



# Effect of Thermal, and Microwave Treatment on Bioactive Compound, Antioxidant Activity, and Sensory Quality of Lime-Flavored Dragon Fruit RTS Beverage

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** The aim of the current study was to investigate the effect of lime juice standardization on sensory evaluation, and changes in betacyanin content, antioxidant activity, and sensory attributes of lime-flavored dragon fruit RTS beverage processed with thermal, and microwave processing.

**Study Design:** CRD and Factorial CRD.

**Place and Duration of Study:** College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, between October 2021 to July 2023.

**Methodology:** The dragon fruit RTS beverage (12% fruit juice, 0.1% citric acid, and 12°Brix TSS) was incorporated with 3% lime juice on the sensory evaluation basis was subjected to thermal, and

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microwave processing. The thermal treatment was carried out for 5, 10, and 15 min at 70, 80, and 90°C, while microwave treatment was done at fixed power density of 900 W for 30, 60, 90, 120, and 150 sec for lime-flavored dragon fruit RTS beverage.

**Results:** The RTS processed for 5 min at 70°C was found best among all thermal treatments on the basis of better betacyanin content retained in RTS (23.80 mg/L), and 73.43% antioxidant activity, whereas 30 sec of exposure time at 900 W power density had better betacyanin content (29.87 mg/L), and 54.31% antioxidant activity than other microwave treatments.

**Conclusion:** The betacyanin retention was higher for microwave processing than thermal processing, whereas antioxidant activity was reported higher for thermal treatment.

**Keywords:** Antioxidant activity; betacyanin; dragon fruit RTS; lime-flavored; microwave; thermal.

## 1. INTRODUCTION

The small exotic fruit originated from forests areas of Mexico, central America, and south America, well known as “pitaya” or “pitahaya” in many countries was a member of cactus family, and scientifically known as “*Hylocereus polyrhizus*” gaining more popularity in South Asian countries such as India, because of its health promoting, and nutritionally fulfilled profile [1,2]. Hossain et al. [3] suggested that the dragon fruit contains good amounts of essential fatty acids, hydroxycinnamates, betalain compounds, flavonoids, and other pigments such as carotenoids (beta-carotene), lycopene, anthocyanin, and their derivatives. Taste of dragon fruit depends upon variety, and chemical composition of that variety, So, it is very good idea to blend dragon fruit with local fruit of Gujarat, India such as Lime (*Citrus aurantifolia*) [4]. Very short life span, and relatively higher cost of dragon fruit leads the processing of juice in different valuable products such as RTS beverage, jelly, jam, etc. The ready to serve beverage is non-fermented fruit or vegetable beverage containing minimum 10% juice/pulp content, 10°Brix total soluble solids, and maximum 0.3% citric acid. To increase the shelf life of product thermal, and non-thermal preservation techniques are used. According to study carried out by Feng et al. [5] on strawberry-apple-lemon blend juice, total phenols was decreased non-significantly heated for one min at 86°C, and approximately increase was 18% for high hydrostatic pressure, and 7% for ultrasound treatment. Prickley pear juice at the flow rate of 6.6 L/h heated for 3 min at 95°C resulted in approximately 22% ascorbic acid reduction [6]. According to Kumar et al. [7], heat pasteurization for 15 sec at 90°C had greater impact on total phenols (from 710 to 690.5 mg of GAE/L), and Vit. C content (from 67.71 to 52.42 mg/100ml) of pomelo (*Citrus maxima*) juice than microwave treatment. Microwave treatment at 90°C had

705.3 mg of GAE/L of total phenols, and 54.29 mg/100ml of ascorbic acid content. According to Liaotrakoon et al. [8], the retained betacyanin content was approximately 32.35% after thermal treatment for 60 min at 90°C, while antioxidant activity of dragon fruit puree was increased upto 34 µg of GA/g.

