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Effect of Storage on Chemical Composition and Microbial Count of Carbonated Ready to Serve (RTS) Beverage from Jamun (*Syzygium cumini* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was undertaken at Fruit and Vegetable Processing Unit of College of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist-Ratnagiri (Maharashtra State) during the period of May 2023 to January 2024. This study aims to evaluate the effects of carbonation levels and juice-sugar percentage on chemical composition and microbial count of carbonated jamun beverages during storage. Now-a-days there is increase in demand for health-conscious products, driven by evolving consumer preferences and increasing awareness regarding the impact of diet on health. Synthetic drinks which are more popular commercially, are not so healthy or nutritive. So, considering medicinal and nutritive properties of jamun the experiment was undertaken. The experiment was carried out in Factorial Completely Randomized Design (FCRD) with two factors having sixteen treatment combination replicated thrice which consisting of four carbonation levels *viz*. C_1- 40 psi, C_2-50 psi, C_3-60 psi and C_4-70 psi and four juice-sugar levels percentage *viz*. J_1- jamun juice 25% + sugar 75%, J_2- jamun juice 30% + sugar 70%, J_3- jamun juice 35% + sugar 65% and J_4- jamun juice 40% + sugar 60%. From the study it was found that during six months of storage of carbonated RTS beverage from jamun, Total Soluble Solids (TSS), reducing sugars, total sugars and titratable acidity increased from 14.00 to14.68°Brix, 2.34 to 6.37 per cent, 10.19 to 10.98 per cent, 0.20 to 0.33 per cent, respectively whereas pH and anthocyanin decreased from 2.31 to 2.23 and 5.68 to 3.62 mg/100 ml, respectively. During six months of storage, microbial count like fungal and bacterial count increased slightly from 0.00 to 0.15 x 10^{-4} CFU/ml and 0.00 to 0.11 x 10^{-4} CFU/ml, respectively.

Keywords: Jamun; carbonated beverage; storage; microbial count.

1. INTRODUCTION

Jamun (*Syzygium cumini* L.) is an evergreen tropical tree. It is a member of Myrtaceae family. It is an important minor and dry land fruit of India. Though this fruit is considered as a minor fruit crop because of its high nutritional value and excellent processing qualities, it is now commercially cultivated in sub-tropics and in most arid regions of the country. In India, the maximum number of jamun trees are found scattered throughout the tropical and subtropical regions. Maharashtra State is the largest Jamun producer followed by Uttar Pradesh, Tamil Nadu, Gujarat, Assam and others. Jamun is used in numerous conventional structures like Unani, Homeopathic, Siddha and Ayurveda [1]. Various studies shows that Jamun is used as anti-inflammatory, anti-arthritic, anti-nociceptive, anti-genotoxin, antispasmodic and to improve memory. Jamun contains Jamboline, a type of glucose, having major role to control the conversion of starch into sugar and helps to maintain blood sugar in normal range [2]. Carbonation is the term used to identify the dissolution of CO_2 gas in the water mixture utilizing pressure and temperature. Now-a-days there is increase in demand for health-conscious products, driven by evolving consumer preferences and increasing awareness regarding the impact of diet on health. Fruit juices occupy a unique position among those products classified as beverages. Being a source of energy, vitamins and minerals, juices are not only indispensable for the maintenance of health but also considered as the beverages of refreshment which quenches thirst and encourages liquid intake. In order to improve the social and nutritional status of people and also to make an impact in export promotion, it is meaningful to increase the production of high quality, delicious and variable processed products from underutilized fruits in present day value addition programme. Fruit juice beverages also hold an important position due to their richness in essential minerals, vitamins and other nutritive constituents. Synthetic drinks which are more popular commercially, are not so healthy or nutritive compared to fruit based beverages. So, research on the preparation of carbonated RTS beverage from jamun juice is important which can offer a healthier alternative to traditional carbonated beverages.

