverage samples were hot filled into pre-sterilized glass bottles up to the brim and sealed immediately. Sealed bottles were pasteurized in hot water (in-bottle pasteurization) at 68 ± 2 °C for 30 minutes (Ayers and Johnson, 1914) followed by cooling to room temperature and drying.

**Fig 2.4: Apple based RTS beverage**

**2.4 Optimization of papaya leaf extract level in apple based RTS beverage**

For optimization of papaya leaf extract level in the prepared beverage, four different concentrations of aqueous extract (2%, 3%, 4% and 5% w/w) were added to the beverage by substituting with the corresponding quantity of water. After addition, the beverage was mixed thoroughly for even distribution of extract throughout the beverage.

All the beverage samples containing different extract concentrations were prepared in triplicate. Subsequently, evaluation of samples was conducted against a control sample (apple based RTS beverage without papaya leaf extract) for various sensory attributes i.e., appearance, taste, mouthfeel and overall acceptability. Based on the sensory analysis, the beverage sample with best attributes was selected and further evaluated for its physico-chemical characteristics and shelf life.

**2.5 Observation Recorded**

The physicochemical properties were analyzed according to the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990).

### 2.5.1 Moisture Content

Approximately 2.0 grams of the samples were transferred into pre-weighed dried crucibles and then transferred into a previously weighed crucible. The crucible was subsequently placed in a drying oven at 105°C for 5 hours. Following this, it was removed and placed in a desiccator to cool. Once cooled, the crucible was re-weighed (Shehu et al. 2022).

$$Moisture\ content\ (\%) = \frac{Loss\ in\ weight\ (g)}{Weight\ of\ the\ sample\ (g)} \times 100$$

### 2.5.2 Ash

Accurately weigh approximately 3 grams of the material in a dish that has been previously dried in an air-oven and weighed. Initially, gently heat the dish over a flame, followed by strong heating in a muffle furnace at 550±10°C until it turns into grey ash. Allow the dish to cool in a desiccator and then weigh it. Heat the dish again at 550±10°C for 30 minutes, cool it in a desiccator, and weigh it. Repeat this process of heating for 30 minutes, cooling, and weighing until the difference between two successive weighings is less than 1 mg. Record the lowest weight obtained.

$$Ash\ (\%) = \frac{Weight\ of\ the\ residue\ (g)}{Weight\ of\ the\ sample\ (g)} \times 100$$

### 2.5.3 Crude Fibre

The 2g of ground sample was taken and mixed with petroleum ether to remove fat. Then it was heated slowly between 35°C and 38°C, and then let it reach 52°C. If the fat content is less than1% then we can skip this step. After that, the sample we have left was boiled with another liquid called sulfuric acid for half an hour, using bumping chips to maintain heating temperature. Then it was filtered out through a muslin cloth and was washed with a hot water until it's not acidic anymore. Then it was boiled again with sodium hydroxide solution. It was filtered again and was washed with 1.25% H2SO4,

three portions (50ml) water, and 25ml alcohol.Then, the residue was removed and kept into a ashing dish and was weighed (W1). Then the residue was dried at 130 ± 2°C for 2 hours and then cooled down in a desiccator,and weighed again. After that, it was heated for 30 min at 600 ± 15°C and kept fir cooling in a desiccator and weighed again (Maynard, 1970). The result was calculated by the formula:

$$Crude\ fiber\ (\%) = \frac{Weight\ of\ the\ crude\ fiber\ residue\ (g)}{Weight\ of\ the\ sample\ (g)} \times 100$$

### 2.5.4 Carbohydrate

Carbohydrate determination was conducted using the Anthrone method. Initially, 100 mg of the sample was placed into a boiling tube and subjected to hydrolysis in a boiling water bath for three hours with 5 mL of 2.5

