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Betacyanin, Antioxidant Activity and Shelf-life Evaluation of Thermally, Microwave, and Chemically Processed Lime-flavored Dragon Fruit Ready to Serve Beverage

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DP and SB designed the study. Author DP performed analyses of the study, statistical analysis, wrote the protocol, wrote the first draft of the manuscript, and comments corrections. Author SB managed the literature searches, manuscript checking, and submission. Both authors read and approved the final manuscript.

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ABSTRACT

The exploration regarding the utilization of cacti family fruits into the directly consumable drink is limited. So, study was conducted to explore utilization of local fruit lime for preparation of lime-flavored dragon fruit ready to serve beverage, and processed with thermal, microwave, and chemical treatments to increase the shelf-life of beverage. Standardizing lime juice in dragon fruit ready to serve (RTS) beverages, optimizing thermal, microwave, and chemical treatments, and studying shelf life were the goals of this study. Dragon fruit RTS beverage was made with 12% fruit

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juice, 12°Brix total soluble solids (TSS), and 0.1% citric acid after a preliminary sensory study. Dragon fruit RTS with 3% lime juice tasted best. The standardized RTS beverage was thermal (70, 80, and 90°C for 5, 10, and 15 min), microwave (900 W power density for 30, 60, 90, 120, and 150 sec), and chemical (500, 1000, and 1500 ppm ascorbic acid incorporation) treated to optimize betacyanin content, antioxidant activity, and sensory evaluation. RTS beverage thermally treated at 70°C for 5 min, a 30-second microwave treatment at 900 W, and RTS beverage with 500 ppm ascorbic acid were found best on betacyanin, antioxidant activity, and sensory basis. The shelf life of optimized RTS beverage was tested at room temperature and refrigerated. During storage at the both temperatures, there were significant variations in pH, TSS, betacyanin content, and sensory qualities. The best retention of betacyanin content was reported on the 6th day of ambient storage for lime-flavored dragon fruit RTS beverage with 500 ppm (10.04 mg/L) with overall acceptability of 5.57. For refrigerator storage, lime-flavored dragon fruit RTS beverage with 500 ppm ascorbic acid retained 24.26 mg/L betacyanin and 5.68 overall acceptability on the 60th day. Additionally, the lime-flavored dragon fruit RTS beverage with 500 ppm ascorbic acid retained betacyanin better than other treatments during ambient and refrigerated storage.

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

1. INTRODUCTION

The member of *Cactaceae* family recognized as “Buah-naga” in Malaysia, and scientifically recognized as “*Hylocereus polyrhizus*” [1]. Among more than sixteen varieties of pitahaya fruit were cultivated, and marketed in exotic fruit market of Europe [2]. A nutritional rich profile of pitahaya is abundant source of some minerals, phenols, bioactive compounds, essential fatty acids [3,1,4]. It is good source of polyphenols, flavanols, flavonoids, tannins, and betacyanin [5]. It containing flavonoids such as myricetin, rutin, and quercetin, polyphenols such as gallic acid, caffeic acid, and protocatechuic acid, bioactive compounds such as betanin, indicaxanthin, isobetanin, phyllocactin, isophyllocactin, hylocerenin, and isohylocerenin [5,6]. The member of *Rutaceae* family, lime fruit scientifically known as “*Citrus aurantifolia*’ or “*Citrus latiflia*” was originated in East India or Malaysia [7]. Lime is a good source of Vit C, potassium, calcium, iron, copper, manganese, and zinc [8]. Neembu-pani is most lovable drink in summers made from lime or lemon due to combination of sour and sweet taste, and beneficial effect in maintaining the blood pressure, and diarrhea. So, the incorporation of lime juice into a newly introduced fruit RTS in INDIA will enhance the sensorial acceptability of light sweetened dragon fruit RTS.