2. MATERIALS AND METHODS

The experiment was undertaken at Fruit and Vegetable Processing Unit of College of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist-Ratnagiri (M.S.) during the period of May 2023 to January 2024. The experiment was carried out in Factorial

Completely Randomized Design (FCRD) with two factors having sixteen treatment combinations replicated thrice which consisting of four carbonation levels *viz*. C_1- 40 psi, C_2- 50 psi, C_3- 60 psi and C_4- 70 psi and four juice-sugar levels *viz*. J_1- jamun juice 25% + sugar 75%, J_2- jamun juice 30% + sugar 70%, J_3- jamun juice 35% + sugar 65% and J_4- jamun juice 40% + sugar 60%.

Jamun fruits of local types used for the experiment were obtained from orchard of Mr. Akshay Gurunath Karnik, farmer from Vengurla, Dist.- Sindhudurg. Fully matured, healthy, perfectly ripe and disease-free fruits were selected and washed thoroughly to remove dirt. After proper selection and washing fruits were used for extracting juice. The juice was extracted mechanically with the help of Jamun pulper. Initially pulp was separated from seed with the help of jamun pulper. Then the juice was extracted by pressing pulp in muslin cloth. As per the treatment details juice and sugar required for the preparation syrup was calculated and taken into a stainless-steel vessel. Then the quantity of sugar was added in the juice as per treatment. Slight heating was done to dissolve sugar into the juice. Then acidity of syrup was maintained to 1.5 per cent by adding appropriate quantity of citric acid in the syrup. In the syrup, 700 mg sodium benzoate was added per litre syrup. The quantity of syrup required (35 g) for 200 ml bottle was weighed and added into each pre-sterilized bottle. For preparation of carbonated RTS beverage, potable water was passed through sand filter and chilled at 4°C temperature. Then this water was passed through the carbonator at 40, 50, 60 and 70 psi CO_2 pressure as per the treatments and finally mixed with the syrup taken into the 200 ml capacity glass bottles and immediately sealed with sterilized crown caps. The carbonated RTS bottles were then stored at cold storage at 12 ± 2°C. The prepared carbonated RTS beverage from jamun was evaluated for different chemical parameters at three months interval from day of preparation of carbonated RTS beverage from jamun up to 6 months.

2.1 Chemical Analysis

Total soluble solids (TSS) of fresh jamun juice and carbonated RTS beverage were determined with the help of Hand Refractometer (Erma Japan, 0 to 32°Brix). Reducing sugars and total sugars of fresh jamun juice and carbonated RTS beverage were estimated by using procedure [3] with suggested modifications (Ranganna, 1986). Titratable acidity and anthocyanin content of fresh jamun juice and carbonated RTS beverage were determined as per standard analytical methods [4]. The pH of fresh jamun juice and carbonated RTS beverage was determined with the help of pH meter (Model Systronics µ pH system 361).

2.2 Microbial Analysis

To estimate the microbial growth in carbonated RTS beverage, serial dilution and pour plate technique was followed using potato dextrose agar medium for fungi and nutrient agar medium for bacteria. For this technique water blanks were prepared by taking 9 ml of distilled water in each sterilized test tube. 1 ml sample of carbonated RTS beverage was transferred to first water blank to obtain 10^{-1} dilution. The content was homogenized by shaking. After 1 minute, 1 ml of aliquot from 10^{-1} dilution was transferred to second water blank to obtain 10^{-2}, likewise the dilution was made to 10^{-4} dilution. For inoculation, from last (10^{-4}) dilution 1 ml of aliquot was transferred in sterilized petri-plates and prepared medium was poured over the sample. Such inoculated plates were then incubated at 27±2°C and observed for colony development.

2.3 Statistical Analysis

The collected data were analyzed statistically following the method suggested [5]. The standard error of the mean (SEM) was calculated and the critical difference (C.D.) at the 1 per cent level of significance was determined whenever the results showed significance.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Fresh Jamun Juice

The data presented in Table 1 shows the mean values for different parameters of fresh jamun juice. The TSS of fresh jamun juice was 15°B, reducing sugars were 9.80 per cent, total sugars were 13.50 per cent, titratable acidity was 0.87 per cent, pH was 3.32 and anthocyanin content was 104 mg/100 ml. Similar results were recorded in jamun fruits [6,7,8] (Patil et al., 2015).

