

# Studies on bio-chemical attributes of spiced beetroot Ready-to-Serve (RTS) beverage

## Abstract

The study could be useful in determining how to use beetroot for juice production in order to reduce loss owing to underutilization, and the addition of spices can provide a distinct flavor while masking the earthy Odor of beetroot. The study was conducted at Post Harvest Laboratory, Department of Horticulture, School of Agriculture, ITM University, Gwalior. The experiment was laid in a Completely Randomized Design with fourteen treatment combinations and three replications in each treatment. The treatments consisted of several combinations of flavour with spices including various concentrations of Cumin, Cardamon, Cinnamon and Mint. Organoleptic attributes are the sensory parameters based on which, one can justify the taste, flavour and overall appearance of the product. From the conducted research trail, it has been observed that out of 11 treatments, T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), showed least results. In the aspects of taste T<sub>0</sub> ranked with the value of 5.67 followed by T<sub>2</sub> 6.67 at 0<sup>th</sup> day. In the aspect of taste, T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) ranked 8.67 in 0<sup>th</sup> day, simultaneously after 2 days interval it has been observed that in taste categories, T<sub>2</sub> recorded 4.33. From the conducted experimental trail, it has been observed that at final day the TSS was recorded highest value with 11.78 °B in T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)). From the conducted experiment it has been found that Vit-C was recorded highest in T<sub>9</sub> and T<sub>10</sub>, with the value of 6.13 and 6.43 mg/100ml. it has been assumed that by the addition of spices like cumin and cinnamon the vit-C content of the RTS was increased, which was future countered by the fermentation process at 3<sup>rd</sup> final day. The synergy between beetroot's betalains and mint's bioactive compounds could potentially amplify the overall health-promoting effects of the juice. Overall, mixing mint with Beetroot RTS juice results in a delightful, nutritious beverage that appeals to both the taste buds and the health-conscious consumer.

**Keywords:** Ready-to-serve (RTS); Beetroot (*Beta vulgaris* L.); TSS, Ascorbic acid, Nutrition; Health

## 1. Introduction

Beetroot (*Beta vulgaris* L.) includes a high concentration of biologically active chemicals such as betalains, carotenoids, phenols, B-vitamins (B1, B2, B3, B6, and B12), folate minerals, fibers, low-energy carbohydrates, and inorganic nitrate. All parts of this plant have medical properties, including anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative, hepatoprotective, and cardiovascular health protector (Kale *et al.*, 2018). Other stated benefits include suppression of lipid peroxidation and chemo preventive actions. (Wrusset *al.* (2015); Babarykinet *al.* 2019)

Beetroot's red hue is used to make jam, ketchup, sweets, sauce, desserts, juices, burgundy wine, and other products (Kumar and Kumar 2015). Beetroot was utilized to prepare and standardize beetroot-based goods such as halwa and lassi (Dwivedi *et al.* 2017). A slight increase in the regular consumption of antioxidant and polyphenol-rich beverages, such as beetroot juices mixed with other spices or sauces, could have a major favorable impact on public health. Blended ready-to-serve beverages allow the general population to drink beetroot juice with simplicity and convenience, potentially contributing to increased consumption of polyphenol-rich vegetables.

Cumin (*Cuminum cyminum*) seeds are utilized in culinary applications. It contains a lot of iron and can help you lose weight and lower cholesterol. It can also treat moderate digestive problems such as diarrhea, dyspepsia, gas, dyspeptic headaches, and bloating (Johri 2011). Mint (*Mentha arvensis*) can help with irritable bowel syndrome, stomach difficulties, indigestion, and respiratory illnesses. Its essential oils exhibit antifungal, antibiofilm, and cytotoxic properties (Stringaro *et al.*, 2018). Cardamom (*Amomum sublatum* Roxb.) is a medicinal plant from the Zingiberaceae family that grows as a perennial herb with subterranean rhizomes at elevations ranging from 765 to 1675 meters above sea level. The larger Nepalese cardamom, also known as huge cardamom (*Amomum sublutum* Roxb.), is native to the eastern Himalaya.

Cinnamon has been shown to offer substantial health advantages, particularly as an anti-inflammatory, antitumor, anticancer, antidiabetic, and anti-hypertriglyceridemia agent, owing to its phytochemical contents, including phenolic and volatile chemicals. (Jeevanthi *et al.*, 2020).

The study could be useful in determining how to use beetroot for juice production in order to reduce loss owing to underutilization, and the addition of ginger can provide a distinct flavor while masking the earthy odor of beetroot. In today's world, people are becoming more concerned of nutrition and diet in all foods, so commercial production of this juice could result in a significant economic benefit to the nation. Beetroot and spices both contain significant levels of antioxidants and minerals, making them extremely nutritious. Despite its numerous health benefits, the majority of people are completely unaware of its relevance. Hence, this work might also provide an enthusiastic market for beetroot which would also help the economy of people involved in its cultivation, production and marketing, ultimately uplifting their living standards.

## **2. Materials and Methods**

The study was conducted at Post Harvest Laboratory, Department of Horticulture, School of Agriculture, ITM University, Gwalior. The experiment was laid in a Completely Randomized Design with fourteen treatment combinations and three replications in each treatment. The treatments consisted of several combinations of flavour with spices including various concentrations of Cumin, Cardamom, Cinnamon and Mint.