Foke et al. [9] prepared dragon fruit RTS having 12% sugar, 0.01% potassium metabisulphite, 12% fruit juice content, and 0.4% citric acid content, The pH was decreased from 2.5 to 2.08, Vit C also decreased from 8.3 to 3.6 mg/100ml, while TSS was increased from 14.2°Brix to 14.9°Brix upto 50 days of storage. According to Bassama et al. [10], the betacyanin content of cactus pear juice heated for 36 sec at 90°C was decreases from 0.9 g/kg to 0.2 g/kg for juice stored at 45°C, and 0.8 g/kg for juice stored at 4°C after 40 days. The pitahaya juice storaged without light exposure can retent more betacyanin content [11]. Increase in betacyanin content from 0.005 to 0.112 µg/g for xoconostole juice heated for 30 min at 67-70°C. the antioxidant activity, phenols, and bioactive compounds for tomato juice were more preserved in storage after treated with potassium metabisulphite than sodium benzoate, and thermal processing [12]. The total phenolic content of pineapple juice was reduced during ambient storage after mild heat treatment (65°C for 15 min), and thermal processing for 15 min at 85°C; Also, reduction was observed in pH, while TSS was increased with storage period [13]. According to Woo et al. [14], light exposure was the major reason for reduction in betacyanin content. Heating of pitahaya juice for 1 h at 85°C leads to isoindicaxanthin, indicaxanthin formation in juice, while approximately 91% of pigment retention was observed in thermally treated juice adjusted at 4 pH, and incorporated with 1% ascorbic acid [15].

The utilization of local fruit juice (lime) into non-fermented beverage of dragon fruit prepared using addition of different concentration of sugar citric acid, and additivces (Ready to Serve beverage) had more sensorial acceptance with the lower price of beverage.

The current study was conducted to standardize the lime juice in dragon fruit RTS beverage, to

optimize the thermal, microwave, and chemical treatment, and to analyze the effects of storage conditions on the parameters such as pH, TSS, betacyanin content, microbial quality, and sensory evaluation of thermally, microwave, and chemically treated lime-flavored dragon fruit RTS beverage.

2. MATERIALS AND METHODS

Tables & figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. It is suggested that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text.

Tables should be explanatory enough to be understandable without any text reference. Double spacing should be maintained throughout the table, including table headings and footnotes. Table headings should be placed above the table. Footnotes should be placed below the table with superscript lowercase letters.

Each figure should have a caption. The caption should be concise and typed separately, not on the figure area. Figures should be self-explanatory. Information presented in the figure should not be repeated in the table. All symbols and abbreviations used in the illustrations should be defined clearly. Figure legends should be given below the figures.

2.1 Lime-flavored Dragon Fruit RTS Preparation

Freshly procured dragon fruit from farmer of Kachchh district of Gujarat, India was used to make dragon fruit RTS beverage. The primary trials were carried out to standardize the 12°Brix TSS, 0.1% citric acid, and 12% fruit juice content for preparation of dragon fruit RTS beverage on sensory basis. The 1%, 2%, 3%, and 4% lime juice incorporation into dragon fruit RTS beverage was also standardized on the basis of sensory evaluation. The standardized lime juice incorporated dragon fruit RTS beverage was processed further with thermal, microwave, and chemical treatment, and optimized on the basis of betacyanin content, antioxidant activity, and sensory evaluation. The processing steps for making dragon fruit RTS beverage was presented in Fig. 1.

2.2 Thermal, Microwave, and Chemical Processing of Lime-flavored Dragon Fruit RTS Beverage

For the thermal treatment RTS was processed for 5, 10, and 15 min at 70, 80, and 90°C, and best treatment was optimized on the basis of betacyanin content, antioxidant activity, and sensory evaluation. The best microwave treatment at 900 W power density for 30, 60, 90, 120, and 150 sec was optimized. Similarly, best treatment from RTS incorporated with 500. 1000, and 1500 ppm ascorbic acid was optimized. The one optimized treatment from each processing along with control RTS beverage were stored at ambient, and refrigeration storage conditions for shelf-life study.

2.3 pH

A digital pH meter (Made: Systronics) was used to calculate the hydrogen's potential of lime-flavored dragon fruit RTS beverage after being calibrated with pH 4, and 9.2.

2.4 TSS (total soluble solids)

A hand refractometer (ATC, Erma Inc., Tokyo, Japan) was used to determine TSS of the lime-flavored dragon fruit RTS beverage after being calibrated with distilled water.

2.5 Betacyanin Content

The betacyanin content of lime-flavored dragon fruit RTS beverage was measured using the Spectrometric method mentioned by Naderi et al. [16] with minor modifications.

