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Bacteriological and sensory stability in dragon fruit (*Hylocereus polyrhizus*) pulp during storage: As influenced by chemical treatments

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Abstract
The effort was made during the present investigation to evaluate the consequence of sodium benzoate (SB), potassium sorbate (PS) and ascorbic acid at various concentrations on microbiological and sensory stability of red fleshed dragon fruit pulp during storage at -20 ± 0.5 °C. The inhibitory effect of chemical preservatives and ascorbic acid on microbial and sensory attributes was analysed periodically at initial, first, second and third month after storage. The experiment was carried out in Completely Randomized Design (CRD) with ten treatments and three replications. The outcomes of the research noted that significant inhibition of the total bacterial count was observed on treating chemical preservatives, however ascorbic acid was shown to be more inhibitory. Organoleptic parameters of the dragon fruit pulp samples were affected negatively on addition of chemical preservatives however, superior sensory quality was obtained in the sample preserved with ascorbic acid at 1000 ppm and accepted by the sensory panelist even after three months of storage.

Keywords: Ascorbic acid, total bacterial count, sensory evaluation

Introduction
Dragon fruit (*Hylocereus polyrhizus*) is a vine falls under the family Cactaceae. The crop originated from the tropical and subtropical regions of Mexico, including Central and South America. Dragon fruit has different vernacular names throughout the world like Pitaya, Queen of the night, Noble woman *etc.* to name some. The fruits are covered with attractive rosy-red skin decorated with green bracts and mellow mouth-melting red pulp with a black colour edible seed embedded in the pulp along with tremendous nutritive property (Le-Bellec *et al*., 2006) [13]. The dragon fruit was introduced to India during the late 90's but the area under dragon fruit is still scanty and limited to few areas of Karnataka, Tamil Nadu, Maharashtra, Gujarat, Kerala and Andhra Pradesh. The total area under dragon fruit cultivation in India is has been increased to 500 to 750 hectares and Karnataka is fast emerging state as the country's leader in dragon fruit cultivation, which is spread over 1,000 acres in the state during 2019 (Anon., 2019).
Dragon fruit exhibits non-climacteric pattern of respiration (Enciso *et al*., 2011) [6], having short shelf life and seasonality in nature (July to November). To overcome this problem and to make the fruits available at every corner of the country and throughout the year, which can be achieved through various preservation and processing methods and preparing various value-added products.
The fruits when moved in and out of cold storage, shelf life is drastically reduced, the skin of stored fruits become thinner as water is moved into the flesh from skin, but they have moderate sugar levels although the taste will be less. Marketing of frozen pulp is an alternative method, which is becoming popular, as the product is not subjected to quarantine for fruit fly. The fruit pulp is a versatile semi-processed product that can be converted to different value-added products such as RTS, squash, wine, jam, flavored milk, ice-creams, cake, toffee, bread etc. There is a high demand for frozen pulps in the international market as pulp can be used as an instant raw material in the beverage, bakery, dairy and confectionery industries.
Being the cheapest among several methods of preservation, chemical preservation of fruit pulp is the most common and widely used all over the world. The chemical preservatives are used to prevent the food spoilage due to microbial attack and thus are effectively used along with deep freezing for better preservation.

Sodium benzoate is a salt of benzoic acid, which is practically tasteless and odorless. It affects the permeability of cell membrane of the microorganism so the cellular fluids come out and the microorganism die. yeasts are inhibited to a greater extent than moulds and bacteria. Potassium sorbate is a salt of sorbic acid and used as anti moulds. It affects the enzyme activity (succinic dehydrogenase and fumarase). It is safest of all preservatives. Ascorbic acid is an isomer of isoascorbic or erythorbic acid, their salts are highly soluble in water and safe to use in food. It is exploited for its antioxidant activity, stabilizing ability and bactericidal effect.

Consumer acceptance is mostly relying on colour of the food product (Crisosto *et al*., 2003) [4] while the "perception of sweetness and flavor can even evoke emotional feelings in humans" (Bayarri *et al*., 2001) [3]. Sensory quantification of fruit pulps and food products is a very important instrument to correlate physicochemical characteristics with human perception. By keeping in view, the above-mentioned facts dragon fruit pulp was stored in laminated aluminium pouches by adding food preservatives individually and studied the microbial and sensory stability analysis of pulp during storage.