According to Cortez-Garcia et al. [9] on xoconostle, microwave treated xoconostle for 5.5 min at 297 W had non-significant increase for total phenols (from 13.08 to 13.19 mg GAE/g), significant decrease in betacyanin content (from 27.98 to 23.70 mg betanin/100g), and significant increase in antioxidant activity (from 4.94 to 5.81%) as per DPPH assay. As per research conducted by Saikia et al. [10]; the initial total phenolic content (145 mg GAE/100ml) was increased to 158, 650, and 700 mg GAE/100ml for thermal (3 min at 75°C), and microwave pasteurization at 600 W, and 900 W, respectively for carambola juice. Ravichandran et al. [11] reported increase in antioxidant activity, betalain content of microwave treated beet. Increase in betalain content for microwave treatment for 30 sec was 19% at 1800 W, and 7% at 900 W power density, however heat treatment such as boiling, and roasting decreases the betalain content of beet. The betacyanin content was grew from 18.5 to 34.6 mg/g, whereas betaxanthin content was dropped from 45.2 to 37.3 mg/g for microwave treated beet root for 12 min at 450 W, according to study of Slavov et al. [12]. The decrease in anthocyanin content of blackberry juice is directly in corelation with antioxidant activity reduction, as per study conducted by Zhang et al. [13]. The antioxidant activity was 80.68% at 60°C extraction time for roselle drink, whereas 72.59% at 100°C for drink prepared with dragon fruit peel [14].

The aim of the study was to standardize the lime juice into dragon fruit RTS beverage, and to optimize the best treatment from thermal, and

microwave treatment. The current study was conducted to evaluate the effect of thermal, and microwave treatment on betacyanin content, antioxidant activity, and sensory evaluation of prepared RTS beverage.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Lime-Flavored Dragon Fruit RTS Beverage

Dragon fruit precured from kachchh district, Gujarat, India was used to prepare RTS beverage having different concentration of sugar level, fruit juice content, and citric acid content. Among all the selected combinations, 12% fruit juice, 12°Brix TSS, and 0.1% citric acid liked most on preliminary sensory trials. The step wise flowchart for preparation of dragon fruit RTS beverage was mentioned below in Fig. 1.

The lime fruits were purchased from local market of Anand, and lemon squeezer was used extract the lime juice. The 1, 2, 3, and 4% lime juice was incorporated in prepared dragon fruit RTS, and standardized on the basis of sensory evaluation.

### 2.2 Thermal, and Microwave Treatment

The lime-flavored dragon fruit RTS beverage poured in glass beaker was thermally treated at 70, 80, and 90°C for 5, 10, and 15 min in waterbath placed at treatment cabinet (Made: Thermolab Scientific Equipments). After treatment RTS was cooled, packed in PET bottles, and stored at refrigerated conditions for further analysis.

The lime-flavored dragon fruit RTS beverage poured in glass beaker was microwave treated at 900 W power density for 30, 60, 90, 120, and 150 sec. RTS was cooled, packed in PET bottles, and stored at refrigerated conditions for further analysis.

### 2.3 Determination of Betacyanin Content

According to Naderi et al. [15], a photometric approach with a minor modification was used to measure the betacyanin content. With deionized water, the RTS samples were 50 times diluted. Using a spectrophotometer (Made: Varian 50Bio), samples' absorbance was measured after 20 min of equilibration. The following formula was used to determine the betacyanin content:

$$\text{Betacyanin content (mg/L)} = \frac{A \times DF \times MW \times 1000}{\epsilon \times L}$$

Where,

A = absorption value at  $\lambda_{\text{max}}$  (540 nm)

DF = dilution factor

MW = molecular weight of betanin (550 g/mol)

$\epsilon$  = molar extinction coefficient of betanin ( $\epsilon = 60,000 \text{ L mol}^{-1} \text{ cm}^{-1}$  in  $\text{H}_2\text{O}$ )

L = pathlength of cuvette (1 cm)