N HCl. Subsequently, solid sodium carbonate was added until effervescence ceased for neutralization. The volume was then adjusted to 100 mL, and centrifugation was employed to separate any precipitates. After collecting the supernatant, 0.5 and 1 mL aliquots were extracted for analysis. Standard solutions ranging from 0 to 1 mL were prepared using the working standard solution, each diluted to 1 mL with distilled water. Following this, 4 mL of anthrone reagent was added to each tube, and the mixture was heated in a boiling water bath for eight minutes. After rapid cooling, the green to dark green color was measured at 630 nm. Utilizing a standard graph plotting concentration against absorbance facilitated the calculation of carbohydrate content in the sample tube based on its absorbance value, (Hedge and Hofreiter, 1962 ).The value was calculated by given formula:

$$Carbohydrate = \frac{mg\ of\ glucose}{vol.of\ test\ sample}\ x\ 100$$

### 2.5.5 Protein

Protein content was assessed using the Kjeldahl method. Initially, a 5g sample was mixed with concentrated sulphuric acid and digested by heating for 8 hours. Following digestion, 5 ml of the sample was treated with 40 ml of NaOH and neutralized over 15 minutes until approximatly 40 ml of distillate was obtained in boric acid solution. The ammonia collected in the boric acid solution was titrated against a standard 0.1 N sulphuric acid solution (P. Manjushs et al. 2022). The calculation was done by given formula:

$$Protein\ (\%) = \frac{Titrate\ vol.of\ the\ sample\ -\ Titarte\ vol.of\ the\ blank\ \times N\ \times 14\ \times\ 100\ \times 6.25}{Weight\ of\ the\ sample\ (mg)}$$

### 2.5.6 Titratable Acidity

To make 0.1N NaOH solution, 0.4g NaOH was dissolved in 100ml of distilled water and filled into a burette for titration. Then the sample weighing 10ml was taken in a beaker and added 90ml distilled water to make it known volume (100ml). From this aliquot 10ml was taken in a conical flask and 1-2 drops of phenolphthalein indicator was added into this aliquot. Titration was done against 0.1N solution till light pink coloured was observed. The reading was recorded and the acidity was was calculated by given formula:

$$Titratable\ Acidity\ \% = \frac{Titrate\ \times\ Normality\ of\ alkali\ \times\ Volume\ made\ up\ \times\ Equivalent\ weight\ of\ acid\ \times\ 100}{Wt./Vol.of\ sample\ (W)\ \times\ Vol.of\ aliquot\ \times\ 1000}$$

### 2.5.7 Total Soluble Solids (TSS)

The TSS was measured with hand refractometer ranging 0-20 °Brix. The drop of a sample was put on to the glass surface of refractometer prism, and gently covers with a lid and observation was taken against light.

### 2.5.8 Reducing and Total Sugars

Sample weighed 100-200 ml and sugar extraction was performed using 80% hot ethanol. It was repeated two or three times, with each repetition using 5 ml of ethanol. Collect and pool the supernatants and evaporate by keeping it on a hot water bath at 80±2° C. Water was added (10ml) and by using Cyclomixer the sugar was dissolved. The aliquots of 0.1, 0.2, 0.5ml was separated out in a test-tubes. With distilled water the volume was made 2ml. The 2ml was pipette out as a blank. In blank and the sample tubes 1ml of Alkaline Copper Tartarate reagent was added. The test-tubes was boiled in a water-bath for 10min. Under the running water the tubes was cooled and 1ml of Arsenomolybdate reagent was added to all the tubes. With distilled water volume made up of 10ml and kept for 10 min. At 620 nm the absorbance of blue colour was read. Then the amount of sugars presented was calculated by given formula:

$$\%\ Reducing\ Sugar\ =\ Absorbance\ of\ Sample\ \times\ Z\ \times\ Dilution\ Factor$$

### 2.5.9 Ascorbic Acid

The sample weights 10-20 ml and makes up to 100ml with 2% $HPO_3$ or 4% oxalic acid and was filtered out through filter paper. After that 2-3ml of aliquot was taken out in a test tube and with 2% $HPO_3$ 5ml was made, or 4% oxalic acid. A blank was prepared with 5ml of 2% $HPO_3$ or 4% oxalic acid and 10ml water. Dye solution (10ml) was added to all the samples tubes and was shaken immediately in a shaker. The absorbance of red colour (pink) was recorded at 518 nm within 15-20 sec. The standard curve was plotted and the ascorbic acid concentration (X) of the sample was calculated from it.

$$Asorbic\ Acid\ (\frac{mg}{100}ml) = \frac{0.5\ mg}{V_1} \times \frac{V_2 ml}{5\ ml} \times \frac{100\ ml}{weight\ of\ the\ sample} \times 100$$