Materials and Methods

Sample preparation

Selected fruits were washed and cut into two halves then the outer cover was peeled manually and homogenous pulp was extracted through mechanical pulper machine under hygienic condition. The obtained pulp was treated with chemical preservatives as mentioned in the treatment details (Table 1) and packed in laminated aluminium pouches of size 6 × 8 inches. The packaged samples were stored at -20 ± 0.5 °C temperature in the deep freezer for further studies.

Total Bacterial Count (CFU g -1)

The total microbial count in dragon fruit pulp was taken at monthly intervals of storage as per the method of Harrigan and Mccance (1996) [8]. Samples were prepared by taking ten grams of representative sample from three replications in each treatment. 10 grams of sample was mixed with 90 ml sterilized water blank in a conical flask and serial dilution technique was carried out to evaluate the total bacterial load in the dragon fruit pulp. The dilution 10-5 was used for bacterial counting. One ml of aliquot from respective dilution was transferred to Petri plates in duplication and sterilized lukewarm molten nutrient agar were poured to the respective plates for the isolation of bacteria. After solidification, the plates were incubated at 37 ± 1 ºC for three days and colony counts were recorded, tabulated and expressed as CFU/g of sample.

Sensory Evaluation

The pulp was evaluated for sensory attributes *viz*., colour and appearance, taste, texture, flavor and overall acceptability by a panel of 15 trained judges consisting of teachers and postgraduate students of Kittur Rani Channamma College of Horticulture, Arabhavi. They evaluated pulp samples during 90 days of storage at room temperature. Each panelist assessed every sample over 30 days interval at sensory evaluation laboratory of the post-harvest technology department by using individual booths with daylight and ambient room temperature. Each consumer evaluated the samples using nine-point hedonic scale (Table 2) as described by Ranganna (2003) [17] where 1 represents extremely disliked and 9 represent extremely liked.

Statistical analysis

The data recorded on the organoleptic characteristics and total bacterial count were subjected to statistical analysis in completely randomized block design. Analysis done using Web Agri. Stat. Package 2 developed by ICAR research complex, Goa. Examination of the data was determined in accordance with Panse and Sukhatme (1985) [15].

Results and Discussion

Total Bacterial count

Microbial spoilage is a major limitation in enhancing storability of pulp. They multiply and infect the pulp when congenial conditions prevail. Among the microorganisms, bacteria were significantly affecting the pulp during storage. As evident of the mean values, total bacterial count showed an increasing trend from fresh (2.90×105 CFU/g) to three months after storage (4.76×105 CFU/g). However, such a marginal increase did not affect the wholesomeness of the product (Table 3)

The treatment T_7 (1250 ppm potassium sorbate) has shown high bacterial load compared to other treatments after three months of storage. The least microbial load was recorded for T_8 (1000 ppm ascorbic acid) which might be due to the effect of ascorbic acid. Deep freeze storage was also effective in inhibiting microbial growth. According to Jangir *et al*., (2017) [11], lower bacterial growth in a deep freezer (-20 °C) might be due to minimum physiological activities and respiration rate of microbes and ice crystal formation in cells. The similar study was reported by Yadav *et al*. (2017) [19] in guava pulp and Jaishankar (2018) [10] in custard apple pulp.

Sensory evaluation. The merit of any consumer product depends not only on its composition and nutritional value but also on the sensory quality. Evaluation of sensory quality of a product is an important tool for deciding the consumer acceptability. Therefore, organoleptic evaluation of dragon fruit pulp involving various treatments was carried out to assess its consumer acceptability by a panel of trained judges.

Colour and appearance: The data related to the colour and appearance of dragon fruit pulp as guided by treatments and storage period are furnished in Table 4. The mean value of the data indicated decrease in colour and appearance values during the storage period irrespective of treatments (8.13 to 7.17). However, the treatments did not show any significant differences at initial as well as three months after storage. This may be due to higher stability of betacyanin pigment at -20 °C for different chemical preservatives used in this experiment.