Table 1. Chemical composition of fresh jamun juice

Sr. No.	Parameters	Content
1.	TSS (ºBrix)	15
2.	Reducing sugars (%)	9.80
3.	Total sugars (%)	13.50
4.	Titratable acidity (%)	0.87
5.	pH	3.32
6.	Anthocyanin (mg/100 ml)	104

3.2 Changes in Chemical Composition of Carbonated RTS Beverage from Jamun during Storage

The results regarding the effect of carbonation levels, juice-sugar levels and interaction between these two on chemical composition of carbonated RTS beverage from jamun during storage is given below:

The results regarding changes in TSS, reducing sugars, total sugars, titratable acidity, pH and anthocyanin of carbonated RTS beverage from jamun during six months of storage are presented in Tables 2, 3, 4, 5, 6 and 7 respectively. All the chemical parameters differ significantly throughout the storage period. TSS, reducing sugars, total sugars and titratable acidity of carbonated RTS beverage from jamun increased from 0 month to 6 months during storage, irrespective of carbonation and juice-sugar levels. The increase in TSS and total sugars during storage might be due to the conversion polysaccharides like starch and pectin into sugars. Similar findings were reported in jamun carbonated beverage during two months of storage [9]. The increase in reducing sugars might be due to gradual inversion of non-reducing sugar like sucrose into reducing sugars (glucose and fructose) by the process of hydrolysis during storage. Similar results were reported in carbonated RTS beverage from Nagpur Mandarin orange during six months of storage [10]. The increase in titratable acidity during storage might be due to formation of organic acids by degradation of ascorbic acid as well as degradation of pectic substances into soluble solids might have contributed towards increase in the acidity. Similar observations were recorded in jamun carbonated beverage during two months of storage (Joshi et al., 2010). pH and anthocyanin content of carbonated RTS beverage from jamun decreased from 0 month to six months during storage, irrespective of carbonation and juice-sugar levels. The decrease in value of pH during storage might be due to degradation of pectin substances which increases acidity. Therefore, as the acidity increases the concentration of hydronium ions also increases and hence, the pH decreases. Similar results were recorded in carbonated jamun beverage during 6 months of storage at low temperature [11]. Decrease in anthocyanin content throughout the storage might be due to hydrolysis of protective 3-glucoside linkages to give unstable anthocyanin which leads to degradation. Similar results were reported in jamun carbonated beverage [9].

It is observed from the table that with respect to carbonation levels, results regarding TSS, reducing sugars, total sugars, titratable acidity, pH and anthocyanin were found to be significant throughout the storage period of six months. In case of carbonation level, TSS, reducing sugars, total sugars and anthocyanin were increased from C_1 to C_3 and then slightly decreased at C_4 throughout the storage, irrespective of juice-sugar levels. Similar observations were recorded in carbonated beverage from cashew apple syrup [12] and kokum during six months of storage period [13]. Titratable acidity and pH were increased from C_1 to C_4 with increase in CO_2 pressure throughout the storage, irrespective of juice-sugar levels. With increase in carbonation level, there is increase in formation of carbonic acid, produced due to carbonation which might be the reason for increase in titratable acidity. Similar results were recorded in the carbonated RTS beverage from kokum during six months of storage period [13].