2.6 Total Plate Count, Yeast & Mold Count, and Coliform Count

TPC, Y&M, and Coliform count were analyzed using method given by Ranganna [17].

2.7 Sensory Evaluation of Lime-flavored Dragon Fruit RTS Beverage

A semi-trained panel member used nine-point hedonic scale for sensory evaluation of lime-flavored dragon fruit RTS beverage. The attributes color, taste, body, flavor, and overall acceptability were utilized to evaluate sensory acceptance of RTS beverage.

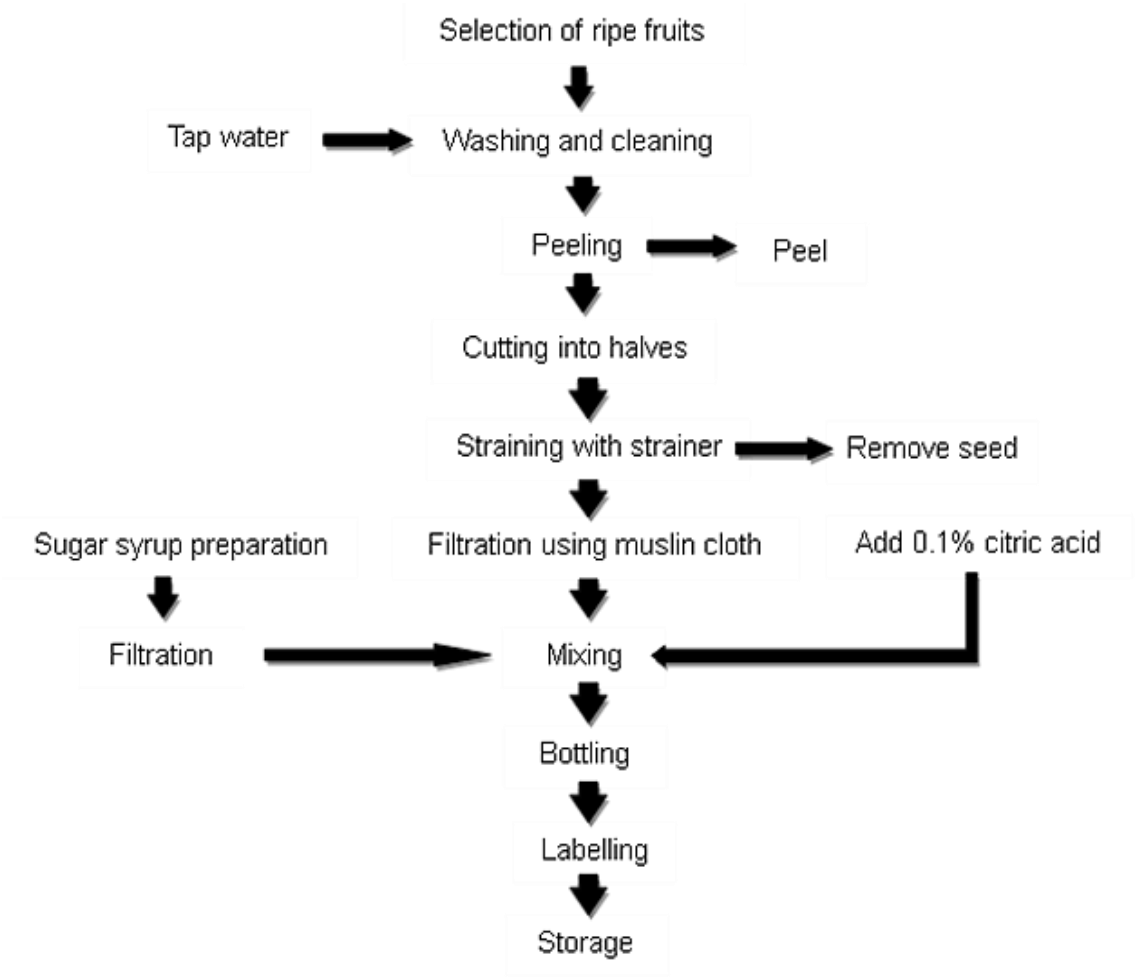

Fig. 1. Process flowchart for preparation of RTS beverage

2.8 Shelf-life Study

Thermally, microwave, and chemically treated lime-flavored dragon fruit RTS beverage packed in PET bottles, and stored at ambient, and refrigerated storage condition for further shelf-life study. RTS beverage was analyzed for pH, TSS, betacyanin content, total plate count, yeast & mold count, coliform count, and sensory evaluation at ambient temperature after each three days for interval, and at refrigerated temperature after each 15 days of interval.