Taste and Flavor: The results on taste and flavor of dragon fruit pulp as influenced by different treatments and storage period are furnished in Table 4. The mean value of the data indicated decrease in taste and flavor values during the storage period irrespective of treatments (8.57 to 7.27). The data revealed non-significant difference with respect to taste and flavor among the treatments during initial and first months after storage. However, the data showed a significant difference after second and third months of storage, with maximum score for T_8 - Ascorbic acid 1000 ppm (8.50 and 8.00) followed by T_9 (8.33 and 7.83) and minimum score was found in T_4 - sodium benzoate 750 ppm (6.67 and 6.50).

Texture: The results on texture of dragon fruit pulp as influenced by different treatments and storage period are furnished in Table 5. The data reveals significant difference

with respect to texture among the treatments and during the storage period. The mean score for texture decreased irrespective of storage period varied between 8.48 at initial to 7.22 at three months after storage. The maximum score was recorded for treatment T_8 (Ascorbic acid 1000 ppm) and T_9 - Ascorbic acid 1500 ppm (9.00 each) and minimum score was recorded for T_1 (control), T_4 and T_7 (8.00 each) during initial stage of storage. After first, second and third months of storage, significantly maximum score was observed in T_9 - Ascorbic acid 1500 ppm (8.67, 8.17 and 7.83) followed by T_8 (8.50, 7.83 and 7.67) and minimum score was observed for T_7 - potassium sorbate 1250 ppm (7.00, 6.67 and 6.33).

Overall acceptability: The results of overall acceptability as affected by different treatments and storage period are presented in Table 5. The data on overall acceptability of dragon fruit pulp reveals that there was significant difference among treatments and mean values decreased from 8.08 to 7.12 from initial to three months after storage. The maximum overall acceptability was recorded in T_8 - Ascorbic acid 1500 ppm (8.83, 8.67, 8.17 and 7.83) followed by T_9 (8.67, 8.50, 8.00 and 7.67) and minimum score was observed in T_4 - sodium benzoate 750 ppm (7.17, 7.00, 6.67 and 6.33) respectively at initial, first, second and third months after storage. In the present investigation, the treatments whereas with respect to parameters like taste and flavor, texture and overall acceptability, ascorbic acid 1000 ppm followed by ascorbic acid 1500 ppm treated pulp had scored maximum and was same throughout the period of storage. This might be due to less degradation of physicochemical properties and very low microbial load and mainly attributed to the combined effect of ascorbic acid along with deep freezing storage. Sodium benzoate 750 ppm followed by potassium sorbate 1250 ppm treated pulp had scored least with respect to all parameters which might be due to high bacterial load and their higher concentration may alter the flavor of dragon fruit pulp. Quality aspects of fruit pulp and food products such as colour and appearance, taste, flavor and overall acceptability generally reduces with the increase in storage period. In the present experiment also, sensory scores for colour and appearance, taste and flavor, texture and overall acceptability of pulp decreased in all the treatments as the storage period progressed. A similar decrease in sensory attributes during storage of fruit pulp has been described by Gamage *et al.* (1997) [7] in custard apple, Hashmi *et al.* (2007) [9] in mango pulp, Akhtar *et al.* (2009) [1] in mango, Durrani *et al.* (2010) [5] in apple pulp, Kamble and Soni (2010) [12] and Pawar *et al.* (2010) [16] in custard apple pulp, Muhammad *et al.* (2011) [14] in apple pulp, Swetha (2016) [18] in custard apple, Yadav *et al.* (2017) [19] in guava and Jaishankar (2018) [10] in custard apple pulp.

Conclusion

Dragon fruit pulp treated with ascorbic acid 1000 ppm (T_8) + freeze storage (-20 ± 0.5 °C) had scored better result with respect to microbial stability and organoleptic traits up to three months of storage. The results obtained during the present investigation indicate that good quality pulp with optimum physicochemical and sensory quality along with very low microbial load, which demonstrates the great potential for the use of such pulp in the food industry.

