

Changes in chemical composition and microbial count of carbonated RTS beverage from jamun (*Syzygiumcumini*L.) during storage

ABSTRACT

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 compared to fruit based ones. So, considering medicinal and nutritive properties of jamun 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. C₁- 40 psi, C₂-50 psi, C₃-60 psi and C₄-70 psi and four juice-sugar levels percentage viz. J₁- jamun juice 25% + sugar 75%, J₂- jamun juice 30% + sugar 70%, J₃- jamun juice 35% + sugar 65% and J₄- jamun juice 40% + sugar 60%. From the study it was found 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.

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

1. INTRODUCTION

Jamun (*Syzygiumcumini*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 show that Jamun is used as anti-inflammatory, anti-arthritic, anti-nociceptive, anti-genotoxin, antispasmodic and improvement of 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 CO₂ 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

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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 ones. 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 consist of four carbonation levels *viz.* C₁- 40 psi, C₂- 50 psi, C₃- 60 psi and C₄- 70 psi and four juice-sugar levels *viz.* J₁- jamun juice 25% + sugar 75%, J₂- jamun juice 30% + sugar 70%, J₃- jamun juice 35% + sugar 65% and J₄- 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 CO₂ 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 [4]. Titratable acidity and anthocyanin content

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offresh 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 onto the sample. Such inoculated plates were then incubated at $27 \pm 2^\circ\text{C}$ and observed for colony development.

2.3 STATISTICAL ANALYSIS:

The data obtained was analyzed statistically as per the method suggested[5]. The standard error of mean (S.Em.) was worked out and the critical difference (C.D.) at 1 per cent level of significance was calculated whenever the results were found significant.

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][9].

Table 1. Chemical composition of fresh jamun juice

Sr. No.	Parameters	Content
1.	TSS ($^\circ\text{Brix}$)	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

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3.2 Chemical composition of carbonated RTS beverage from jamun

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 Table 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 [10][8]. 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 are reported in carbonated RTS beverage from Nagpur Mandarin orange during six months of storage [13]. 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 [10]. 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 [15]. 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 [10].

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 C₁ to C₃ and then slightly decreased at C₄ throughout the storage, irrespective of juice-sugar levels. Similar observations were recorded in carbonated beverage from cashew apple syrup^[11] and kokum during six months of storage period [12]. Titratable acidity and pH were increased from C₁ to C₄ with increase in CO₂ 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 [12].

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-

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sugar level, TSS and total sugars were decreased from J₁ to J₄ throughout the storage. This decreasing trend might be due to decrease in sugar concentration from J₁(75% sugar) to J₄(60% sugar). Similar results were recorded in carbonated RTS beverage from kokum during six months of storage period [12]. Reducing sugars, titratable acidity, pH and anthocyanin were increased from J₁ to J₄. This increase in reducing sugars might be due to increase in native reducing sugars with increase in juice concentration from J₁ to J₄. Similar results were recorded by in carbonated beverage from pomegranate fruits cv. Mridula during three months of storage [14]. The increase in titratable acidity might be due to presence of original acids in juice which increases from J₁ (25% juice) to J₄ (40% juice). Similar observations were recorded in carbonated RTS beverage from kokum [12] and guava carbonated drink [16]. The increase in anthocyanin content might be due to presence of original anthocyanin in jamun juice. 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 C₃J₁ recorded the highest TSS (15.73°B) and total sugars (12.10%). In case of six months of storage, C₃J₁ recorded the highest TSS (15.87°B) and was at par with C₄J₁ (15.73°B) and C₂J₁ (15.60°B). Interaction C₃J₁ recorded the highest total sugars (12.76%) at six months of storage and was significantly superior over other interactions. Interaction C₃J₄ recorded the highest reducing sugars (7.46%) and was at par with C₁J₄ (7.39%) and anthocyanin (5.26 mg/100 ml) and at par with C₄J₄ (5.24 mg/100 ml) at six months of storage. Interaction C₄J₄ recorded the highest titratable acidity (0.37%) which was at par with C₄J₃ (0.36%), C₁J₄ and C₂J₄ (0.35%) at six months of storage. Significantly lowest pH was recorded by interaction C₁J₁ (2.07) at six months of storage over other interactions.

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

CO ₂ levels	TSS (°Brix)														
	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

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C₂	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
C₃	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
C₄	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
	S.Em.±		C.D. at 1%		S.Em.±		C.D. at 1%		S.Em.±		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

	Reducing sugars (%)														
	0 Months					3 Months					6 Months				
	Juice-sugar levels					Juice-sugar levels					Juice-sugar levels				
CO₂ levels	J₁	J₂	J₃	J₄	Mean	J₁	J₂	J₃	J₄	Mean	J₁	J₂	J₃	J₄	Mean
C₁	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
C₂	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
C₃	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
C₄	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
	S.Em.±		C.D. at 1%		S.Em.±		C.D. at 1%		S.Em.±		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				

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

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CO ₂ levels	Total 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	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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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

CO ₂ levels	Titratable acidity (%)														
	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	0.16	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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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		

during storage

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

CO ₂ levels	pH														
	0 Months					3 Months					6 Months				
	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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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

CO ₂ levels	Anthocyanin (mg/100 ml)														
	0 Months					3 Months					6 Months				
	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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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		

during storage

3.3 Microbial studies on 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 the storage, multiplication of fungus might have taken place in the product which might have increased the fungal count. Similar observations were recorded in carbonated RTS beverage from kokum during six months of storage period [12]. 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 C₁ (40 psi) to C₄ (70 psi), irrespective of juice-sugar levels. This reduction in fungal growth might be due to increase in CO₂ 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 [12] and Nagpur Mandarin orange during six months of storage [13].