2.9 Statistical Analysis

The factorial Completely Randomized Design was used to analyze data of shelf-life study. Triplicate data for all experiments were analyzed at agriculture statistics department, Anand Agricultural University, Anand, Gujarat, India.

3. RESULTS AND DISCUSSION

3.1 Lime Juice Standardization in Dragon Fruit RTS Beverage

Among 1%, 2%, 3%, and 4% addition of lime juice in prepared dragon fruit RTS beverage, 3% lime juice incorporation was selected best on the sensory basis. The significantly higher taste score (7.94), flavor score (7.86), and overall acceptance (7.92) was reported for Dragon fruit RTS incorporated with 3% lime juice.

3.2 Optimization of Thermal, Microwave, and Chemical Processed Lime-flavored Dragon Fruit RTS Beverage

The thermal processing for 5 min at 70°C (T_1t_1) had higher betacyanin content (23.80 mg/L), antioxidant activity (73.43%), and overall acceptance (7.55).

The RTS microwaved at 900 W for 30 sec (MW_1) had higher antioxidant activity (54.31%), while 29.87 mg/L of betacyanin content was observed.

The RTS being incorporated with 500 ppm ascorbic acid (C_1) had maximum antioxidant activity of 68.37%, whereas non-significant change was noticed for betacyanin content (38.00 mg/L).

An individual effect of above-mentioned treatments was found best on the basis of betacyanin content, antioxidant activity, and overall sensory acceptance for lime-flavored dragon fruit RTS beverage. The lime-flavored dragon fruit RTS beverage being thermally processed for 5 min at 70°C, microwave processed for 30 sec, and incorporated with 500 ppm ascorbic acid were optimized, and packed in PET bottles for further shelf-life study.

3.3 Effect of Ambient Storage Condition on Developed Lime-flavored Dragon Fruit RTS Beverage

3.3.1 pH

Summary of the effect of ambient storage conditions on the pH of developed lime-flavored dragon fruit RTS beverage is presented in Table 1. (Fig. 2.). The pH of newly produced lime-flavored dragon fruit RTS beverages was significantly influenced by each individual treatment, the number of storage days, and the interaction of treatment with storage days. The pH of the RTS beverage significantly decreased while being stored in ambient conditions. Similar findings for dragon fruit RTS beverage was reported by Foke et al. [9]. The pH of the lime-flavored dragon fruit RTS beverage decreased as a result of chemical reactions that occurred during storage, and produced organic acid. Percent decrease in pH was 15.40, 15.48, 15.99, and 16.11%, respectively for control sample, thermal, microwave, and chemical treated sample stored at ambient conditions.

3.3.2 TSS

The effect of ambient storage conditions on the TSS of developed lime-flavored dragon fruit RTS beverage is presented in Table 1. (Fig. 2.). On the TSS of newly developed lime-flavored dragon fruit RTS beverages, the individual effects of treatment, storage days, and interaction of treatment with storage days were significant. Significant increase in TSS of RTS beverage was reported during ambient storage conditions. Similar results were reported by Foke et al. [9]. Chemical reactions during storage causes conversion of complex polysaccharides into simple sugars resulted into increase in TSS of lime-flavored dragon fruit RTS beverage. TSS was significantly enhanced during storage with increasing storage days for developed lime-flavored dragon fruit RTS beverages. On 6th day, percent increase in TSS was 10.75, 15.21, 11.29, and 12.63%, respectively for control sample, thermal, microwave, and chemical treated sample stored at ambient conditions.

