

Development of Functional RTS Beverage from Jamun (*Syzygium cumini* L.) and *Melastoma malabathricum*

Nandita Barman¹ and Mridula Saikia Barooah²

¹Department of Food Science and Technology, Assam Agricultural University, Jorhat, Assam, INDIA

²Department of Food Science and Nutrition, Assam Agricultural University, Jorhat, Assam, INDIA

E-mail: ¹nanditabarman278@gmail.com, ²msbarooah@gmail.com

Abstract—Fruits of *Melastoma malabathricum* are rich in phytochemicals such as polyphenols, flavanoids and tannins which attribute to a strong free radical scavenging activity. The present study was designed to develop and standardize a blended RTS beverage from Jamun and *M. malabathricum*. The extracted juice of Jamun and *M. malabathricum* fruits were analyzed for its physico-chemical parameters and free radical scavenging activity. Six formulation of the beverages were prepared by blending the Jamun and *M. malabathricum* fruit juice (T_1 – 50% Jamun + 50%*Melastoma*, T_2 – 60%Jamun + 40%*Melastoma*, T_3 – 70%Jamun + 30%*Melastoma*, T_4 – 80%Jamun + 20%*Melastoma*, T_5 – 90%Jamun + 10%*Melastoma*, T_6 – 100%Jamun without *Melastoma* extract as control). The prepared RTS beverages were kept at an ambient temperature for a period of 3 months and analyzed for different physico-chemical parameters, free radical scavenging activity and subjected to sensory evaluation at an interval of 1 month. *M. malabathricum* fruit juice supplementation enhanced the bioactive composition of the beverages in terms of increased ascorbic acid and antioxidant activity. During storage period, the physico-chemical parameters like acidity, reducing sugars and total soluble sugars increased continuously, while TSS and pH decreased. Among the blends, T_2 having formulation of 60% Jamun and 40% *Melastoma* was found to be optimum for overall acceptability. Overall, it can be concluded that fruits of *M. malabathricum* can be used as a valuable ingredient for the development of functional RTS beverages with important bioactive compounds and the standardized RTS beverage can have an immense role in health benefactors.

Keywords: Jamun; *Melastoma malabathricum*; RTS; Antioxidant; Sensory evaluation.

1. INTRODUCTION

Beverages are consumed regularly around the world and therefore constitute an important pillar in the daily diet of humans. Their popularity is due mostly to the variety of pleasant taste and sensation, such as sweet, cool or refreshing [1]. However, beverages are also consumed for health reasons. Functional beverages are an excellent means for nutrient and bioactive compounds including vitamins,

minerals, antioxidants, omega-3 fatty acids, plant extracts, fibre, prebiotics and probiotics.

Fruit and vegetable juices are predominately rich source of antioxidants. Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [2].

Syzygium cumini (L.), commonly known as Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc is an important medicinal plant. It showed considerable high antioxidant activity, which constitute anthocyanins, tannins and flavonols [3]. The ripe fruits are used for preparation of health drinks, making preserves, squashes, jellies, RTS and wine as it is effective in the treatment of diabetes mellitus, inflammation, ulcers and diarrhea [4].

Melastoma malabathricum Linn. is a shrub that belongs to the family Melastomataceae and it comes with beautiful pink or purple flowers. The fruits are encapsulated and contain many non-endospermous seeds with small embryos inside purplish pulps. The fruits of *M. malabathricum* are rich sources of several secondary metabolites such as flavonoid and phenolic compounds such as anthocyanins which possess antioxidant, phytoalexin, antibacterial activities and therapeutic activities such as anticancer which are used for the treatment of various human ailments. Besides being beneficial to health, the fruits also have potential as natural food colourant [5].

Hence, in the light of above research facts, the present investigation was under taken with objective to incorporate antioxidant rich fruit juice extracts of *Melastoma malabathricum* for the development of delicious and nutritious blended functional RTS beverage that could therapeutically help in improving the health of consumers and to study the physicochemical, free radical scavenging activity and sensory quality in storage period at an ambient temperatures.