### **2.1 Treatment Details**

#### **List 1. Treatment Details**

<b>Major Source Crop (RTS)</b>	Beetroot ( <i>Beta vulgaris</i> L.)
<b>year</b>	2023-24
<b>Experimental Design</b>	CRD (Completely randomized design)
<b>No. of treatments</b>	11
<b>No. of replication</b>	3
<b>Date of conducted experiment</b>	17-02-2024
<b>List of Spices taken for RTS</b>	Cardamom ( <i>Elettaria cardamomum</i> ) Cumin ( <i>Cuminum cyminum</i> ) Mint ( <i>Mentha spp.</i> ) Cinnamon ( <i>Cinnamomum verum</i> )
<b>Treatments</b>	<b>Treatment Combination</b>
<b>T<sub>0</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml)
<b>T<sub>1</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (5 ml)
<b>T<sub>2</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)
<b>T<sub>3</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cinnamon extract (2 ml)
<b>T<sub>4</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cinnamon extract (4 ml)
<b>T<sub>5</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cardamom extract (2 ml)
<b>T<sub>6</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cardamom extract (4 ml)
<b>T<sub>7</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cumin extract (1 ml)
<b>T<sub>8</sub></b>	Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cumin extract (2 ml)
<b>T<sub>9</sub></b>	Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml)
<b>T<sub>10</sub></b>	Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml)

## 2.2 Experimental Details

### 2.2 Analytical procedure

TSS, pH, acidity, reducing sugar, total sugar, and ash of the improved product were measured. The completed product was then stored at room temperature for 28 days, with analysis performed every 7 days to determine TSS, acidity, vitamin C, and browning index.

### 2.2.1 Sensory evaluation of formulated products

The sensory evaluation approach was used to select the best of 20 created RTS samples. Ranganna (1986) used a 9-point hedonic scale to evaluate sensory perception. The panelists were ITM University research students and teachers with previous experience (semi-trained) in sensory evaluation.

The sensory evaluation criteria were chosen to be appearance/color, smell/odor, taste, mouth feel, and overall acceptability. Panelists were asked to rate the samples on a scale of 1 to 9, with 1 representing severely hated and 9 representing extremely liked samples. Sensory evaluation was carried out in an individual booth with enough lighting and no irritating odors. Each panelist was given coded samples with random numbers and an evaluation card.

## 2.3 Chemical Analysis

### 2.3.1 Titrable acidity

Titration was carried out by titrating 10 ml clear juice with standard N/10 NaOH and result was expressed as percentage citric acid.

$$\% \text{ Acidity} = \frac{\text{vol. of titrant} \times \text{strength of NaOH} \times \text{eqv. wt of citric acid}}{\text{wt of sample} \times 1000} \times 100$$

### 2.3.2 Total soluble solid

Total soluble solid was determined with hand refractometer (0-30 °Bx) and values were expressed as degree brix according to Ranganna (1986). Total Soluble Solids (TSS) is an important quality parameter for Ready-to-Serve (RTS) beverages. It is typically measured using a refractometer, which gives a reading in degrees Brix, indicating the sugar content of the solution.

### 2.3.3 pH

pH was directly measured by using pH meter which was standardized by using buffer solution of pH 7 and 4 at the temperature required.

### 2.3.4 Reducing sugar

Reducing sugar and total sugar of RTS was determined by using Lane and Eynon as described in Ranganna (1986).

### 2.3.5 Vitamin C

Ascorbic acid was determined by 2,6-dichloro-indophenol titration method as given in Ranganna (1986).

Use the formula:

$$\text{Ascorbic Acid (mg/100 mL)} = \frac{V_{\text{sample}} \times C_{\text{std}} \times D \times 100}{V_{\text{std}} \times V_{\text{sample used}}}$$

where:

- $V_{\text{sample}}$  is the volume of DCPIP used for the sample.
- $C_{\text{std}}$  is the concentration of the standard ascorbic acid solution.
- $D$  is the dilution factor, if any.
- $V_{\text{std}}$  is the volume of DCPIP used for the standard.
- $V_{\text{sample used}}$  is the volume of the sample used for titration.

<p><b>1. Sample Preparation</b></p> <ul style="list-style-type: none"> <li>└─ Homogenize beetroot RTS beverage</li> <li>└─ Filter (if necessary)</li> </ul> <p><b>2. Standard Solutions Preparation</b></p> <ul style="list-style-type: none"> <li>└─ Prepare 1 mg/mL standard ascorbic acid solution</li> <li>└─ Prepare 0.1% DCPIP solution</li> <li>└─ Prepare 3% metaphosphoric acid and 8% acetic acid solution</li> </ul> <p><b>3. Extraction of Ascorbic Acid</b></p> <ul style="list-style-type: none"> <li>└─ Mix 10 mL beetroot RTS with 10 mL metaphosphoric acid-acetic acid solution</li> <li>└─ Filter the mixture to obtain a clear extract</li> </ul> <p><b>4. Titration of Sample</b></p> <ul style="list-style-type: none"> <li>└─ Pipette 5 mL of filtered extract into a conical flask</li> <li>└─ Titrate with DCPIP until persistent pink color appears</li> <li>└─ Record volume of DCPIP used</li> </ul> <p><b>5. Standardization of DCPIP Solution</b></p> <ul style="list-style-type: none"> <li>└─ Pipette 5 mL of standard ascorbic acid solution into a conical flask</li> <li>└─ Titrate with DCPIP until persistent pink color appears</li> <li>└─ Record volume of DCPIP used</li> </ul> <p><b>6. Calculation</b></p>
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**Chart-1 Detailed Estimation of Vitamin C**

### 2.3.6 Betalain

Light absorption measured at 538 nm and 476 nm was used to calculate the betanin and betaxanthin concentrations, respectively. In addition, the absorption at 600 nm was measured and used to correct for small amounts of impurities. The results were expressed as betacyanin (calculated in terms of betanin) and betaxanthin (calculated in terms of violaxanthin-I). The total Betalain concentration is expressed as the sum of the betacyanins and betaxanthins.