### 2.4 Determination of Antioxidant Activity

According to Nadeem et al. [16], a photometric approach with a minor modification was used to quantify the percent inhibitory activity. Methanol was used to create a sample blank solution that contained 0.02 g/L of DPPH. One ml of RTS sample was mixed with two ml of a 0.02 g/L DPPH solution. The sample mixture was incubated for 30 min in a dimly lit environment. The sample was then analyzed using a UV-Visible spectrophotometer (Made: Varian 50Bio) at a wavelength of 517 nm. The formula used to calculate antioxidant activity as follow.

$$\% \text{ inhibition activity} = \frac{(A_{\text{SB}} - A_{\text{S}})}{A_{\text{SB}}} \times 100$$

Where,

$A_{\text{SB}}$  = Absorption of sample blank

$A_{\text{S}}$  = Absorption of sample

### 2.5 Sensory Evaluation

Using a nine-point hedonic scale, a semi-trained panel of ten judges evaluated the samples' sensory quality at sensory evaluation lab, Department of Food Processing Technology, Anand Agricultural University, Anand. The attributes color, taste, body, flavor, and overall acceptance were utilized to evaluate sensory quality of the RTS beverage. The critical difference (C.D.) at 5%, standard error of mean (SEm), and co-efficient of variation (CV) values were mentioned in respective tables.

### 2.6 Statistical Analysis

The data collected on different characteristics were analyzed with the help of factorial design in CRD using the software.

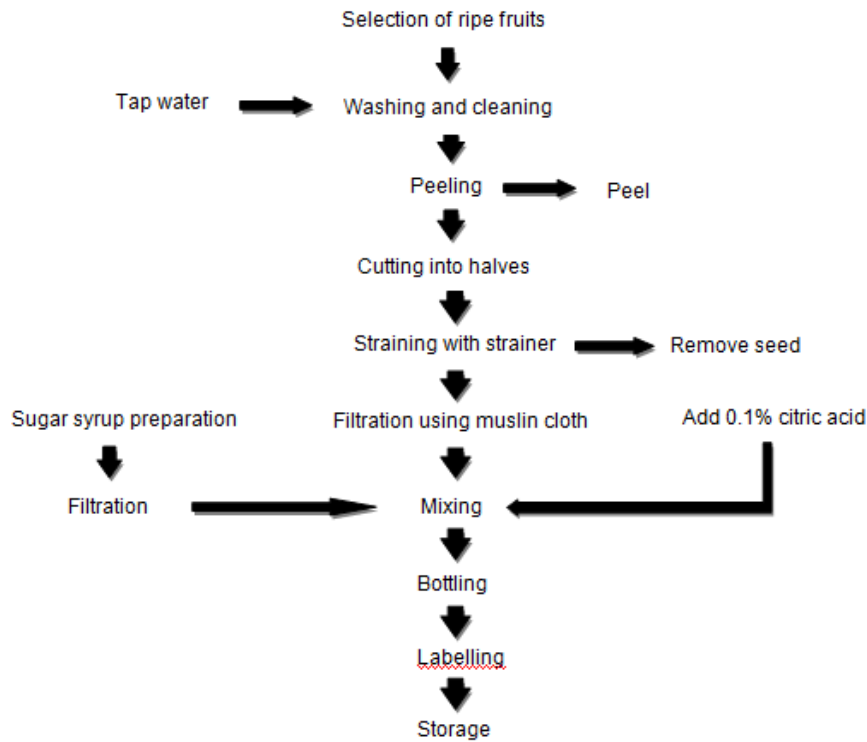


Fig. 1. Process flowchart for preparation of RTS beverage

### 3. RESULTS AND DISCUSSION

#### 3.1 Standardization of Lime Juice in Dragon Fruit RTS Beverage

The color, and body score for lime-flavored dragon fruit RTS beverage was non-significant; However, the taste (7.94), flavor (7.86), and overall acceptability (7.92) score was significantly higher for dragon fruit RTS beverage incorporated with 3% lime juice (Table 1.). So, the 3% lime juice was incorporated in dragon fruit RTS beverage, and processed with thermal, and microwave treatment for further study.