2. MATERIALS AND METHODS

2.1. MATERIALS

The present study was conducted in the year 2016-17 at Quality Control Laboratory, Department of Horticulture, Assam Agricultural University, Jorhat, Assam.

The fresh Jamun and *Melastoma malabathricum* fruits were collected from a selected locality of Assam Agricultural University, Jorhat, Assam. Other materials like sugar were procured from a local market.

All the chemicals used in this study were of analytical grade.

2.1.1. Extraction of Jamun juice. The fruit juice was extracted as per the procedure outlined by Srivastava and Kumar [6]. The fresh and well ripe fruits were selected.

2.1.2. Extraction of *M. malabathricum* juice. The fresh and well ripe fruits were cleaned and washed and fruit juice was extracted by a standardized method.

The extracted juice of Jamun and *M. malabathricum* fruits were analyzed initially for its physico-chemical parameters and free radical scavenging activity.

2.1.3. Preparation of blends. Six formulation of the beverages were prepared by blending the Jamun and *M. malabathricum* fruit juice (T₁ – 50%Jamun + 50%Melastoma, T₂ – 60%Jamun + 40%Melastoma, T₃ – 70%Jamun + 30%Melastoma, T₄ – 80%Jamun + 20%Melastoma, T₅ – 90%Jamun + 10%Melastoma, T₆ – 100%Jamun without Melastoma extract as control) and the observations were recorded for different blends.

2.1.4. Preparation of ready-to-serve (RTS) beverage. RTS was prepared by using the respective blends by addition of sugar syrup and permitted quantity of preservatives. The TSS was adjusted to 10° Brix. Citric acid (0.3%) was added to maintain the acidity and Sodium benzoate (100ppm) was used as preservative.

The RTS was prepared as per the procedure outlined by Srivastava and Kumar [6]. The prepared RTS beverages were kept at an ambient temperature for a period of 3 months and analyzed for different physico-chemical parameters, free radical scavenging activity and subjected to sensory evaluation at an interval of 1 month.

2.2. DETERMINATION OF p^H

The pH was directly measured in a digital pH meter (Denver Instruments, Model 215) coupled with a combined electrode.

2.3. DETERMINATION OF TSS

The total soluble solids was determined by using Erma Tokyo's (0-32°C) hand refractometer and expressed in °Brix.

2.4.DETERMINATION OF TITRABLE ACIDITY

The titrable acidity was estimated by the volumetric method [7]. Percentage of acid content was calculated by using the formula: % Acidity (as citric acid) = Titre value × Normality of alkali × Equivalent wt. of acid × Vol. made up × 100

Volume of sample taken for estimation × Wt or Vol. of sample taken × 1000

2.5. DETERMINATION OF REDUCING SUGAR AND TOTAL SOLUBLE SUGAR

Reducing sugar content was estimated by DNS method [8] and Total sugar content was estimated by anthrone method [9] with slight modification.

2.6. DETERMINATION OF ASCORBIC ACID CONTENT

Vitamin C was determined by the 2,6-dichlorophenolindophenol dye method [10]. Percentage of ascorbic acid content was calculated by using the following formula: Ascorbic acid (mg/100g/ml) = Titre value × Dye factor × Vol. made up × 100

Wt. or Volume × Volume of sample
of sample taken taken for estimation

2.7. DETERMINATION OF ANTIOXIDANT ACTIVITY

Free radical scavenging activity of the RTS was measured by the method with slight modifications [11]. The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on the 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical. An aliquot (100 µl) of the beverages was mixed with 2.9 ml of 0.05 mM DPPH solution prepared in 99.5% methanol. The mixture was thoroughly vortex-mixed and incubated in the dark for 30 min. The discoloration of DPPH was measured against control at 517nm. Methanol was used as blank and DPPH methanolic solution was used as control.

Calculation:

$$\% \text{ inhibition} = (A_b - A_s) / A_b \times 100$$

where,

A_b is the absorbance of control sample

A_s is the absorbance of sample

2.8. SENSORY ANALYSIS

Sensory evaluation of the beverages was done by a panel of 20 people of different age groups at 9 Point Hedonic Scale in order to find out the consumer preference for overall acceptability [12].