#### *Calculation of Betanin Concentration*

Step 1: Use the Beer-Lambert Law to calculate the betanin concentration in mol/L.

Step 2: Convert the concentration from mol/L to mg/L using the molar mass of betanin.

To estimate the betanin content in beetroot juice, you can use the Beer-Lambert Law, which relates the absorbance of light to the concentration of the absorbing substance (in this case, betanin). Here's the formula and explanation:

#### *Formula*

The Beer-Lambert Law equation is:

$$A = \epsilon \cdot c \cdot l$$

Where:

**A** is the absorbance of the solution at a specific wavelength (538 nm for betanin).

$\epsilon$  is the molar absorptivity (extinction coefficient) of betanin at 538 nm, given in units of  $L \cdot mol^{-1} \cdot cm^{-1}$ .

- **c** is the concentration of betanin in the solution, expressed in mol/L.
- **l** is the path length of the cuvette that the sample is measured in, typically 1 cm.

#### **Steps to Estimate Betanin Content**

1. **Measure Absorbance:** Use a spectrophotometer to measure the absorbance (A) of the beetroot juice or its dilution at 538 nm. Ensure the spectrophotometer is calibrated with distilled water as the blank.
2. **Calculate Concentration (c):**
  - Rearrange the Beer-Lambert Law equation to solve for

$$c = \frac{A}{\epsilon l}$$

3. **Convert Concentration to Desired Units:** Typically, you may need to convert the concentration from mol/L to mg/L (or other units) based on the application. This involves multiplying the concentration ccc by the molar mass of betanin (approximately 550.43 g/mol).

**Constants:**

- **Molar Absorptivity ( $\epsilon$ ):** For betanin at 538 nm, the molar absorptivity is approximately  $60,000 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ .

**2.4 Data analysis**

Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The data were analysed for one -way ANOVA, mean ANOVA (at 1% level of significance), CRD at 1% significance level were obtained to determine whether the sample were significantly different from each other and to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in Appendix A. The mean was compared using CRD method. Standard deviation and means were also analysed from the same statistical tool.

**3. Result and Discussion**

**3.1 Organoleptic Attributes**

**3.1.1 Taste**

Organoleptic attributes are the sensory parameters based on which, one can justify the taste, flavour and overall appearance of the product. From the conducted research trail, it has been observed that out of 11 treatments, T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), showed least results. In the aspects of taste T<sub>0</sub> ranked with the value of 5.67 followed by T<sub>2</sub> 6.67 at 0<sup>th</sup> day. In the aspect of taste, T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) ranked 8.67 in 0<sup>th</sup> day, simultaneously after 2 days interval it has been observed that in taste categories, T<sub>2</sub> recorded 4.33. T<sub>9</sub> and T<sub>10</sub> recorded 7.67 and 7.89 ranking in the category of taste. Ingham *et al*, (1995) reported that Beetroot has a strong, earthy taste that can be off-putting to some people. Mint leaves have a refreshing and cooling flavour that can help mask this earthiness, making the juice more palatable. Mint leaves contain aromatic oils, such as menthol, which release pleasant volatile compounds. These compounds can significantly improve the aroma of beetroot RTS juice, making it more attractive and enjoyable to consumers. Spices such as cloves, black pepper, and cumin can add bitterness or pungency to the juice. In high concentrations, these flavors can become unpleasant, reducing the overall sensory appeal (Shahidi, 2003).

**3.1.2 Color and appearance**

Color and appearance are also the major parameter under sensory evaluation. Under this category, T<sub>3</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cinnamon extract (2 ml)) and T<sub>7</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Cumin extract (1 ml)) ranked best with value 8.00. At the same time T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), recorded with the minimum ranking of 5.33. T<sub>9</sub> (Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml)) and T<sub>10</sub> (Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml)) recorded 6.67 at 0<sup>th</sup> day and similarly at 3<sup>rd</sup> day 4.33 and 4.00 (Table 1.). Beetroot has a distinctive earthy and sweet flavor. Adding too many spices can overpower this primary flavor, leading to a loss of the characteristic taste of beetroot. At 3<sup>rd</sup> day it has been observed that overall appearance showed reduction in the raking as per the taste. Mint leaves contain aromatic oils, such as menthol, which release pleasant volatile compounds. These compounds can significantly improve the aroma of beetroot RTS juice, making it more attractive and enjoyable to consumers (Eccles, 1994).

### 3.1.3 Flavour

In the category of flavour, it has been recorded that T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)), has maximum ranking with 8.43 at 0<sup>th</sup> day, but due to fermentation it has reduced to 3.33 at final day. T<sub>9</sub> (Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml)) and T<sub>10</sub> (Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml)), recorded the ranking with 7.33 and 7.00 at 0<sup>th</sup> day, whereas 3.33 and 2.33 at final day. Excessive use of spices can lead to sensory overload, where the complexity and intensity of the flavors and aromas become overwhelming. This can cause sensory fatigue, making the juice less enjoyable over time (Lawless and Heymann, 2010). It has been noted that due to mixing of two major spices in beetroot RTS, masking the flavour of each other's and after 2 days interval due to fermentation the parameter showed reduction in the ranking. The combination of beetroot and mint creates a complex and layered flavor profile. The cool, refreshing flavor of mint complements the earthy and sweet flavors of beetroot, resulting in a more sophisticated and enjoyable drink (Spence, 2015).