#### 3.2 Effect of Thermal Treatment on the Lime-Flavored Dragon Fruit RTS Beverage

##### 3.2.1 Betacyanin content

Effect of thermal treatment on betacyanin content of lime-flavored dragon fruit RTS beverage was given in Table 2. (Fig. 2.). Significant variation for betacyanin content was observed for processing temperature, while non-significant variation was observed for processing time. Additionally, a non-significant change was seen for the

interaction between time and temperature. The betacyanin content of lime-flavored dragon fruit RTS beverage decreased with increasing processing temperature as well as time.

So, betacyanin content was decreased with processing time, and temperature. Similar findings were given by Liaotrakoon et al [8]; and Moussa-Ayoub *et al.* [6]. Betacyanin content decrease as it is unstable at higher temperature. Betacyanin content in RTS beverage was more stable at 5 min. In comparison to other time-temperature combinations, the betacyanin content was greater at 70°C for 5 min processing time. T<sub>1</sub>t<sub>1</sub> had higher betacyanin content (23.80 mg/L) among all treatment combinations.

##### 3.2.2 Antioxidant activity

The effect of thermal treatment on the antioxidant activity of a lime-flavored dragon fruit RTS beverage was summarized in Table 3. (Fig. 3.). For the antioxidant activity of lime-flavored dragon fruit RTS beverage, non-significant variation was seen for processing temperature, processing time, and the interaction of time with temperature. With longer processing times, the antioxidant potential of the lime-flavored dragon fruit RTS beverage reduced.

**Table 1. Effect of lime juice incorporation in dragon fruit RTS beverage on sensory score**

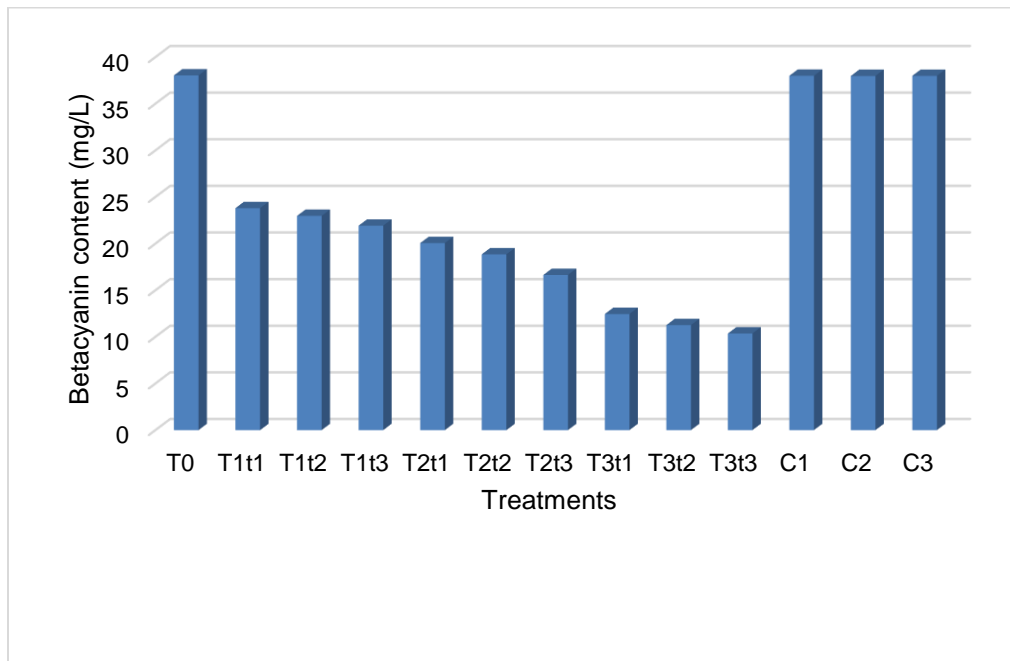
Treatment	Color	Taste	Body	Flavor	Overall acceptability
T <sub>1</sub>	8.10 ± 0.07	7.22 ± 0.17 <sup>c</sup>	7.80 ± 0.10	7.36 ± 0.19 <sup>b</sup>	7.24 ± 0.13 <sup>c</sup>
T <sub>2</sub>	8.08 ± 0.11	7.54 ± 0.09 <sup>b</sup>	7.83 ± 0.11	7.51 ± 0.07 <sup>b</sup>	7.48 ± 0.04 <sup>b</sup>
T <sub>3</sub>	8.15 ± 0.08	7.94 ± 0.15 <sup>a</sup>	7.85 ± 0.13	7.86 ± 0.16 <sup>a</sup>	7.92 ± 0.17 <sup>a</sup>
T <sub>4</sub>	8.05 ± 0.10	7.17 ± 0.11 <sup>c</sup>	7.80 ± 0.11	7.27 ± 0.17 <sup>b</sup>	7.12 ± 0.15 <sup>c</sup>
C.D. (5%)	NS	0.18	NS	0.21	0.18
SEm	0.04	0.06	0.05	0.07	0.06
CV%	1.17	1.18	1.47	2.05	1.80