2.9. STATISTICAL ANALYSIS

The data obtained was subjected to statistical analysis using "Completely Randomized Design" with 3 replications. The results were statistically evaluated by one-way analysis of variance (ANOVA). The significance difference of the treatment values was determined at the 0.05 probability level by using LSD test using SPSS software. Values are means of three (3) replications \pm standard deviation.

3. RESULTS AND DISCUSSION

The Physico-chemical compositions and free radical scavenging activity of studied Jamun and *Melastoma malabathricum* fruit juices were presented in (see Table 1).

"TABLE 1. Analysis of Jamun and *M. malabathricum* juices"

Parameters	Jamun	<i>Melastoma malabathricum</i>
Ph	4.1 \pm 0.03	4.3 \pm 0.02
Total Soluble Solids ($^{\circ}$ Brix)	9 \pm 0.02	6 \pm 0.04
Acidity (%)	0.81 \pm 0.02	0.62 \pm 0.01
Ascorbic acid (mg/100g)	22.6 \pm 0.05	15.16 \pm 0.03
Total Sugar (g/100g)	13.92 \pm 0.06	6.8 \pm 0.03
Reducing Sugar (g/100g)	5.59 \pm 0.02	3.38 \pm 0.01
DPPH inhibition (%)	92.20 \pm 0.5	96.36 \pm 0.13

Values are average of three replicates \pm SD

The study reveals that the free radical scavenging activity of *Melastoma malabathricum* (96.36%) fruit is higher than Jamun (92.20%), but however it is comparatively lower in physico-chemical parameters like TSS, acidity, ascorbic acid, p^H , total sugar and reducing sugar is than Jamun.

3.1. TITRABLE ACIDITY

Data relating to change in acidity during storage are furnished in Table 2. which reveals that there was an increasing trend in acidity in all the developed RTS in storage conditions. Across storage, highest acidity was recorded in T6 which increased from (0.341%) to (0.378%) and lowest acidity was recorded in T1 which increased from (0.304%) to (0.338%). The increase in acidity might be due to the formation of organic acids by the degradation of ascorbic acid [13]. The slight growth of micro-organisms in the beverages may leads to the increase in titrable acidity [14].

"TABLE 2. Changes in Titrable acidity (%) of RTS during storage"

RTS Blends	0 day	30 days	60 days	90 days
T ₁	0.304	0.311	0.327	0.338
T ₂	0.309	0.315	0.332	0.341
T ₃	0.312	0.321	0.339	0.344
T ₄	0.324	0.331	0.345	0.357
T ₅	0.338	0.345	0.353	0.362

T ₆	0.341	0.352	0.361	0.378
SD (\pm)	0.08	0.08	0.01	0.05
CD=(0.05)	0.34	0.34	0.04	0.21

Values are average of three replicates \pm SD

3.2. p^H

The pH values of all the RTS are presented in Table 3. From the table, it was found that during storage at an ambient condition, a decreasing trend in the pH was observed in all the beverages. During storage, highest pH was recorded in T1 (3.91%) and lowest was recorded in T6 (3.31%).

The increase in acidity of the drinks attributed to the increase in release of hydrogen ions during the storage. Therefore the corresponding decrease was noticed in pH [15].

"TABLE 3. Changes in pH of RTS during storage"

RTS Blends	0 day	30 days	60 days	90 days
T ₁	4.45	4.32	4.00	3.91
T ₂	4.41	4.14	3.94	3.73
T ₃	4.39	3.89	3.75	3.68
T ₄	4.36	4.13	3.79	3.62
T ₅	4.35	3.90	3.63	3.47
T ₆	4.23	3.96	3.77	3.31
SD (\pm)	0.09	0.03	0.2	0.06
CD=(0.05)	0.38	0.12	0.86	0.25

Values are average of three replicates \pm SD

3.3. TOTAL SOLUBLE SOLIDS

TSS content during the storage period is presented in Table 4. which shows a decreasing trend with increase in the storage time. From the table, it was observed that the initial TSS range for all the beverages was maintained at 10 $^{\circ}$ Brix and at the end of the storage period, the highest TSS range was maintained in T1 (10 $^{\circ}$ Brix) and lowest TSS was observed in T6 (9 $^{\circ}$ Brix) which does not show any significant difference during storage and similar result was reported by Vidhya [16].