### 3.1.4 Aroma

Aroma is the vital approach in any sensory parameters. It helps to increase the customer appeals towards the products. Beetroot RTS is having dusky flavour, after adding the species like mint, cinnamon, cumin and cardamom, helps to enhance the aroma of the RTS. As per the olfactory attributes shown in Table-1, it can be noticed that T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), recorded least ranking in aroma with the value of 3.00 at 0<sup>th</sup> day and 1.67 at 3<sup>rd</sup> day. Mint is having strong flavour and aroma, as per the previous references, it can be noted that at 3<sup>rd</sup> day T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) ranked 2.67, but in 0<sup>th</sup> day highest value with 8.50. T<sub>10</sub> (Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml)) recorded 1.67 in the category of aroma at 3<sup>rd</sup> day. The aromatic oils in mint leaves, particularly menthol, provide a pleasant fragrance that enhances the sensory experience of the juice (Gholamipourfard *et al*, 2021).

### 3.1.5 Overall appearance

Comparing the overall appearance of the product RTS, it has been recorded that T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) ranked best with the value of 8.33 followed by T<sub>4</sub> and T<sub>6</sub>. T<sub>0</sub>(Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), recorded least value at 0<sup>th</sup> day with 5.00 and T<sub>10</sub> recorded minimum value at 0<sup>th</sup> day with 1.67 Fig-1. The aroma of mint can enhance the overall sensory appeal of the juice. A pleasant aroma is a critical factor in the overall drinking experience, as it can influence taste perception and enjoyment. Mint leaves can help balance the natural sweetness of beetroot juice, making it more palatable without the need for added sugars. This balance can make the juice more appealing to a wider audience (McKay and Blumberg, 2006)

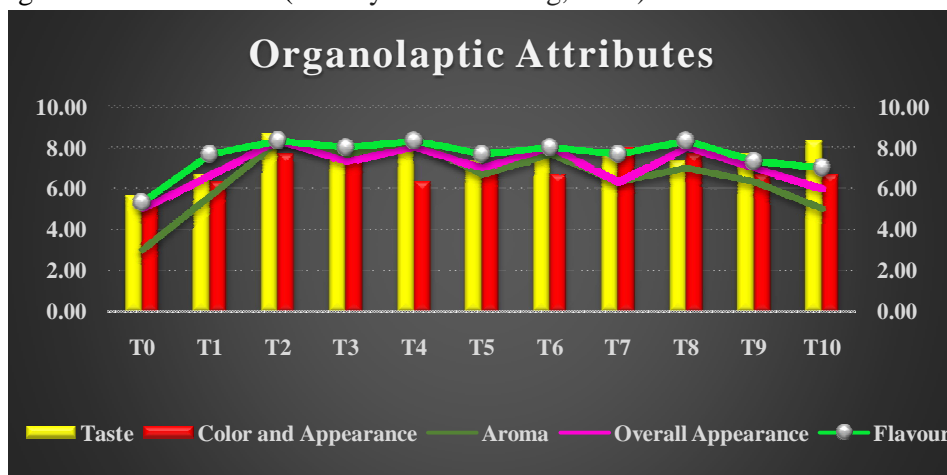


Fig-1 Graphical representation of Organoleptic attributes

### 3.2 Bio-chemical Attributes

#### 3.2.1 TSS

Total Soluble Solids (TSS) refer to the combined content of all soluble substances in a liquid. In the context of RTS (Ready-to-Serve) juice, TSS primarily includes sugars, acids, vitamins, minerals, and other soluble compounds that contribute to the juice's flavor, sweetness, and nutritional value. From the conducted experimental trail, it has been observed that at final day the TSS was recorded highest value with 11.78 °B in T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)). T<sub>0</sub> control (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), which is having normal juice of beetroot was recorded 10.08 °B (Table 2.). TSS gradually increasing from T<sub>0</sub> to T<sub>2</sub>, after that it reduces till T<sub>10</sub> with the value of 8.19 °B. T<sub>1</sub> and T<sub>2</sub> are at par with the value of 11.02 and 11.78 °B (Fig 2). Mint leaves contain some soluble compounds such as essential oils, flavonoids, and small amounts of sugars. These contribute to the TSS when added to the juice (Satputeet *et al.* 2018).

#### 3.2.2 Total Sugar

Research has been conducted on the Food tech lab, department of Horticulture, School of Agriculture. From the conducted experiment it has been observed that T<sub>1</sub> Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (5 ml), recorded good total sugar% as after T<sub>0</sub>. Control recorded the higher sugar % as compared to the others treatments, this is due to no addition of spices, by the addition of spices the sugar level reduces as per the availability. T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)), recorded total sugar% value 11.22. T<sub>9</sub> Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) +

Sugar (10 g) + Water (80 ml) and T<sub>10</sub>Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml) recorded the minimum level of sugar at 3<sup>rd</sup> final day observation with the value 8.02 and 8.04. Additionally, the combination of spices and sugar can improve the antioxidant properties of beetroot juice, as evidenced by research indicating that sugar can stabilize the bioactive compounds in the presence of spices (González-Molina *et al.*, 2010). Thus, the total sugar percentage is crucial not only for flavor balance but also for enhancing the functional benefits of beetroot RTS juice when spices are added.