With respect to juice-sugar levels, significant difference in TSS, reducing sugars, total sugars, titratable acidity, pH and anthocyanin was found during storage. In case of juice-sugar level, TSS and total sugars were decreased from J_1 to J_4 throughout the storage. This decreasing trend might be due to decrease in sugar concentration from J_1 (75% sugar) to J_4 (60% sugar). Similar results were recorded in carbonated RTS beverage from kokum during six months of storage period [13]. Reducing sugars, titratable acidity, pH and anthocyanin were increased from

J_1 to J_4. This increase in reducing sugars might be due to increase in native reducing sugars with increase in juice concentration from J_1 to J_4. Similar results were recorded in carbonated beverage from pomegranate fruits cv. (cultivated variety) Mridula during three months of storage [14]. The increase in titratable acidity may be attributed to the presence of organic acids found in jamun juice, such as citric acid, malic acid and tartaric acid. These acids contributed to the overall acidity levels, which rise from J_1 (25% juice) to J_4 (40% juice). Similar observations were recorded in guava carbonated drink [15]. The increase in anthocyanin content may be attributed to the presence of original anthocyanins in jamun juice, primarily delphinidin and cyanidin. Similar results were recorded in carbonated beverage from pomegranate fruits cv. Mridula [14].

The interaction between carbonation and juice-sugar levels showed significant results for TSS, reducing sugars, total sugars, titratable acidity, pH and anthocyanin during entire storage period. At 0 month storage, interaction C_3J_1 recorded the highest TSS (15.73°B) and total sugars (12.10%). In case of six months of storage, C_3J_1 recorded the highest TSS (15.87°B) and was at par with C_4J_1 (15.73°B) and C_2J_1 (15.60°B). Interaction C_3J_1 recorded the highest total sugars (12.76%) at six months of storage and was significantly superior over other interactions. Interaction C_3J_4 recorded the highest reducing sugars (7.46%) and was at par with C_1J_4 (7.39%) and anthocyanin (5.26 mg/100 ml) and at par with C_4J_4 (5.24 mg/100 ml) at six months of storage. Interaction C_4J_4 recorded the highest titratable acidity (0.37%) which was at par with C_4J_3 (0.36%), C_1J_4 and C_2J_4 (0.35%) at six months of storage. Significantly lowest pH was recorded by interaction C_1J_1 (2.07) at six months of storage over other interactions.

3.3 Changes in Microbial Count of Carbonated RTS Beverage from Jamun during Storage

The results regarding changes in fungal and bacterial count of carbonated RTS beverage from jamun during six months of storage are presented in Table 8 and Table 9 respectively. The results showed that fungal and bacterial count increased slightly during six months of storage, irrespective of carbonation and juice-sugar levels. During storage, the conditions may have facilitated fungal growth in the product, potentially leading to an increase in the fungal count. Similar observations were recorded in carbonated RTS beverage from kokum during six months of storage period [15]. Generally, bacteria are very sensitive to carbon dioxide but increase in bacterial count was observed which may be due to multiplication of acid tolerant bacteria in the product during the storage. Similar findings were recorded in carbonated beverage from pomegranate fruits cv. Mridula during three months of storage [14].

In case of carbonation levels, no any fungal and bacterial growth was detected initially at 0 month of storage. The results regarding carbonation levels were found significant at 3 and 6 months of storage. From the data, it was observed that fungal and bacterial count decreased from C_1 (40 psi) to C_4 (70 psi), irrespective of juice-sugar levels. This reduction in fungal growth might be due to increase in CO_2 pressure. The effectivity of carbon dioxide against microorganisms generally increases with increase in concentration. Generally, carbon dioxide under higher pressure has ability to inhibit the bacterial growth. Inclusion of carbon dioxide, excludes the oxygen and it affects metabolic activities of bacteria. Similar findings were recorded in carbonated RTS beverage from kokum [13] and Nagpur Mandarin orange during six months of storage [10].

It is seen from the table that with respect to juice-sugar levels, results regarding fungal and bacterial count were found non-significant at 3 and 6 months of storage. At 0 month of storage, no any fungal and bacterial growth was detected. In case of juice-sugar levels, fungal and bacterial count increased from J_1 to J_4, irrespective of carbonation levels. This increase in fungal count might be due to increase in juice concentration and decrease in sugar concentration from J_1 (25% juice + 75% sugar) to J_4 (40% juice + 60% sugar). Generally, high juice and low sugar concentration favours the potential for spoilage due to microorganisms. Similar observations were recorded in carbonated RTS beverage from kokum during six months of storage [13] and pomegranate fruits cv. Mridula during three months of storage [14].