3.3.3 Betacyanin content

The effect of ambient storage conditions on the betacyanin content of the developed lime-flavored dragon fruit RTS beverage is shown in Table 1. (Fig. 3.). The betacyanin level of developed lime-flavored dragon fruit RTS beverages was significantly impacted by the individual effects of treatment, storage days, and interaction of treatment with storage days. During ambient storage conditions, a significant decrease in the betacyanin level of RTS beverage was noted. Similar findings were reported by Liaotrakoon et al. [4], Herbach et al. [15], and Bassama et al. [10]. The betacyanin content of lime-flavored dragon fruit RTS beverage decreased as a result of the betacyanin being degraded by heat, light, and pH. Percent retention of betacyanin was 11.73%, 17.27%, 12.63%, and 26.42%, respectively for control sample, thermal, microwave, and chemical treated sample stored at ambient conditions.

3.3.4 Sensory evaluation

A panel of judges used a nine-point hedonic scale to evaluate developed lime-flavored dragon fruit RTS beverages for color, taste, body, flavor, and overall acceptability. Table 2. summarizes the effect of ambient storage conditions on the color score of a produced lime-flavored dragon fruit RTS beverage. On the color score of newly produced lime-flavored dragon fruit RTS beverages, the individual effects of treatment, storage days, and interaction of treatment with storage days were significant. Due to the loss of coloring pigment from the beverage, a significant fall in the color score of the RTS beverage was noted during ambient storage conditions. For produced lime-flavored dragon fruit RTS beverages, the color score was noticeably diminished as storage days increased.

The effect of ambient storage conditions on taste score of the dragon fruit RTS beverage is

discussed in Table 2. The developed lime-flavored dragon fruit RTS beverages had a significant individual effect of treatment, storage days, and interaction of treatment with storage days. RTS beverage taste rating significantly decreased under ambient storage conditions, according to reports. For developed lime-flavored dragon fruit RTS beverages, the taste score was noticeably worse with longer storage times. The control sample's maximum score (7.88) was recorded on day 0, and dropped to 5.08 on day 6 of storage.

The impact of ambient storage conditions on the body score of a developed lime-flavored dragon fruit RTS beverage is tabulated in Table 2. The body score of developed lime-flavored dragon fruit RTS beverages was significantly impacted by the individual effects of treatment, storage days, and interaction of treatment with storage days. RTS beverage's body score was reported to have significantly decreased during ambient storage conditions. For developed lime-flavored dragon fruit RTS beverages, the body score drastically decreased as storage days increased.

The effect of ambient storage conditions on the flavor rating of a developed lime-flavored dragon fruit RTS beverage is presented in Table 3. The flavor score of specially produced lime-flavored dragon fruit RTS beverages was significantly influenced by the individual effects of treatment, storage days, and combination of treatment with storage days. With longer storage times, a significant decline in flavor score of RTS beverage was noted.

The effect of ambient storage conditions on the developed lime-flavored dragon fruit RTS beverage's overall acceptability score is summarized in Table 3. (Fig. 3.). The developed lime-flavored dragon fruit RTS beverages' overall acceptability score was significantly influenced by the individual effects of treatment, storage days, and treatment's interaction with storage days. With longer periods of storage under ambient conditions, a significant decline in overall acceptability score of RTS beverage was noted.

3.3.5 Microbial quality

As per the FSSAI regulations, TPC and yeast & mold count for RTS beverage should not more than 50, and 2 cfu/ml, respectively, and coliform should not present in 100ml sample. Control, thermal, microwave, and chemically treated samples evaluated for TPC, coliform and yeast & mold count during storage study. Total plate count, and yeast & mold count were below the permissible limit given by FSSAI, and coliform was absent in control, thermal, microwave, and chemically treated samples.

The optimized lime-flavored dragon fruit RTS beverage incorporated with 500 ppm ascorbic acid had higher betacyanin content (10.04 mg/L), and overall acceptability score (5.57) on 6th day as compared to control, thermal, and microwave treatment stored at ambient storage conditions. So, 500 ppm ascorbic acid incorporated lime-flavored dragon fruit RTS beverage was found best at ambient storage conditions.