### 2.5.10 Specific Gravity

The temperature of the sample and the distilled water was recorded at the room temperature. The specific gravity bottle was cleaned and dried before use. The tare weight of the specific gravity bottle was recorded along with the stopper. The specific gravity bottle was filled with distilled water up to the brim and stopper was placed tightly so as to exclude extra water. With tissue paper the exteriors of the bottle was dried. Weight of bottle and water (A) was recorded. Similarly, fill the same specific gravity bottle with test sample and record the weight of bottle and sample (B). The specific gravity was calculated by given formula

$$Specific\ gravity\ =\ \frac{B\ -\ T}{A\ -T}$$

### 2.5.11 pH

The calibration of pH meter was done with pH 7 and pH 4 buffer solution. The electrode was placed in a buffer solution with pH of 7 and was let to stable and reading was recorded after stabilizing. Then the pH meter was

set to the pH of buffer solution by pressing a measure button. Then the electrode was rinsed with distilled water. The electrode was then placed in a buffer of pH 4 and kept for stabilize and set to 4. The electrode was then rinsed with distilled water and dried with tissue paper.

For measuring the pH of the sample the pH meter was set to pH mode and the temperature was adjusted to 25° C. The electrode was placed in the sample to be tested and the pH was shown in the display. After stabilizing the reading was taken. The electrode was rinsed and kept back in the storage solution.

### 2.5.12 Total Phenols

The total phenolic content was assessed using Folin-Ciocalteu reagent, with a method adapted from (Ainsworth and Gillespie, 2007), with gallic acid serving as a reference standard to construct the calibration curve. Different concentrations (10, 20, 40, 80, and 100 µg/ml) of gallic acid solutions (0.5 ml aliquots) were combined with 2 ml of diluted Folin-Ciocalteu reagent (1:10 with deionized water) and then neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The resulting mixture was left at room temperature for 30 minutes with periodic shaking to develop the color. The absorbance of the resulting blue color was measured at 765 nm using a single-beam UV-VIS spectrophotometer (UV mini-1240). This procedure was replicated for both the aqueous and ethanolic extracts. Each analysis was conducted in triplicate. The total phenolic content was calculated from the linear equation of the standard curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry extract (Adusei et al.2019).

### 2.5.13 Flavonoids

The total flavonoid content was assessed using the Aluminum chloride method as described by (Boham 1974), with quercetin utilized as a standard. A 1 ml aliquot of the test sample was combined with 4 ml of water in a 10 ml volumetric flask. Subsequently, 0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% Aluminum chloride were added after a 5-minute interval. Following a 6-minute incubation at room temperature, 1 ml of 1 M Sodium hydroxide was introduced into the reaction mixture. The final volume was promptly adjusted to 10 ml with distilled water. Absorbance readings of the sample were taken against the blank at 510 nm using a spectrophotometer. Each experiment was conducted thrice for accuracy, and the results were expressed as mean ± standard deviation, denoted as flavonoid content (Quercetin equivalent, QE) per gram of dry weight (Mathur and Vijayvergia, 2017).

### 2.5.14 Antioxidant Activity

A DPPH solution was prepared by measuring 7.89 mg of DPPH using a chemical balance, dissolving it in 100 ml of 99.5% ethanol, and then storing it in darkness for 2 hours. In a test tube, 1,000 µl of the DPPH solution was combined with 800 µl of Tris-HCl buffer (pH 7.4). Subsequently, 200 µl of the test sample solution was quickly added and mixed. Then the mixture was left at room temperature for 30 minutes, and the absorbance was measured at 517 nm. For the blank, a mixture containing 1,200 µl of ethanol and 800 µl of Tris-HCl buffer (pH 7.4) was utilized. The inhibition ratio was calculated using the following formula (Xiao et al.2020):

$$Inhibition\ ratio\ (\%) = (A1 - A2) \times 100/A1$$

Where, A1 = the absorbance when ethanol is added instead of the test sample,

A2 = the absorbance of the test sample solution.

The antioxidant activity was determined by calculating the percentage inhibition of oxidation compared to the control sample without added flavonoids, utilizing the following equation (Burda & Oleszek, 2001).