### **3.2.3 Reducing Sugar**

Reducing sugars play a critical role in the quality and characteristics of beetroot Ready-to-Serve (RTS) juice. These sugars, such as glucose and fructose, are essential for balancing the flavor profile by enhancing the natural sweetness and masking any earthy or bitter notes inherent in beetroot. From the conducted experiment it can be recorded that T<sub>2</sub>(Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)), recorded the maximum reducing sugar % as compared to the other treatments after T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), with the value of 5.41 and 5.81 respectively. By the addition of two or more spices the level of reducing sugar is decreasing as shown among the treatments(Fig 3).

### **3.2.4 Ascorbic Acid (mg/100ml)**

Vitamin C plays a crucial role in Beetroot RTS (Ready-to-Serve) juice by not only enhancing its nutritional profile but also contributing to its shelf life and sensory qualities. Ascorbic acid (mg/100ml) was recorded in 3<sup>rd</sup> final day as a biochemical parameter and statistically analysed. From the conducted experiment it has been found that Vit-C was recorded highest in T<sub>9</sub>Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml) and T<sub>10</sub>Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml), with the value of 6.13 and 6.43 mg/100ml (Fig 4). It has been assumed that by the addition of spices like cumin and cinnamon the vit-C content of the RTS was increased, which was future countered by the fermentation process at 3<sup>rd</sup> final day. T<sub>0</sub> Beet root (20 ml) + Sugar (10 g) + Water (80 ml), recorded the least Vit-C value with 3.65 followed by T<sub>1</sub> and T<sub>2</sub>. Studies have indicated that spices can act synergistically with the ingredients in beetroot juice, potentially stabilizing Vitamin C and preventing its degradation during processing and storage (Siddiquiet *al.*, 2014)

### **3.2.5 pH**

The pH level plays a crucial role in the quality and stability of Beetroot RTS (Ready-to-Serve) juice. Beetroot juice typically has a natural pH range of around 4.5 to 5.5. pH affects various aspects of the juice, including its taste, color, shelf-life, and nutritional content. From the conducted trail, it has been observed that T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), recorded highest pH with the value of 5.86, then decreases in T<sub>1</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (5 ml)) than again increases in T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)). It has been observed from the data table, that pH was decreasing gradually within the treatment. T<sub>9</sub> Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml) and T<sub>10</sub> Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml), recorded acidic pH with the value of 3.60 and 3.61 respectively.

### **3.2.6 Betalain mg/100ml**

From the conducted trail it has been observed that Betalain was recorded highest in T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)), as compared to the others treatments, with the value 64.14 mg/100ml. T<sub>1</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (5 ml)) and T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) are at par with the value of 61.79 and 61.48 mg/100ml. by the addition of the cardamom, Cumin and mint together in the last treatment the amount of Betalain reduced. T<sub>9</sub> Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml) and T<sub>10</sub> Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml), recorded the value 52.83 and 52.16 mg/100ml respectively (Fig 5).

### 3.2.7 Titratable acidity (%)

From the conducted trail it has been clearly observed that titratable acidity is increasing with the mixing of spices. T<sub>0</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml)) recorded 0.217 % of titratable acidity. As compared to the other attributes T<sub>2</sub> recorded 0.277 % titratable acidity followed by T<sub>1</sub> with the value of 0.250. It has been noticed from the data table that, T<sub>9</sub> Beet root (20 ml) + Mint extract (5 ml) + Cardamom extract (2 ml) + Sugar (10 g) + Water (80 ml) and T<sub>10</sub> Beet root (20%) + Mint extract (5 ml) + Cinnamon extract (2 ml) + Sugar (10 g) + Water (80 ml), recorded the value 0.427 and 0.430% respectively (Fig 6). However, specific studies focusing on the direct impact of these spices on the titratable acidity of Beetroot RTS juice are limited. Understanding these interactions could provide insights into optimizing flavor profiles and enhancing the nutritional attributes of functional beverages (Lin *et al.*, 2010).