"TABLE 4. Changes in Total Soluble Solids ($^{\circ}$ Brix) of RTS during storage"

RTS Blends	0 day	30 days	60 days	90 days
T ₁	10	10	10	10
T ₂	10	10	10	9
T ₃	10	10	10	9
T ₄	10	10	10	9
T ₅	10	10	9	9
T ₆	10	10	9	9
SD (\pm)	0.01	0.01	0.02	0.02
CD=(0.05)	0.04	0.04	0.04	0.04

Values are average of three replicates \pm SD

3.4. ASCORBIC ACID

It was observed in Table 5. that the ascorbic acid content of the beverages decreased significantly from initial day to 90

days of storage period. The highest ascorbic acid content was observed in T₁ which decreases from (16.02 mg/100 ml) to (15.58 mg/100 ml) during the storage period and the lowest ascorbic acid content was observed in T₆ which decreases from (14.72 mg/100 ml) to (14.57 mg/100 ml).

Ascorbic acid content of blended RTS beverages decreased continuously during the period of storage. This reduction might be due to oxidation of ascorbic acid into dehydro-ascorbic acid or hydroxyl methyl furfural at room temperature, due its sensitive nature [17]. Degradation of ascorbic acid into other organic acids could lead to decrease in pH as it was confirmed by the results on pH in the present study.

“TABLE 5. Changes in Ascorbic acid (mg/100ml) of RTS during storage”

RTS Blends	0 day	30 days	60 days	90 days
T ₁	16.02	15.98	15.91	15.85
T ₂	15.94	15.91	15.87	15.83
T ₃	15.72	15.65	15.60	15.56
T ₄	15.53	15.47	15.42	15.39
T ₅	14.98	14.83	14.78	14.74
T ₆	14.72	14.66	14.62	14.57
SD (±)	0.28	0.12	0.09	0.01
CD=(0.05)	1.20	0.51	0.38	0.04

Values are average of three replicates ± SD

3.5. REDUCING SUGARS

Among the treatments, as shown in Table 6. the highest amount of reducing sugars was observed in T₁ which increases from (4.99g/100ml) to (5.33g/100ml) during the storage period and lowest was observed in T₆ which increases from (4.83g/100 ml) to (5.15 g/100 ml). The analysis revealed that the reducing sugars increased steadily and significantly from the initial day of preparation to the maximum at 90 days of storage.

The increase in reducing sugars might be due to the conversion of non-reducing sugars into reducing sugars in presence of citric acid [14].

“TABLE 6. Changes in Reducing sugars (g/100ml) of RTS during storage”

RTS Blends	0 day	30 days	60 days	90 days
T ₁	4.99	5.13	5.21	5.33
T ₂	4.93	5.11	5.19	5.28
T ₃	4.91	5.09	5.18	5.25
T ₄	4.86	4.95	5.16	5.21
T ₅	4.87	4.91	5.03	5.18
T ₆	4.83	4.95	5.10	5.15
SD (±)	0.02	0.02	0.05	0.05
CD=(0.05)	0.08	0.08	0.21	0.21

Values are average of three replicates ± SD

3.6. TOTAL SUGARS

Among the treatments as shown in Table 6. the highest value of total sugars was observed in T₁ which increases from (6.13g/100ml) to (6.28g/100ml) during the storage period. The RTS prepared from the jamun juice 100% (T₆) was found to have the least quantity of total sugars which increases from (5.82g/100ml) to (5.94g/100ml). The percentage of total sugars increased significantly from the day of preparation to 90 days after storage. However, the increasing trend in total sugars was observed by earlier workers and was ascribed due to inversion of sugars and hydrolysis of polysaccharides into simple sugars [18].