Antioxidant activity % = $100 \times [1 - (A_s^0 - A_s^{120}) / (A_c^0 - A_c^{120})]$

Where, $A_s^0$ = absorbance of sample at 0 min

$A_s^{120}$ = absorbance of sample at 120 min

$A_c^0$ = absorbance of control sample at 0 min

$A_c^{120}$ = absorbance of control sample at 120 min

**2.6 Microbial analysis of beverage**

Total plate count (TPC) was done by using Nutrient Agar Media for bacterial count by the method recommended (Harrigan and McCance , 1966). Nutrient agar media was prepared, and the samples were subjected to serial dilution, reaching a dilution factor of 10^-5. Subsequently, 0.25 ml of the samples, suspended in 0.9 N saline solutions, was inoculated onto respective petri dishes containing nutrient agar media. Multiple replicates were prepared for each dilution. The inoculated petri dishes were then placed in a BOD incubator and incubated for 48 hours at 37±1ºC for microbial growth assessment (Singh et al. 2018). The bacterial colonies were calculated by given formula:

*TPC (cfu/ml) = No. of colonies × dilution factor / 0.25*

**2.7 Sensory Evaluation of beverage**

The sensory evaluation of samples was done on a 9 point Hedonic rating scale, where 9 was “like extremely” and 1 was “dislike extremely” (Amerine et. al. 1965). The prepared formulation was judged by 10 semi-trained panelists (consisting of faculty members of the department) on different sensory attributes *viz.*, colour, taste, odour, mouthfeel, and overall acceptability.

**2.8 Storage Study**

The prepared sample was filled in a glass bottle with crown cap and kept at refrigerated temperature (4 -7° C) to evaluated the changes in acidity, pH, microbial load (cfu/ml), and sensory observations over a 15-day period.

## 3. Result and Discussion

Detailed explanation of experimental results pertaining to the investigation, along with appropriate discussions in light of the available literature is given hereunder.

### 3.1 Nutritional characteristics of apple pulp and papaya leaf extract

The apple pulp and papaya leaf extract used for the study were subjected to nutritional analysis and the findings are presented in Table 3.1.

**Table 3.1 Nutritional composition of apple pulp and papaya leaf extract**

| Component | Apple Pulp (Mean ± SD) | Papaya Leaf Extract (Mean ± SD) |
|---|---|---|
| **TSS (°Brix)** | 11.3 ± 0.12 | 5.10 ± 0.02 |
| **Acidity (%)** | 0.24 ± 0.11 | 0.05 ± 0.04 |
| **pH** | 3.63± 1.2 | 5.7± 1.21 |
| **Total sugars (g/100g)** | 17.09 ± 0.40 | 3.04± 2.13 |
| **Reducing Sugar (g/100g)** | 10.04 ± 0.30 | 3.02 ± 2.11 |
| **Non-reducing sugar (mg/100g)** | 1.63 ± 0.51 | 0.04 ± 1.76 |
| **Total phenols (mg/100g GAE)** | 402.47± 29.9 | 424.89 ± 0.22 |
| **Total Flavonoids (mg/100gCAE)** | 21.91 ± 2.55 | 47.16 ± 2.15 |
| **Ascorbic acid (mg/100ml)** | 6.357± 2.16. | 1.214±0.81 |
| **Antioxidant activity $IC_{50}$ (µg/ml)** | 59.81±2.76 | 78.03 ± 1.33 |

The TSS, acidity, and pH of apple pulp were found to be 11.3 ± 0.12, 0.24 ± 0.11, 3.63 respectively. The acidic pH of apple pulp could be attributed to naturally present malic acid in the fruit. The total sugar, reducing sugar and non-reducing sugar of the pulp were observed to be 17.09 ± 0.40, 10.04 ± 0.30, 1.63 ±0.51 respectively and were in good agreement with the data reported in the available literature. The values obtained for phytochemical analysis of pulp were 402.47 ± 29.9 total phenols, 21.91 ± 2.55 total flavonoid, 6.357 ascorbic acid, and 59.81 ± 2.76 antioxidant activity. These values were well within the range as per the available literature.

Papaya leaf extract on the other hand, illustrated better phytochemical profile with 424.89 ± 0.22 total phenols, 47.16 ± 2.15 total flavonoid, 1.214 ± 0.81 ascorbic acid, and 78.03 ± 1.33 antioxidant activities. These values indicated the potential richness of the extract with essential bioactive compounds that could be used for

enhancing the nutritional value of processed foods.