\*n: Mean of five repetitions; T<sub>1</sub>: 1% lime juice; T<sub>2</sub>: 2% lime juice; T<sub>3</sub>: 3% lime juice; T<sub>4</sub>: 4% lime juice; NS: non-significant

**Table 2. Effect of thermal treatment on betacyanin content (mg/L) of lime-flavored dragon fruit RTS beverage**

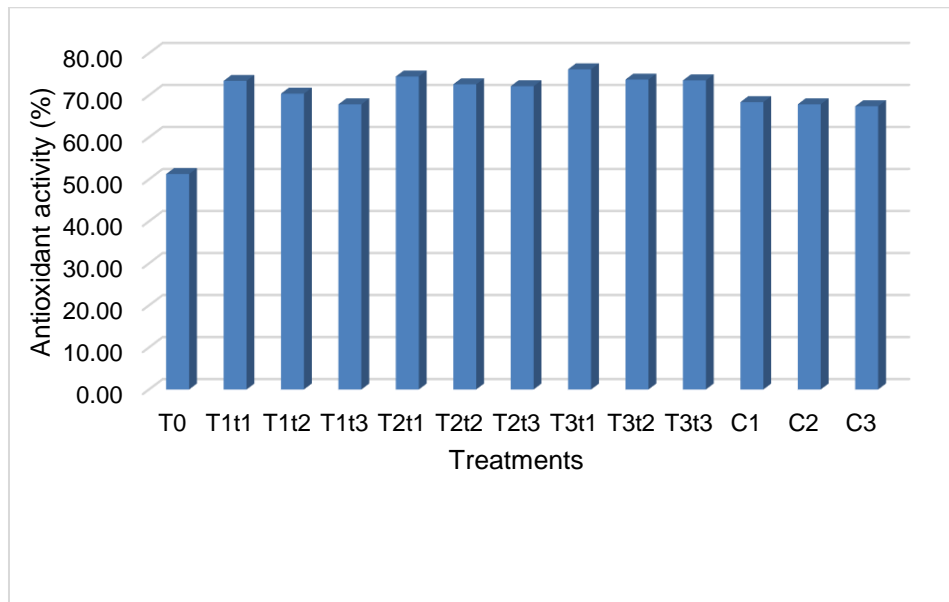
Temperature (°C)	Time (min)			Mean T
	t <sub>1</sub>	t <sub>2</sub>	t <sub>3</sub>	
T <sub>1</sub>	23.80	22.98	21.92	22.90
T <sub>2</sub>	20.06	18.85	16.64	18.52
T <sub>3</sub>	12.45	11.26	10.36	11.36
Mean t	18.77	17.70	16.31	
Factor	T	t	T × t	
C.D. (5%)	2.27	NS	NS	
SEm	0.77	0.77	1.33	
CV%	13.05			