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Table 1: Treatment details

T_1	Control (without any preservative)
T_2	Pulp + Sodium benzoate 250 ppm
T_3	Pulp + Sodium benzoate 500 ppm
T_4	Pulp + Sodium benzoate 750 ppm
T_5	Pulp+ Potassium sorbate 750 ppm
T_6	Pulp + Potassium sorbate 1000 ppm
T_7	Pulp + Potassium sorbate 1250 ppm
T_8	Pulp + Ascorbic acid 1000 ppm
T_9	Pulp + Ascorbic acid 1500 ppm
T_{10}	Pulp + Ascorbic acid 2000 ppm

Table 2: Hedonic scale

Hedonic scale	Colour and appearance	Texture	Taste and Flavor	Overall acceptability
Like Extremely	9	9	9	9
Like very much	8	8	8	8
Like moderately	7	7	7	7
Like slightly	6	6	6	6
Neither like nor dislike	5	5	5	5
Dislike slightly	4	4	4	4
Dislike moderately	3	3	3	3
Dislike very much	2	2	2	2
Dislike extremely	1	1	1	1

Table 3: Effect of chemical preservatives and ascorbic acid on total bacterial count of dragon fruit pulp during storage

Treatment	Total bacterial count ($10^5 \times$ CFU g^{-1})			
	Months after storage			
	0	1	2	3
T_1	3.45	4.62	5.18	6.36
T_2	3.00	3.24	3.61	3.96
T_3	2.75	2.92	3.36	3.72
T_4	2.91	3.22	5.86	7.33
T_5	2.86	3.05	3.24	3.46
T_6	2.65	2.98	3.41	3.56
T_7	3.45	4.87	6.67	8.82
T_8	2.62	2.83	3.06	3.34
T_9	2.63	2.75	2.92	3.45
T_{10}	2.65	2.96	3.25	3.64
Mean	2.90	3.34	4.06	4.76
S.Em±	0.051	0.046	0.1	0.11
C.D. @ 1%	0.20	0.19	0.42	0.44

Table 4: Effect of chemical preservatives and ascorbic acid on colour and appearance and taste and flavor of dragon fruit pulp during storage

Treatment	Colour and appearance				Taste and flavor			
	Months after storage							
	0	1	2	3	0	1	2	3
T_1	7.67	7.33	7.00	6.50	8.00	7.67	7.00	6.67
T_2	7.83	7.50	7.17	6.83	8.67	8.00	7.67	7.33
T_3	8.33	8.00	7.67	7.33	8.67	8.33	8.00	7.67
T_4	8.17	7.83	7.50	7.17	8.33	7.33	6.67	6.50
T_5	7.83	7.67	7.33	7.00	8.67	8.00	7.67	7.00
T_6	7.67	7.50	7.00	6.83	8.33	7.67	7.33	7.50
T_7	7.67	7.33	6.83	6.67	8.00	7.33	7.00	6.83
T_8	9.00	8.67	8.33	8.00	9.00	8.83	8.50	8.00
T_9	8.83	8.5	8.16	7.83	9.00	8.50	8.33	7.83
T_{10}	8.33	8.00	7.83	7.50	9.00	8.33	8.00	7.33
Mean	8.13	7.83	7.48	7.17	8.57	8.00	7.62	7.27
S.Em±	0.28	0.30	0.31	0.22	0.23	0.28	0.25	0.26
C.D. @ 1%	NS	NS	NS	NS	NS	NS	1.02	1.04

NS: Non-Significant

Table 5: Effect of chemical preservatives and ascorbic acid on texture and overall acceptability of dragon fruit pulp during storage

Treatment		Texture			Overall acceptability			
					Months a fter storage			
	0	1	2	3	0	1	2	3
T_1	8.00	7.00	6.83	6.50	7.83	7.33	7.00	6.83
T_2	8.67	8.00	7.67	7.50	8.17	7.83	7.67	7.33
T_3	8.83	7.83	7.50	7.33	8.33	8.00	7.83	7.50
T_4	8.00	7.50	7.00	6.83	7.17	7.00	6.67	6.33
T_5	8.67	8.00	7.67	7.50	7.83	7.50	7.50	7.00
T_6	8.33	7.50	7.33	7.00	8.00	7.67	7.00	6.67
T_7	8.00	7.00	6.67	6.33	7.67	7.33	6.83	6.50
T_8	9.00	8.50	7.83	7.67	8.83	8.67	8.17	7.83
T_9	9.00	8.67	8.17	7.83	8.67	8.50	8.00	7.67
T_{10}	8.33	8.00	7.83	7.50	8.33	8.00	7.83	7.50
Mean	8.48	7.80	7.45	7.20	8.08	7.78	7.45	7.12
S.Em±	0.22	0.19	0.25	0.27	0.24	0.25	0.27	0.27
C.D. @ 1%	0.87	0.79	1.02	1.08	0.97	1.01	1.10	1.10

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