### 3.2 Sensory evaluation of RTS beverage

The sensory evaluation results of different samples are summarized in the Table---. The sensory attributes evaluated included colour, taste, odour, mouthfeel, and overall acceptability. The mean scores and standard deviations (Mean ± SD) for each attribute were calculated to assess the sensory quality of each sample.

**Table 3.2 Sensory scores of different beverage samples**

| Sample[#] | Sensory score* (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Colour | Taste | Odour | Mouthfeel | Overall acceptability |
| **Control** | 7.80 ± 0.17 | 8.14 ± 0.76 | 7.79 ± 0.39 | 8.17 ± 0.28 | 7.98 ± 0.87 |
| **$T_1$** | 7.94 ± 0.27 | 8.02 ± 0.27 | 7.78 ± 0.46 | 8.06 ± 0.17 | 7.95 ± 0.56 |
| **$T_2$** | 8.14 ± 0.31 | 7.97 ± 0.41 | 7.89 ± 0.54 | 8.17 ± 0.10 | 8.04 ± 0.54 |
| **$T_3$** | 8.54 ± 0.14 | 7.93 ± 0.53 | 8.11 ± 0.11 | 8.15 ± 0.59 | 8.18 ± 0.45 |
| **$T_4$** | 8.32 ± 0.98 | 7.19 ± 0.43 | 7.37 ± 0.27 | 8.03 ± 0.55 | 7.72 ± 0.67 |

***Maximum score out of 9 expressed as mean ± standard deviation of ten determinations,*

*[#]Control (RTS beverage with no papaya leaf extract), $T_1$ (RTS beverage with 2% papaya leaf extract), $T_2$ (RTS beverage with 3% papaya leaf extract), $T_3$ (RTS beverage with 4% papaya leaf extract), $T_4$ (RTS beverage with 5% papaya leaf extract)*

#### 3.2.1 Colour

The colour scores ranged from 7.80 to 8.54. Sample $T_3$ (RTS beverage with 4% papaya leaf extract) received the highest colour score (8.54 ± 0.14), indicating that it was most visually appealing to the panellists. This was followed by $T_4$ (5% extract, 8.32 ± 0.98), $T_2$ (3% extract, 8.14 ± 0.31), and $T_1$ (2% extract, 7.94 ± 0.27). The control sample (no papaya leaf extract) had the lowest colour score (7.80 ± 0.17). The relatively high scores for $T_2$ and $T_3$ suggest that the addition of papaya leaf extract enhanced the visual appeal of the beverages compared to the control. Panelists provided comments to explain the high colour score of samples, describing a healthier and organic perception of the beverage.

#### 3.2.2 Taste

Taste scores varied from 7.19 to 8.14. The control sample had the highest taste score (8.14 ± 0.76), indicating that it was preferred in terms of flavor. $T_1$ and $T_2$ had slightly lower scores of 8.02 ± 0.27 and 7.97 ± 0.41,

respectively, while $T_3$ scored 7.93 ± 0.53. $T_4$ received the lowest taste score (7.19 ± 0.43). A slightly bitter aftertaste in the samples was mentioned by the panel as the reason for low taste score, suggesting that a higher concentration of papaya leaf extract (5%) may negatively impact the flavour.

### 3.2.3. Odour

For odour, scores ranged from 7.37 to 8.11. $T_3$ had the highest score (8.11 ± 0.11), indicating a more favourable aroma compared to the other samples. The control, $T_1$, and $T_2$ had similar odour scores of 7.79 ± 0.39, 7.78 ± 0.46, and 7.89 ± 0.54, respectively. $T_4$ had the lowest odour score (7.37 ± 0.27), which might reflect an undesirable change in the aroma due to the highest concentration of papaya leaf extract.

### 3.2.4. Mouthfeel

The mouthfeel scores were closely clustered, with $T_2$ and the control both scoring highest at 8.17 (± 0.10 and ± 0.28, respectively). $T_3$ followed closely with a score of 8.15 ± 0.59. $T_1$ and $T_4$ had slightly lower scores of 8.06 ± 0.17 and 8.03 ± 0.55, respectively. These results indicate that all samples had a generally acceptable mouthfeel, with minimal differences between them.