“TABLE 7. Changes in Total Sugar (g/100ml) of RTS during storage”

RTS Blends	0 day	30 days	60 days	90 days
T ₁	6.13	6.18	6.21	6.28
T ₂	6.11	6.14	6.18	6.25
T ₃	6.05	6.09	6.13	6.21
T ₄	5.89	5.93	5.98	6.03
T ₅	5.86	5.89	5.95	6.00
T ₆	5.82	5.86	5.90	5.94
SD (±)	0.06	0.05	0.06	0.01
CD=(0.05)	0.25	0.21	0.25	0.04

Values are average of three replicates ± SD

3.7. ANTIOXIDANT ACTIVITY

Antioxidant activity in terms of DPPH % radical scavenging activity was found to be higher in all the blended RTS beverages as compared to the RTS (T₆) prepared from 100% jamun juice Table 8. Among the treatments, as shown in Table 8, the highest value of antioxidant activity was observed in T₁ having formulation of 50% Jamun juice and 50% *M. malabathricum* fruit extracts which decreases from (94.19%) to (93.98) and lowest antioxidant activity was observed in T₆ which decreases from (81.28%) to (80.51%) during the storage period.

The fruits of *M. malabathricum* which is underutilized has higher DPPH free radical scavenging activity, which can be related to its higher phenolic content. The higher content of total phenolics, flavonoids and antioxidant capacity of *M. malabathricum* fruits suggests the possibility of its incorporation for development of newer effective *M. malabathricum* preparations [19].

“TABLE 8. Changes in Free radical scavenging activity (%) of RTS during storage”

RTS Blends	0 day	30 days	60 days	90 days
T ₁	94.19	94.12	94.03	93.98
T ₂	92.70	92.64	92.58	92.53
T ₃	91.35	91.30	91.24	91.19
T ₄	87.78	87.65	87.61	87.53
T ₅	84.27	84.19	84.13	84.05

T ₆	81.28	80.72	80.63	80.51
SD (±)	0.5	0.44	0.4	0.13
CD=(0.05)	2.16	1.94	1.73	0.56

Values are average of three replicates ± SD

3.8. OVERALL ACCEPTABILITY

Overall acceptability of RTS also decreased over the time as shown in Table 9. Among the blends, T₂ having formulation of 60% Jamun and 40% *Melastoma* (7.14), scores highest for overall acceptability while lowest score was obtained by T₃ (6.00) during storage. Biochemical changes occurring during the storage might have led to the formation of undesirable colours, flavours and taste, which might have affected the poor acceptability of the products which can further lead to decrease the organoleptic score of the product [20].

“TABLE 9. Changes in Overall acceptability of RTS during storage”

RTS Blends	0 day	30 days	60 days	90 days
T ₁	8.00	7.57	6.85	6.57
T ₂	9.00	8.71	7.57	7.14
T ₃	7.71	6.85	6.28	6.28
T ₄	7.85	7.28	6.71	6.28
T ₅	7.57	7.00	6.42	6.00
T ₆	8.00	7.28	7.00	6.42
SD(±)	0.21	0.24	0.35	0.28
CD=(0.05)	0.53	0.61	0.88	0.71

Values are average of three replicates ± SD

4. CONCLUSION

Among the blends, T₂ having formulation of 60% Jamun and 40% *Melastoma* was found to be optimum for overall acceptability. The above RTS beverages can be stored effectively for three months in an ambient temperature. *M. malabathricum* fruit juice supplementation enhanced the bioactive composition of the beverages in terms of increased ascorbic acid and antioxidant activity. Overall, it can be concluded that the above fruit can be used as a valuable ingredient for the development of functional RTS beverages with all important properties and medicinal characteristics of *M. malabathricum*. Thus, it can be proved that the standardized RTS beverage can be a good health drink with phenolics and antioxidants and can have an immense role in health benefactors.

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