\*T: temperature; T<sub>1</sub>: 70°C; T<sub>2</sub>: 80°C; T<sub>3</sub>: 90°C; t: time; t<sub>1</sub>: 5 min; t<sub>2</sub>: 10 min; t<sub>3</sub>: 15 min; T × t: interaction of temperature, and time; NS: non-significant



**Fig. 2. Effect on betacyanin content of lime-flavored dragon fruit RTS beverage**

To: Control; T1t1: 70°C/5min; T1t2: 70°C/10min; T1t3: 70°C/15min; T2t1: 80°C/5min; T2t2: 80°C/10min; T2t3: 80°C/15min; T3t1: 90°C/5min; T3t2: 90°C/10min; T3t3: 90°C/15min; MW1: 30sec; MW2: 60sec; MW3: 90sec; MW4: 120sec; MW5: 150sec



**Fig. 3. Effect on antioxidant activity of lime-flavored dragon fruit RTS beverage**

To: Control; T1t1: 70°C/5min; T1t2: 70°C/10min; T1t3: 70°C/15min; T2t1: 80°C/5min; T2t2: 80°C/10min; T2t3: 80°C/15min; T3t1: 90°C/5min; T3t2: 90°C/10min; T3t3: 90°C/15min; MW1: 30sec; MW2: 60sec; MW3: 90sec; MW4: 120sec; MW5: 150sec

In relation to an increase in processing temperature, antioxidant activity increased. The higher temperature influences the release of total phenols from cells present in RTS beverage, thereby imparting antioxidant effect. However, prolonged holding time at higher temperature can lead to a decrease in the concentration of phytochemicals released. In the presence of higher temperature, the Maillard browning reaction occurs, resulting in the formation of melanoidins, which possess antioxidant properties [6]. Moussa-Ayoub et al. [6]; and Liaotrakoon et al. [8] observed similar increase for phenolic content, and antioxidant activity. Antioxidant activity for lime flavored dragon fruit RTS beverage was found higher for treatment T3t1 (76.19%).

### 3.2.3 Organoleptic quality of RTS beverage

A nine-point hedonic scale used by panel of judges to evaluate a thermally treated lime-flavored dragon fruit RTS beverage for color, taste, body, flavor, and overall acceptability. The individual temperature, and processing time had non-significant effect on color score. Regarding the interaction of time with temperature for thermal treatment, the color score of the lime-flavored dragon fruit RTS beverage did not change significantly.

The non-significant effect on taste score was observed for individual effect of temperature, and processing time. The interaction of time-temperature combination for thermal treatment did not significantly affect the taste rating of the lime-flavored dragon fruit RTS beverage. Least taste score was assigned for sample T2t3 (7.57).

For lime flavored dragon fruit RTS beverages, the impact of processing time, and temperature on body score was non-significant. Regarding the interaction of time with temperature for thermal treatment, the body score of the lime-flavored dragon fruit RTS beverage did not change significantly.

The non-significant impact on flavor score was reported for individual temperature, and processing time. When thermal treatment was applied, the interaction between time, and temperature did not significantly affect the flavor score of the lime-flavored dragon fruit RTS beverage.

The effect of heat treatment on overall acceptability rating of the lime-flavored dragon fruit RTS beverage was presented in Table 4. On the overall acceptability score, processing time variation was not significant, although processing temperature variation was significant. Also, overall acceptability score of lime-flavored

dragon fruit RTS beverage varied non-significantly with respect to interaction of time with temperature for thermal treatment. Overall acceptability score for sample T<sub>1</sub>t<sub>1</sub>, and T<sub>1</sub>t<sub>2</sub> was 7.55, and 7.60, respectively; However, variation was non-significant. On the basis of the overall acceptability score, treatment T<sub>1</sub>t<sub>1</sub> was determined to be the best treatment.