### 3.2.5. Overall Acceptability

Overall acceptability scores ranged from 7.72 to 8.18. $T_3$ achieved the highest overall acceptability score (8.18 ± 0.45), reflecting its superior performance in sensory attributes combined. The control and $T_2$ had comparable scores of 7.98 ± 0.87 and 8.04 ± 0.54, respectively, indicating that these samples were also well received. $T_1$ had an overall acceptability score of 7.95 ± 0.56, while $T_4$ scored the lowest (7.72 ± 0.67), possibly due to its lower taste and odour scores.

The sensory evaluation results demonstrated that $T_3$ (RTS beverage with 4% papaya leaf extract) was the most preferred sample overall, achieving the highest scores in colour, odour, and overall acceptability. Therefore, RTS beverage prepared using 4% papaya leaf extract was finalized and further evaluated for its physico-chemical and

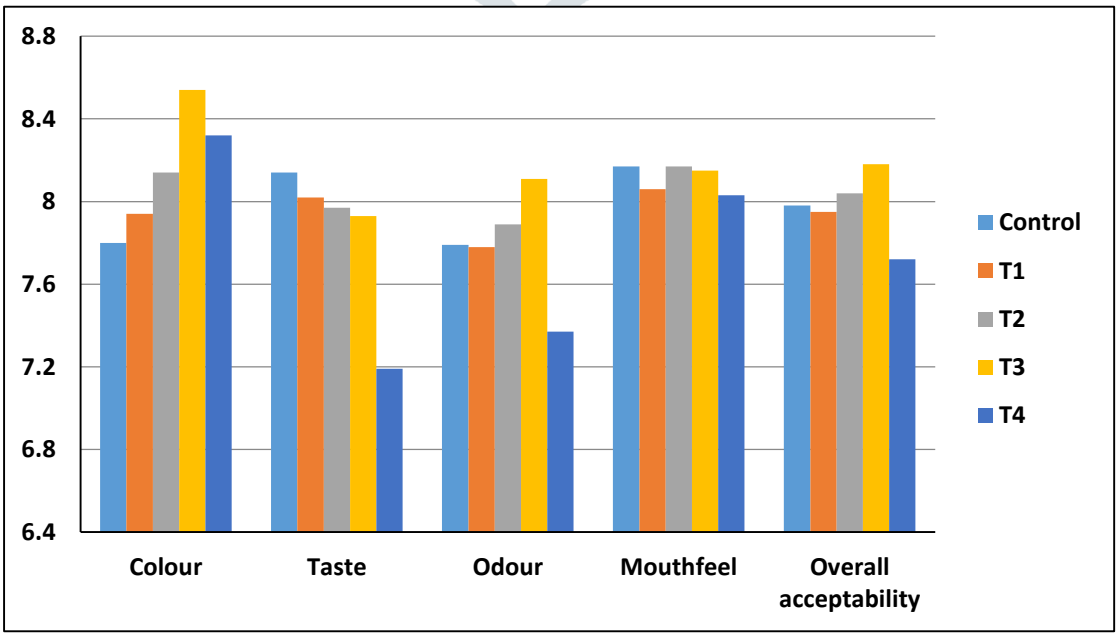


**Fig 3.1 Sensory scores of different beverage samples**

microbiological attributes.

### 3.3 Physico-chemical analysis of optimized papaya leaf extract infused RTS beverage

The optimized beverage based on various sensory attributes was finally evaluated for its physico-chemical attributes. Further a comparison was drawn between the developed product and control sample (RTS beverage with no papaya leaf extract), with respect to these characteristics (Table 5.3)