## Conclusion

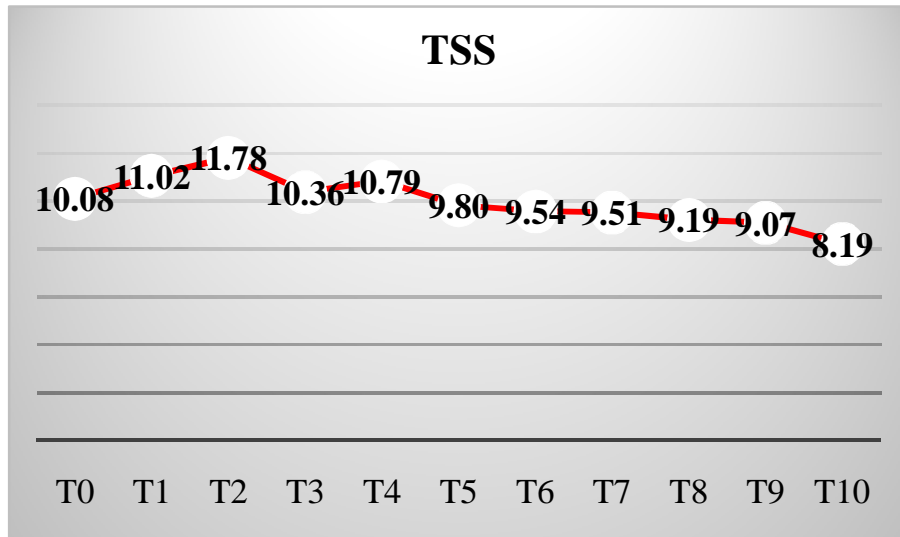
The incorporation of mint into Beetroot RTS (Ready-to-Serve) juice offers a refreshing twist to the beverage, enhancing both its flavor profile and potential health benefits. Mint, known for its aromatic and cooling properties, complements the earthy sweetness of beetroot, creating a more balanced and palatable drink. The addition of mint not only contributes to an invigorating taste but also brings additional nutritional benefits, including improved digestion, anti-inflammatory properties, and enhanced antioxidant capacity. In the aspect of taste, T<sub>2</sub> (Beet root (20 ml) + Sugar (10 g) + Water (80 ml) + Mint extract (10 ml)) ranked 8.67 in 0<sup>th</sup> day, simultaneously after 2 days interval it has been observed that in taste categories, T<sub>2</sub> recorded 4.33. T<sub>9</sub> and T<sub>10</sub> recorded 7.67 and 7.89 ranking in the category of taste. The synergy between beetroot's betalains and mint's bioactive compounds could potentially amplify the overall health-promoting effects of the juice. Overall, mixing mint with Beetroot RTS juice results in a delightful, nutritious beverage that appeals to both the taste buds and the health-conscious consumer.

## References

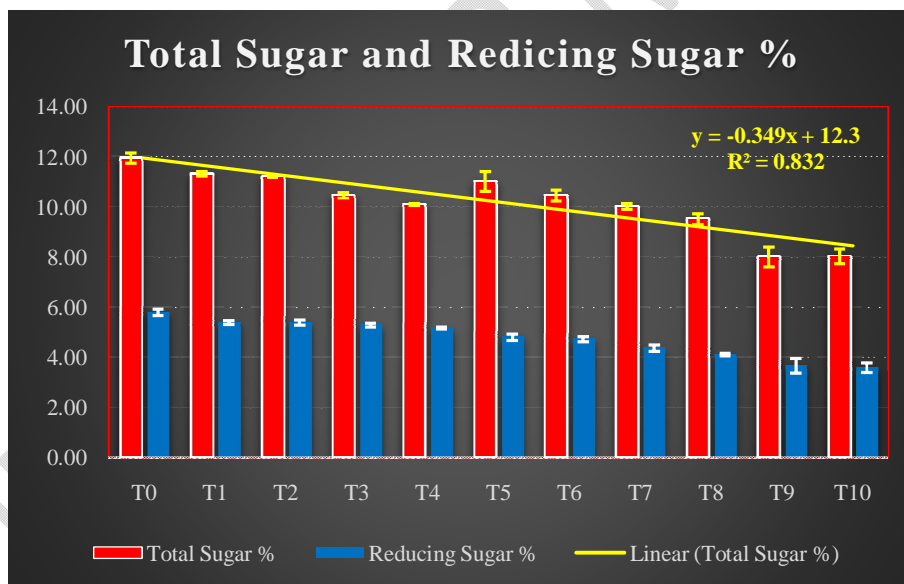
1. Babarykin, D., Smirnova, G., Pundinsh, I., Vasiljeva, S., Krumina, G., & Agejchenko, V. (2019). Red beet (*Beta vulgaris*) impact on human health. *Journal of biosciences and medicines*, **7(3)**, 61-79.
2. Dwivedi, N., Singh, A., Jaiswal, M., & Agrahari, K. (2017). Standardization and development of beetroot-based product. *International Journal of Home Science*, **3(2)**, 26-30.
3. Eccles, R. (1994). Menthol and related cooling compounds. *Journal of Pharmacy and Pharmacology*, **46(8)**, 618-630.
4. Gholamipoufard, K., Salehi, M., & Banchio, E. (2021). *Mentha piperita* phytochemicals in agriculture, food industry and medicine: Features and applications. *South African Journal of Botany*, **141**, 183-195.
5. González-Molina, E., Domínguez-Perles, R., Moreno, D. A., & García-Viguera, C. (2010). Natural bioactive compounds of Citrus limon for food and health. *Journal of Pharmaceutical and Biomedical Analysis*, **51(2)**, 327-345.
6. Ingham, K. E., Linforth, R. S., & Taylor, A. J. (1995). The effect of eating on aroma release from mint-flavoured sweets. *Flavour and fragrance journal*, **10(1)**, 15-24.
7. Johri, R. K. (2011). *Cuminum cyminum* and *Carum carvi*: An update. *Pharmacognosy reviews*, **5(9)**, 63.
8. Kale, R. G., Sawate, A. R., Kshirsagar, R. B., Patil, B. M., & Mane, R. (2018). Studies on evaluation of physical and chemical composition of beetroot (*Beta vulgaris* L.). *International journal of chemical studies*, **6(2)**, 2977-2979.
9. Kumar, S., & Kumar, P. (2015). Rheological modeling of non-depectinized beetroot juice concentrates. *Journal of Food Measurement and Characterization*, **9**, 487-494.
10. Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices*. Springer Science & Business Media.
11. Lin, L.Z., & Harnly, J.M. (2010). Identification of the phenolic components of cheddar cheese and cheese whey by liquid chromatography and detection by ultraviolet absorbance and mass spectrometry. *Journal of Agricultural and Food Chemistry*, **58(2)**, 3608-3613.
12. McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **20(8)**, 619-633.
13. Satpute, D., Padghan, P., Patil, Y., & Suryawanshi, D. (2018). Effect of menthol (*Mentha arvensis*) and beet root extract on physico-chemical properties of paneer whey-based beverage. *International Journal of Food Science and Nutrition*, **3(1)**, 99-105.
14. Shahidi, F., & Naczk, M. (2003). *Phenolics in food and nutraceuticals*. CRC press.
15. Siddiqui, M. W., et al. (2014). "Role of Spices in Protection of Nutritional Quality of Processed Foods." *Journal of Food Science*, vol. 79, no. 4, pp. R501-R508.
16. Spence, C. (2015). Multisensory flavor perception. *Cell*, **161(1)**, 24-35.