### 3.3 Effect of Microwave Treatment on Lime-Flavored Dragon Fruit RTS Beverage

#### 3.3.1 Betacyanin content

The effect of microwave heating on the betacyanin content of a lime-flavored dragon fruit RTS beverage was presented in Table 5. (Fig. 2.). Non-significant variation was found for betacyanin content of microwave treated standardized lime-flavored dragon fruit RTS beverage. Reduction in betacyanin content was non-significant in lime-flavored dragon fruit RTS beverage treated at 900 W power density for 30, 60, 90, 120, and 150 sec. However, betacyanin content decreased in compare to control sample (38.04 mg/L) due to the generation of heat inside the sample. same findings for betacyanin content of microwave treated samples were reported by Slavov et al. [12].

#### 3.3.2 Antioxidant activity

The effect of microwave treatment on antioxidant activity of a lime-flavored dragon fruit RTS beverage was discussed in Table 5. (Fig. 3.). Significant variation for antioxidant activity was observed for microwave treated lime flavored dragon fruit RTS beverage at fixed power density. Antioxidant activity decreased with

increase in processing time for microwave treatment. Maximum antioxidant activity was observed for sample treated at 900 W for 30 sec (MW<sub>1</sub>: 54.31%); However, it was not significantly different from MW<sub>2</sub> (47.94). So, microwave treated sample for 30 sec was considered as best treatment. Total phenol, and ascorbic acid were major compounds for giving radical scavenging activity, decrease in antioxidant activity as a result of breakdown of total phenol, and ascorbic acid. Same results were reported for antioxidant activity by Kumar et al. [7].

#### 3.3.3 Organoleptic quality of RTS beverage

The effect of microwave treatment on the sensory evaluation of a lime-flavored dragon fruit RTS beverage was discussed in Table 6. A panel of semi-trained judges selected to evaluate a standardized lime-flavored dragon fruit RTS beverage for color, taste, body, flavor, and overall acceptability by using a nine-point hedonic scale.

Non-significant variation was found for color, taste, body, flavor, and overall acceptability score for microwave treated lime-flavored dragon fruit RTS beverage. A non-significantly higher overall acceptability score was observed for microwave treated samples for 30, and 60 sec (7.56).

The effect of microwave processing time at fixed power density (900 W) on betacyanin content, sensory attributes like; color, taste, body, flavor, and overall acceptability was non-significant; However, effect of microwave processing time was significant for antioxidant activity of lime-flavored dragon fruit RTS beverage. Antioxidant activity was observed higher (54.31%) for microwave treated sample for 30 sec (MW<sub>1</sub>).

**Table 3. Effect of thermal treatment on antioxidant activity (%) of lime-flavored dragon fruit RTS beverage**

Temperature (°C)	Time (min)			Mean T
	t <sub>1</sub>	t <sub>2</sub>	t <sub>3</sub>	
T <sub>1</sub>	73.43	70.40	67.86	70.56
T <sub>2</sub>	74.48	72.62	72.16	73.07
T <sub>3</sub>	76.19	73.75	73.56	74.50
Mean t	74.69	72.25	71.19	
Factor	T	t	T x t	
C.D. (5%)	NS	NS	NS	
SEm	2.37	2.37	4.11	
CV%	9.80			

\*T: temperature; T<sub>1</sub>: 70°C; T<sub>2</sub>: 80°C; T<sub>3</sub>: 90°C; t: time; t<sub>1</sub>: 5 min; t<sub>2</sub>: 10 min; t<sub>3</sub>: 15 min; T x t: interaction of temperature, and time; NS: non-significant

**Table 4. Effect of thermal treatment on overall acceptability score of lime-flavored dragon fruit RTS beverage**

Temperature (°C)	Time (min)			Mean T
	t <sub>1</sub>	t <sub>2</sub>	t <sub>3</sub>	
T <sub>1</sub>	7.55	7.60	7.53	7.56
T <sub>2</sub>	7.55	7.49	7.37	7.47
T <sub>3</sub>	7.40	7.49	7.36	7.42
Mean t	7.50	7.53	7.42	
Factor	T	t	T × t	
C.D. (5%)	0.09	NS	NS	
SEm	0.03	0.03	0.05	
CV%	1.20			