**Table 3.3 Physico- chemical analysis of papaya leaf extract infused with RTS beverage**

| Responses | Papaya leaf extract infused RTS beverage (Mean ± SD) | Control (Mean ± SD) |
|---|---|---|
| **Specific gravity (g/cc)** | 1.042 ± 0.57 | 0.92 ± 0.46 |
| **TSS (°Brix)** | 10.00 ± 0.32 | 9.23 ± 0.22 |
| **Acidity (%)** | 0.256 ± 3.10 | 0.13 ± 2.91 |
| **pH** | 3.595 ± 0.21 | 2.34 ± 0.11 |
| **Total sugars (g/100g)** | 10.21 ± 1.31 | 8.21 ± 1.11 |
| **Reducing sugars (g/100g)** | 8.12 ± 2.11 | 6.81 ± 1.09 |
| **Non-reducing sugar (mg/100g)** | 1.11 ± 1.16 | 0.91 ± 0.17 |
| **Total phenols (mg/100g GAE)** | 4.12 ± 1.55 | 2.12 ± 1.23 |
| **Total flavonoids (mg/100g CAE)** | 150.23 ± 2.25 | 140.11 ± 1.15 |
| **Ascorbic acid (mg/100ml)** | 3.35 ± 1.16 | 2.31 ± 1.01 |
| **Antioxidant activity $IC_{50}$ (µg/ml)** | 65.23 ± 1.89 | 60.32 ± 1.32 |
| **Total plate count (cfu/ml)** | 2.3 ± 4.6 | 1.1 ± 4.2 |

### 3.4 Storage study of optimized papaya leaf extract infused RTS beverage

The storage study of the Ready-to-Serve (RTS) beverage at refrigerated temperature (4 -7° C) was carried out to evaluated the changes in acidity, pH, microbial load (cfu/ml), and sensory observations over a 15-day period. Results of the storage studies of the beverage are given in Table 3.4

**Table 3.4 Storage study of RTS beverage**

| Days of storage | Acidity (%) | pH | cfu/ml | Observations |
|---|---|---|---|---|
| 0 | 0.25 | 3.595 | 23±5.1 | No off smell, no turbidity |
| 3 | 0.27 | 3.68 | 26±9.3 | No off smell, no turbidity |
| 6 | 0.30 | 3.79 | 29±12.6 | No off smell, no turbidity |
| 9 | 0.32 | 3.56 | 32±7.4 | No off smell, no turbidity |
| 12 | 0.34 | 3.16 | 35±13.2 | Slightly acidic smell, with little turbidity |
| 15 | 0.36 | 3.11 | 37±15.4 | Slightly acidic smell, with little turbidity |

### 3.4.1 Effect on Acidity

The acidity of the RTS beverage increased over the storage period, starting at 2.56 on day 0 and reaching 2.36 by day 15. This gradual increase in acidity suggests that fermentation or other biochemical processes may be occurring, which produce organic acids as by-products. This is a common occurrence in stored beverages, especially those containing natural extracts, as microbial activity can lead to the production of lactic acid, acetic acid, and other organic acids, thereby increasing the overall acidity.

### 3.4.2 Effect on pH

The pH of the beverage initially remained relatively stable, with a slight increase from 3.5958 on day 0 to 3.79 on day 6. However, from day 9 onwards, a significant decrease in pH was observed, dropping to 3.16 by day 12 and further to 3.11 by day 15. This trend inversely correlates with the increase in acidity. The initial slight increase in pH might be due to the buffering capacity of the beverage components. The subsequent sharp decline indicates a substantial accumulation of acidic compounds, overpowering the buffering capacity, likely due to increased microbial activity or chemical reactions producing more acids as the storage period progresses.

### 3.4.3 Effect on sensory characteristics

Throughout the first 9 days, no off smell or turbidity was observed, indicating that the beverage remained stable and free from significant microbial spoilage. However, by day 12 and day 15, a slightly acidic smell and a little turbidity were noted. These changes suggest the onset of spoilage, likely due to microbial growth. The turbidity could be a result of microbial proliferation or the precipitation of components such as proteins or other suspended particles. The slightly acidic smell aligns with the increased acidity and lower pH, indicative of the metabolic activities of spoilage microorganisms.