17. Stringaro, A., Colone, M., &Angiolella, L. (2018). Antioxidant, antifungal, antibiofilm, and cytotoxic activities of *Mentha* spp. essential oils. *Medicines*, **5(4)**, 112.
18. Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., ... &Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, **42**, 46-55.

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**Fig 2 Graphical representation of TSS**



**Fig 3. Graphical representation of Total Sugar and Reducing Sugar %**

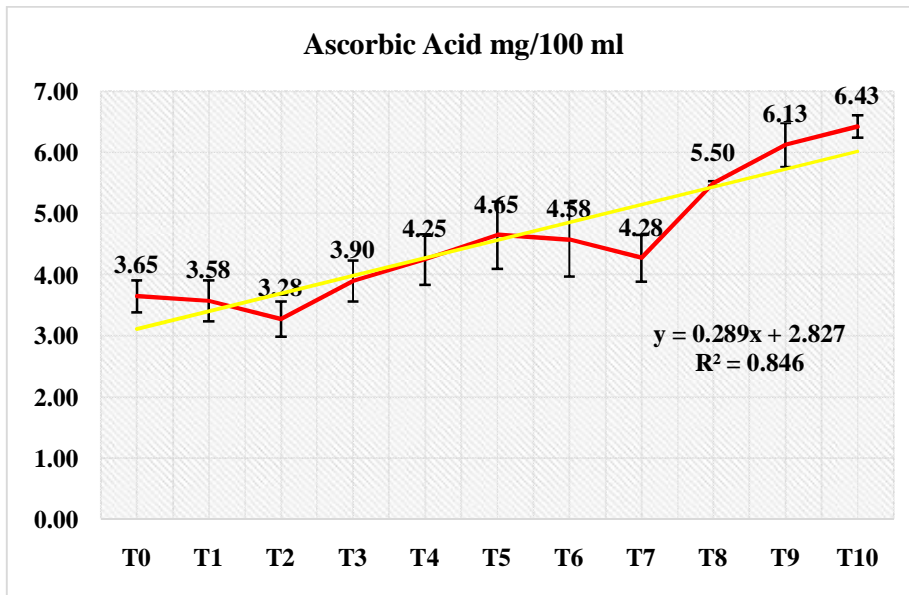


Fig 4. Graphical representation of Ascorbic Acid mg/100ml

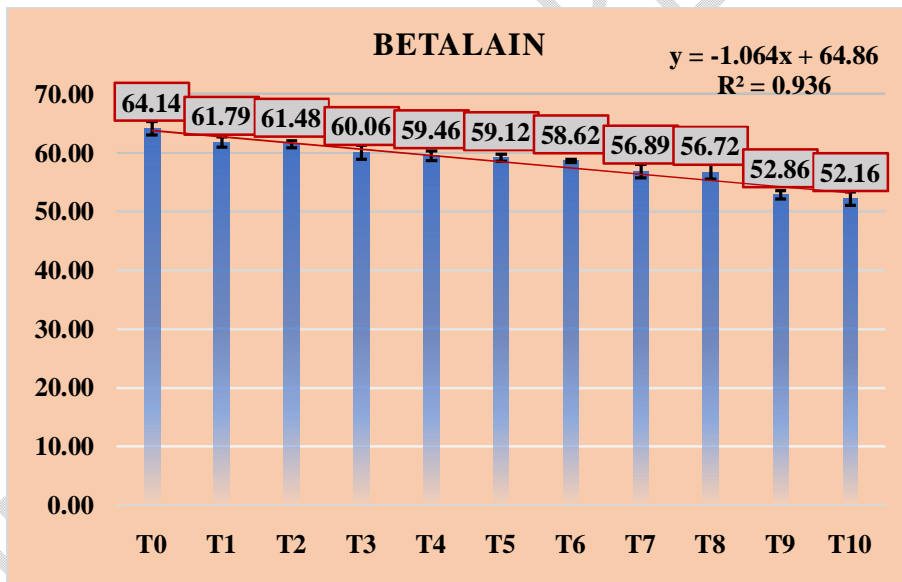


Fig 5. Graphical representation of Betalain mg/100ml

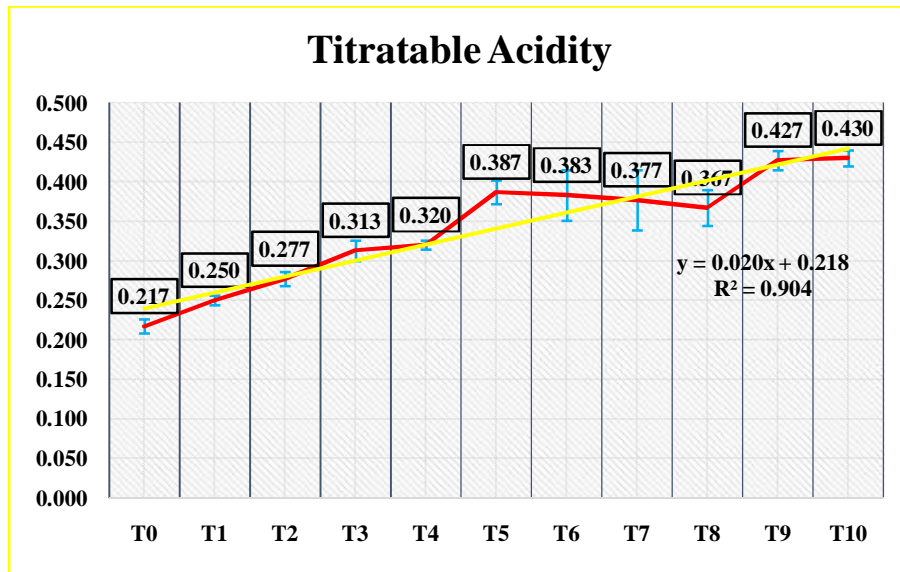


Fig 6. Graphical representation of Titratable acidity %

Table 1. Organoleptic attributes of Spiced Beetroot RTS

	Taste		Color and Appearance		Flavour		Aroma		Overall Appearance	
	0 day	3rd Day	0 day	3rd Day	0 day	3rd Day	0 day	3rd Day	0 day	3rd Day
T <sub>0</sub>	5.67	2.67	5.33	3.67	5.33	2.67	3.00	1.67	5.00	2.33
T <sub>1</sub>	6.67	3.67	6.33	3.67	7.67	3.33	5.67	2.67	6.67	3.67
T <sub>2</sub>	8.67	4.33	7.67	4.33	8.43	3.33	8.50	2.67	8.33	3.33
T <sub>3</sub>	7.67	5.33	8.00	4.67	8.00	3.67	7.67	2.33	7.33	3.33
T <sub>4</sub>	8.33	6.33	6.33	5.33	8.33	5.33	8.33	3.67	8.00	5.33
T <sub>5</sub>	7.33	5.67	7.00	3.33	7.67	4.00	6.67	3.00	7.00	3.67
T <sub>6</sub>	7.67	5.33	6.67	3.67	8.00	2.67	7.67	3.00	8.00	3.33