\*T: temperature; T<sub>1</sub>: 70°C; T<sub>2</sub>: 80°C; T<sub>3</sub>: 90°C; t: time; t<sub>1</sub>: 5 min; t<sub>2</sub>: 10 min; t<sub>3</sub>: 15 min; T × t: interaction of temperature, and time; NS: non-significant

**Table 5. Effect of microwave treatment on betacyanin content (mg/L), and antioxidant activity of lime-flavored dragon fruit RTS beverage**

Treatment	Betacyanin content (mg/L)	% Antioxidant activity
MW <sub>1</sub>	29.87 ± 2.04	54.31 ± 6.08 <sup>a</sup>
MW <sub>2</sub>	28.27 ± 2.83	47.94 ± 5.34 <sup>a</sup>
MW <sub>3</sub>	31.52 ± 1.88	31.74 ± 7.11 <sup>b</sup>
MW <sub>4</sub>	32.43 ± 1.87	21.11 ± 7.27 <sup>c</sup>
MW <sub>5</sub>	31.05 ± 0.85	12.83 ± 3.74 <sup>d</sup>
C.D. (5%)	NS	9.11
SEm	3.00	3.02
CV%	19.61	18.01

\*n: Mean of three replications; MW<sub>1</sub>= 30 sec, MW<sub>2</sub>= 60 sec, MW<sub>3</sub>= 90 sec, MW<sub>4</sub>= 120 sec, MW<sub>5</sub>= 150 sec, NS= non-significant

**Table 6. Effect of microwave treatment on sensory evaluation of lime-flavored dragon fruit RTS beverage**

Treatment	Color	Taste	Body	Flavor	Overall acceptability
MW <sub>1</sub>	8.04 ± 0.10	7.74 ± 0.16	7.49 ± 0.01	7.58 ± 0.12	7.56 ± 0.16
MW <sub>2</sub>	8.09 ± 0.04	7.71 ± 0.19	7.53 ± 0.05	7.51 ± 0.12	7.56 ± 0.11
MW <sub>3</sub>	8.04 ± 0.10	7.73 ± 0.21	7.54 ± 0.07	7.51 ± 0.11	7.51 ± 0.12
MW <sub>4</sub>	8.04 ± 0.10	7.72 ± 0.15	7.50 ± 0.02	7.55 ± 0.12	7.53 ± 0.13
MW <sub>5</sub>	8.09 ± 0.04	7.75 ± 0.12	7.55 ± 0.03	7.55 ± 0.13	7.54 ± 0.13
C.D. (5%)	NS	NS	NS	NS	NS
SEm	0.04	0.08	0.02	0.06	0.07
CV%	1.02	2.16	0.56	1.59	1.74

\*n: Mean of three repetitions; MW<sub>1</sub>= 30 sec, MW<sub>2</sub>= 60 sec, MW<sub>3</sub>= 90 sec, MW<sub>4</sub>= 120 sec, MW<sub>5</sub>= 150 sec, NS= non-significant

#### 4. CONCLUSION

The betacyanin content degradation was increased with increase in temperature, and treatment time duration for thermal treatment. The reduction of betacyanin content was more for thermal treatment than microwave treatment. The antioxidant activity was increased with increasing temperature due to release of phenolic compounds from cells, but at the same time exposure of long time at higher temperature can lead to degradation of that compounds resulted in decrease in antioxidant activity. For

microwave treatment less exposure time can slightly increase antioxidant activity, However, longer exposure time leads to reduction in antioxidant activity. For deep understanding about antioxidant activity response behavior, study related to total phenols is needed. Thermal treatment for 5 min at 70°C was retained 23.80 mg/L of betacyanin content, and 73.43% antioxidant activity, whereas for microwave treatment for 30 sec at 90°C was retained 29.87 mg/L betacyanin, and 54.31% antioxidant activity.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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