**Fig 4.1: Optimized Apple based RTS beverage infused with papaya leaf extract**

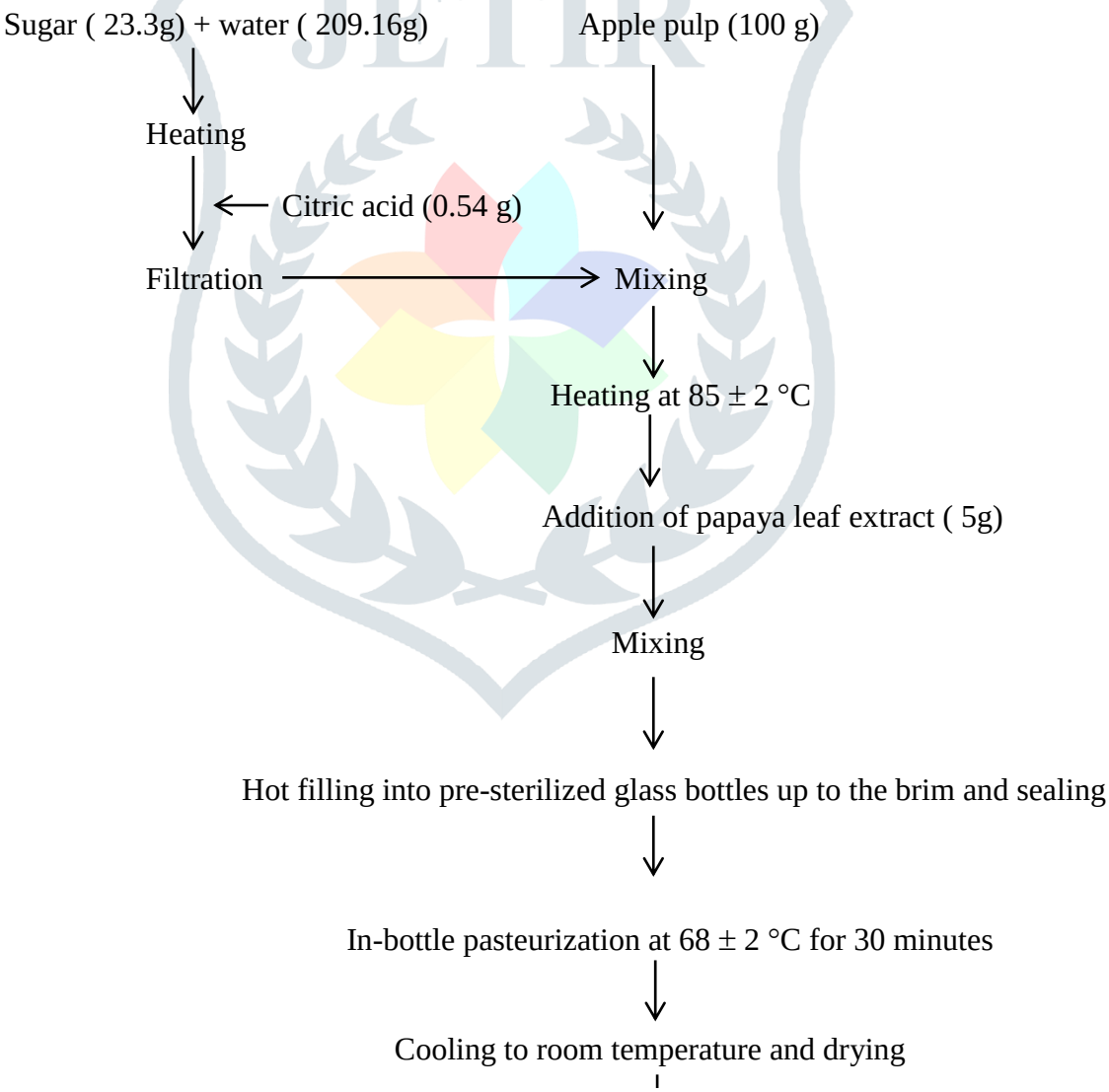


**Fig 4.2: Standard formulation for apple based ready to serve (RTS) beverage infused with papaya leaf extract**

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## 4. Conclusion

In conclusion, the infusion of papaya leaf extract into apple-based ready-to-serve beverages presents a promising avenue for product innovation and enhancement. Through our study, we discovered several key findings that shed light on the potential benefits and implications of this infusion. The sensory evaluation revealed that the addition of papaya leaf extract significantly enhance the taste and flavor profile of the beverage. Moreover, our analysis suggests that the infusion process may contribute additional nutritional benefits to the beverage. Papaya leaf extract is known to contain a variety of bioactive compounds, including antioxidants, vitamins, and enzymes, which could potentially augment the nutritional content of the beverage. While further studies are needed to quantify these effects accurately, our initial findings suggest that the infusion of papaya leaf extract has the potential to elevate the beverage's health profile.

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