T <sub>7</sub>	7.67	5.33	8.00	4.33	7.67	4.00	6.33	2.67	6.33	2.33
T <sub>8</sub>	7.33	5.67	7.67	3.67	8.33	3.67	7.00	2.33	8.00	3.00
T <sub>9</sub>	7.67	4.67	6.67	4.33	7.33	3.33	6.33	2.67	7.00	2.33
T <sub>10</sub>	7.89	4.67	6.67	4.00	7.00	2.33	5.00	1.67	6.00	1.67
C.D. (p=0.01)	0.98	1.10	1.42	1.64	1.68	1.69	1.51	1.35	1.81	1.64
SE(m)	0.33	0.04	0.48	0.55	0.57	0.57	0.51	0.46	0.61	0.55

**Table 2. Biochemical Attributes of Spiced Beetroot RTS**

<b>Treatment</b>	<b>Total Sugar %</b>	<b>Reducing Sugar %</b>	<b>Ascorbic Acid mg/100 ml</b>	<b>pH</b>	<b>Betalin mg/100ml</b>	<b>TSS</b>	<b>Titrateable Acidity</b>
<b>T<sub>0</sub></b>	11.96	5.81	3.65	5.86	64.14	10.08	0.217
<b>T<sub>1</sub></b>	11.34	5.39	3.58	5.02	61.79	11.02	0.250
<b>T<sub>2</sub></b>	11.22	5.41	3.28	5.12	61.48	11.78	0.277
<b>T<sub>3</sub></b>	10.47	5.30	3.90	4.83	60.06	10.36	0.313
<b>T<sub>4</sub></b>	10.11	5.18	4.25	4.85	59.46	10.79	0.320
<b>T<sub>5</sub></b>	11.03	4.81	4.65	4.52	59.12	9.80	0.387
<b>T<sub>6</sub></b>	10.46	4.74	4.58	4.20	58.62	9.54	0.383
<b>T<sub>7</sub></b>	10.04	4.37	4.28	4.05	56.89	9.51	0.377
<b>T<sub>8</sub></b>	9.52	4.12	5.50	3.80	56.72	9.19	0.367
<b>T<sub>9</sub></b>	8.02	3.67	6.13	3.60	52.86	9.07	0.427
<b>T<sub>10</sub></b>	8.04	3.60	6.43	3.61	52.16	8.19	0.430
<b>C.D.(p= 0.01)</b>	<b>0.68</b>	<b>0.41</b>	<b>0.33</b>	<b>1.10</b>	<b>2.75</b>	<b>0.19</b>	<b>0.056</b>
<b>SE(m)</b>	<b>0.23</b>	<b>0.14</b>	<b>0.08</b>	<b>0.37</b>	<b>0.93</b>	<b>0.06</b>	<b>0.019</b>