The interaction between carbonation and juice-sugar levels showed non-significant results for fungal and bacterial count at 3 and 6 months of storage. There was no indication of fungal and bacterial contamination at 0 month of storage. Fungal count was not detected in all the interactions except C_1J_2, C_1J_3, C_1J_4, C_2J_3 and

Table 2. Changes in TSS (ºBrix) of carbonated RTS beverage from jamun during storage

CO₂ levels	TSS (ºBrix)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	15.07	14.13	13.33	13.07	**13.90**	15.33	14.80	13.87	13.47	**14.37**	15.27	14.80	14.20	13.73	**14.50**
C_2	15.40	14.67	13.20	12.93	**14.05**	15.87	15.20	13.80	13.27	**14.53**	15.60	15.33	14.47	13.67	**14.77**
C_3	15.73	14.87	13.73	12.80	**14.28**	15.80	15.40	14.33	13.33	**14.72**	15.87	15.20	14.73	13.53	**14.83**
C_4	15.00	14.47	13.07	12.53	**13.77**	15.67	14.73	14.00	13.20	**14.40**	15.73	14.93	14.40	13.33	**14.60**
Mean	**15.30**	**14.53**	**13.33**	**12.83**	**14.00**	**15.67**	**15.03**	**14.00**	**13.32**	**14.51**	**15.62**	**15.07**	**14.45**	**13.57**	**14.68**
	SEM±			**C.D. at 1%**		**SEM±**			**C.D. at 1%**		**SEM±**			**C.D. at 1%**	
C	0.06			0.24		0.06			0.22		0.05			0.20	
J	0.06			0.24		0.06			0.22		0.05			0.20	
C x J	0.13			0.49		0.12			0.45		0.10			0.40	

Table 3. Changes in reducing sugars (%) of carbonated RTS beverage from jamun during storage

CO₂ levels	Reducing sugars (%)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	2.03	2.13	2.28	2.66	**2.28**	3.15	3.30	3.61	3.81	**3.47**	5.25	5.63	6.74	7.39	**6.25**
C_2	2.14	2.26	2.34	2.50	**2.31**	3.16	3.36	3.75	3.89	**3.54**	5.53	5.76	6.69	7.18	**6.29**
C_3	2.23	2.32	2.56	2.65	**2.44**	3.20	3.60	3.93	3.94	**3.67**	5.54	6.30	6.75	7.46	**6.51**
C_4	2.17	2.31	2.36	2.54	**2.35**	3.21	3.56	3.69	3.93	**3.60**	5.38	6.12	6.97	7.21	**6.42**
Mean	**2.14**	**2.26**	**2.39**	**2.59**	**2.34**	**3.18**	**3.45**	**3.74**	**3.89**	**3.57**	**5.43**	**5.95**	**6.79**	**7.31**	**6.37**
	SEM±			**C.D. at 1%**		**SEM±**			**C.D. at 1%**		**SEM±**			**C.D. at 1%**	
C	0.02			0.07		0.02			0.08		0.03			0.12	
J	0.02			0.07		0.02			0.08		0.03			0.12	
C x J	0.04			0.15		0.04			0.15		0.06			0.24	

Table 4. Changes in total sugars (%) of carbonated RTS beverage from jamun during storage