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 J₁ to J₄, irrespective of carbonation levels. This increase in fungal count might be due to increase in juice concentration and decrease in sugar concentration from J₁ (25% juice + 75% sugar) to J₄ (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 [12] 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 C₁J₂, C₁J₃, C₁J₄, C₂J₃ and C₂J₄ whereas bacterial count was not detected in all the interactions except C₁J₃, C₁J₄, and C₂J₄ at 3 and 6 months of storage. Interactions C₁J₃ and C₁J₄ recorded the highest (0.67×10^4 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 CO₂ pressure and high juice concentration with low sugar concentration, which might be favourable for survival of microorganisms.

Comment [MA25]: Please give logical reason to support the results.

Table 8. Changes in fungal count (CFU/ml at 10⁻⁴ dilution) of carbonated RTS beverage

CO ₂ 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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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		

from jamun during storage

Table 9. Changes in bacterial count (CFU/ml at 10⁻⁴ dilution) of carbonated RTS

CO ₂ 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
	S.Em.±		C.D. at 1%			S.Em.±		C.D. at 1%			S.Em.±		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		

beverage from jamun during storage

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 manuscript.

REFERENCES:

1. Kumawat, M., Damor, J., Kachchhwa, J. and Garg, A. K. (2018). Pharmacological properties and therapeutic. *World J. Pharmacological Research*, **7**(3): 312-322.
2. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Paul, T. A. and Devasagayam, J. (2007). Indian herbs and herbal drugs for the treatment of diabetes. *J. Clinical Biochemistry and Nutrition*, **40**(3): 163-173.
3. Lane, J. H. and Eynon, L. (1923). Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. *J. Soc. Chem. Ind.*, **42**: 32-37.
4. Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*. Second Ed. Tata Mc Graw- Hill Publishing Company Ltd., New Delhi. pp. 9-106.
5. Panse, V. G. and Sukhatme, P. V. (1985). *Statistical methods for agricultural workers*. 4th Ed. Indian Council of Agricultural Research, New Delhi, pp. 97-156.
6. Akhila, H. and Hiremath, U. S. (2018). Physico-chemical properties of jamun (*Syzygiumcumini* L.) fruits and its processed products. *Int. J. Pure App. Biosci.*, **6**(6): 1317-1325.
7. El-Safy, S., Khalifa, A. M., Almashad, A. A., Mohamed Khalil, A. M., Hammad, E. M., Sami, R., Aljahani, A. H., Pareek, S., Helal, M., Alharthi, S. and Taha, I. M. (2023). Utilization of jamun fruit (*Syzygiumcumini* L.) for value added food products. *J. Food Quality*, **2**: 1-10.
8. Phanase, S. S. (2015). Standardization of jamun (*Syzygiumcumini* L.) carbonated beverage. M. Sc. (PHM) thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.)
9. Patil, S. S., Thorat, R. M. and Rajasekaran, P. (2012). Utilization of jamun fruit (*Syzygiumcumini* L.) for production of red wine. *J. Advanced Laboratory Research in Biology*, **3**(3): 200-203.

10. Joshi, H. N., Hiwale, S. S. and Leua, H. N. (2014). Studies on physico-chemical characteristics of jamun (*Syzygiumcumini* L.) beverages during storage. *Madras Agricultural Journal*, **101**(9): 300-303.
11. Gajbhiye, R. C., Khapare, L. S., Kadam, M. B., Deshmukh, S. V., Pawar, S. N., Sawant, B. N., Salvi, S. P. and Talha, P. M. (2021). Standardizing the preparation of carbonated beverage from cashew apple (*Anacardium occidentale*) syrup. *Crop Research*, **56**(6): 317-322.
12. Gharat, R. V. (2023). Studies on carbonated RTS beverage from kokum (*Garcinia indica* Choicy) M. Sc. (Hort.) thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.)
13. Faizi, Z. A. (2022). Studies on preparation of carbonated ready-to-serve beverage of Nagpur Mandarin (*Citrus reticulata* Blanco). *Acta Scientific Nutritional Health*, **6**(1): 67-75.
14. Sandhan, V. S. (2003). Preparation of carbonated beverage from pomegranate (*Punica granatum* L.) fruits cv. Ganesh and Mridula. M. Sc. (Agri.) thesis submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (M.S.)
15. Ilamaran, M., Amutha, S., Meenakshi, V. and Sivasankari, B. (2020). Effect of TSS and CO₂ pressure on bio-chemical and sensory qualities of enzymatic clarified carbonated jamun and pomegranate beverages. *Int. J. Curr. Microbiol. App. Sci.*, **9**(12): 85-92.
16. Rammiya, U. S., Hema, V., Shanmugasundaram, S. and Sinija, V. R. (2019). Study on physiochemical properties on carbonated guava drink. *International J. Chemical Studies*, **7**(4): 958-964.