	Total sugars (%)														
	0 Months					3 Months					6 Months				
CO_2 levels	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	10.37	9.63	9.35	9.29	**9.66**	11.40	10.43	9.87	9.67	**10.34**	11.85	11.01	10.18	9.99	**10.76**
C_2	10.67	9.94	9.62	9.39	**9.90**	11.57	10.83	10.08	9.77	**10.56**	12.09	11.50	10.45	10.08	**11.03**
C_3	12.10	11.60	10.20	9.44	**10.83**	12.40	11.52	9.90	9.66	**10.87**	12.76	11.94	10.49	9.84	**11.26**
C_4	11.75	10.71	9.78	9.27	**10.38**	11.49	10.67	9.93	9.54	**10.41**	12.31	11.21	10.27	9.65	**10.86**
Mean	**11.22**	**10.47**	**9.74**	**9.35**	**10.19**	**11.72**	**10.87**	**9.94**	**9.66**	**10.55**	**12.25**	**11.42**	**10.35**	**9.89**	**10.98**
	SEM±		**C.D. at 1%**			**SEM±**		**C.D. at 1%**			**SEM±**		**C.D. at 1%**		
C	0.07		0.27			0.05		0.18			0.03		0.11		
J	0.07		0.27			0.05		0.18			0.03		0.11		
C x J	0.14		0.54			0.09		0.36			0.06		0.22		

Table 5. Changes in titratable acidity (%) of carbonated RTS beverage from jamun during storage

	Titratable acidity (%)														
	0 Months					3 Months					6 Months				
CO_2 levels	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	0.16	0.18	0.19	0.22	**0.19**	0.25	0.26	0.27	0.31	**0.27**	0.30	0.32	0.32	0.35	**0.32**
C_2	0.19	0.20	0.20	0.22	**0.20**	0.26	0.28	0.29	0.31	**0.28**	0.32	0.32	0.34	0.35	**0.33**
C_3	0.18	0.19	0.22	0.23	**0.20**	0.27	0.28	0.31	0.32	**0.29**	0.31	0.33	0.33	0.34	**0.33**
C_4	0.19	0.20	0.21	0.22	**0.21**	0.28	0.29	0.29	0.30	**0.29**	0.31	0.33	0.36	0.37	**0.34**
Mean	**0.18**	**0.19**	**0.21**	**0.22**	**0.20**	**0.27**	**0.28**	**0.29**	**0.31**	**0.29**	**0.31**	**0.33**	**0.34**	**0.35**	**0.33**
	SEM±		**C.D. at 1%**			**SEM±**		**C.D. at 1%**			**SEM±**		**C.D. at 1%**		
C	0.002		0.01			0.002		0.01			0.002		0.01		
J	0.002		0.01			0.002		0.01			0.002		0.01		
C x J	0.005		0.02			0.004		0.02			0.004		0.02		

Table 6. Changes in pH of carbonated RTS beverage from jamun during storage

CO_2 levels	pH														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	2.18	2.27	2.31	2.35	**2.28**	2.11	2.13	2.26	2.31	**2.20**	2.07	2.11	2.21	2.27	**2.17**
C_2	2.24	2.30	2.33	2.38	**2.31**	2.19	2.26	2.29	2.35	**2.27**	2.13	2.23	2.26	2.30	**2.23**
C_3	2.23	2.32	2.34	2.39	**2.32**	2.16	2.30	2.32	2.34	**2.28**	2.15	2.25	2.28	2.31	**2.25**
C_4	2.26	2.29	2.34	2.41	**2.33**	2.22	2.25	2.31	2.36	**2.29**	2.18	2.26	2.27	2.32	**2.26**
Mean	**2.23**	**2.30**	**2.33**	**2.39**	**2.31**	**2.17**	**2.24**	**2.30**	**2.34**	**2.26**	**2.13**	**2.21**	**2.26**	**2.30**	**2.23**
	SEM±		**C.D. at 1%**			**SEM±**		**C.D. at 1%**			**SEM±**		**C.D. at 1%**		
C	0.003		0.01			0.004		0.02			0.004		0.01		
J	0.003		0.01			0.004		0.02			0.004		0.01		
C x J	0.007		0.03			0.008		0.03			0.007		0.03		

Table 7. Changes in anthocyanin (mg/100 ml) of carbonated RTS beverage from jamun during storage

CO_2 levels	Anthocyanin (mg/100 ml)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	4.07	5.63	6.22	6.75	**5.67**	3.17	4.35	5.20	6.18	**4.73**	2.12	2.97	3.92	4.94	**3.49**
C_2	4.28	5.44	6.33	6.86	**5.73**	3.25	4.24	5.43	6.26	**4.80**	2.06	3.16	4.00	4.96	**3.54**
C_3	4.62	5.38	6.12	6.98	**5.77**	3.43	4.40	5.36	6.43	**4.91**	2.24	3.21	4.26	5.26	**3.74**
C_4	4.26	5.40	5.94	6.53	**5.54**	3.64	4.26	5.25	6.24	**4.85**	2.16	3.17	4.32	5.24	**3.72**
Mean	**4.31**	**5.46**	**6.15**	**6.78**	**5.68**	**3.37**	**4.32**	**5.31**	**6.28**	**4.82**	**2.15**	**3.13**	**4.13**	**5.10**	**3.62**
	SEM±		**C.D. at 1%**			**SEM±**		**C.D. at 1%**			**SEM±**		**C.D. at 1%**		
C	0.05		0.18			0.03		0.12			0.02		0.08		
J	0.05		0.18			0.03		0.12			0.02		0.08		
C x J	0.09		0.35			0.06		0.23			0.04		0.17		

Table 8. Changes in fungal count (CFU/ml at 10^{-4} dilution) of carbonated RTS beverage from jamun during storage

CO₂ levels	Fungal count (CFU/ml at 10^{-4} dilution)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	0.00	0.00	0.00	0.00	**0.00**	0.00	0.33	0.33	0.67	**0.33**	0.00	0.33	0.67	0.67	**0.42**
C_2	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.33	**0.08**	0.00	0.00	0.33	0.33	**0.17**
C_3	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
C_4	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
Mean	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.08**	**0.08**	**0.25**	**0.10**	**0.00**	**0.08**	**0.25**	**0.25**	**0.15**
	SEM±			**C.D. at 1%**		**SEM±**			**C.D. at 1%**		**SEM±**			**C.D. at 1%**	
C	-			-		0.08			0.32		0.09			0.36	
J	-			-		0.08			NS		0.09			NS	
C x J	-			-		0.17			NS		0.19			NS	

Table 9. Changes in bacterial count (CFU/ml at 10^{-4} dilution) of carbonated RTS beverage from jamun during storage

CO₂ levels	Bacterial count (CFU/ml at 10^{-4} dilution)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean	J_1	J_2	J_3	J_4	Mean
C_1	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.33	0.67	**0.25**	0.00	0.00	0.67	0.67	**0.33**
C_2	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.33	**0.08**
C_3	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
C_4	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
Mean	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.08**	**0.17**	**0.06**	**0.00**	**0.00**	**0.17**	**0.25**	**0.11**
	SEM±			**C.D. at 1%**		**SEM±**			**C.D. at 1%**		**SEM±**			**C.D. at 1%**	
C	-			-		0.06			0.23		0.07			0.28	
J	-			-		0.06			NS		0.07			NS	
C x J	-			-		0.12			NS		0.14			NS	

C_2J_4 whereas bacterial count was not detected in all the interactions except C_1J_3, C_1J_4, and C_2J_4 at 3 and 6 months of storage. Interactions C_1J_3 and C_1J_4 recorded the highest (0.67 x 10^{-4} CFU/ml) fungal and bacterial count at six months of storage. Higher fungal and bacterial count recorded by interaction these might be due to comparatively low CO_2 pressure and high juice concentration with low sugar concentration, which might be favourable for survival of microorganisms [16].

4. CONCLUSION

From the above results, it was concluded that during the storage of carbonated RTS beverage from jamun, TSS, reducing sugars, total sugars and titratable acidity were increased, whereas pH and anthocyanin were decreased throughout the storage period of six months. During storage, microbial count increased throughout the storage period of six months. Both fungal and bacterial count were decreased with increase in carbonation levels and increased with increase in juice-sugar levels, at the end of the six months of storage period.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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