3.4 Effect of Refrigerated Storage Condition on Developed Lime-flavored Dragon Fruit RTS Beverage

3.4.1 pH

Summary of the effect of refrigerated storage conditions on the pH of developed lime-flavored dragon fruit RTS beverage is presented in Table 4. (Fig. 4.). On the pH of developed lime-flavored dragon fruit RTS beverages, the individual effects of treatment, storage days, and combination of treatment with storage days were significant. Over a period of 60 days in refrigeration storage conditions, the pH of RTS beverage drastically fell. Similar findings were reported by Foke et al. [9]. The pH of the lime-flavored dragon fruit RTS beverage decreased as a result of chemical reactions that occurred during storage, and produced organic acid.

3.4.2 TSS

The effect of refrigerated storage conditions on the TSS of developed lime-flavored dragon fruit RTS beverage is discussed Table 4. (Fig. 4.). On the TSS of newly developed lime-flavored dragon fruit RTS beverages, the individual effects of treatment, storage days, and interaction of treatment with storage days were significant. Under refrigerated storage conditions, there was a documented significant increase in TSS of RTS beverage. Similar findings were reported by Sharma [18]; and Foke et al. [9], respectively. The TSS of a lime-flavored dragon fruit RTS beverage increased as a result of chemical processes that occurred during storage that resulted in the conversion of complex polysaccharides into simple sugars.

Table 1. Effect of ambient storage condition on pH, TSS, and betacyanin content (mg/L) of developed lime-flavored dragon fruit RTS beverage

pH					TSS					Betacyanin content (mg/L)				
Treatments	**Storage days**			**Mean T**	**Treatments**	**Storage days**			**Mean T**	**Treatments**	**Storage days**			**Mean T**
	0	**3**	**6**			**0**	**3**	**6**			**0**	**3**	**6**	
T_o	3.96	3.49	3.35	3.6	T_o	12.09	12.89	13.39	12.79	T_o	38.04	7.81	4.46	16.77
T_1t_1	3.94	3.46	3.33	3.57	T_1t_1	12.12	13.38	13.96	13.16	T_1t_1	23.8	7.29	4.11	11.73
MW_1	3.94	3.44	3.31	3.56	MW_1	12.05	12.9	13.41	12.78	MW_1	26.69	6.53	3.37	12.2
C_1	3.91	3.4	3.28	3.53	C_1	12	13.01	13.51	12.84	C_1	38	19.19	10.04	22.41
Mean S	3.94	3.45	3.32		Mean S	12.06	13.05	13.57		Mean S	31.63	10.2	5.5	
Factor	C.D. (5%)	SEm	CV%		Factor	C.D. (5%)	SEm	CV%		Factor	C.D. (5%)	SEm	CV%	
T	0.008	0.003	0.181		T	0.018	0.006	0.142		T	0.704	0.24	4.56	
S	0.007	0.002			S	0.016	0.005			S	0.61	0.208		
T X S	0.013	0.005			T X S	0.031	0.011			T X S	1.22	0.415		

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days

Table 2. Effect of ambient storage condition on color, taste, and body score of developed lime-flavored dragon fruit RTS beverage

Color score					Taste score					Body score				
Treatments	**Storage days**			**Mean T**	**Treatments**	**Storage days**			**Mean T**	**Treatments**	**Storage days**			**Mean T**
	0	**3**	**6**			**0**	**3**	**6**			**0**	**3**	**6**	
T_o	8.18	7.03	5.53	6.91	T_o	7.88	7.05	5.08	6.67	T_o	7.86	7.29	5.55	6.9
T_1t_1	8.01	7.1	5.58	6.9	T_1t_1	7.7	7.17	5.18	6.68	T_1t_1	7.54	7.37	5.65	6.85
MW_1	8.06	7.12	5.64	6.94	MW_1	7.78	7.26	5.28	6.77	MW_1	7.49	7.32	5.59	6.8
C_1	7.93	7.57	5.88	7.13	C_1	7.82	7.39	5.44	6.88	C_1	7.87	7.36	5.65	6.96
Mean S	8.05	7.2	5.65		Mean S	7.79	7.22	5.24		Mean S	7.69	7.34	5.61	
Factor	C.D. (5%)	SEm	CV%		Factor	C.D. (5%)	SEm	CV%		Factor	C.D. (5%)	SEm	CV%	
T	0.081	0.028	1.19		T	0.083	0.028	1.25		T	0.059	0.02	0.88	
S	0.007	0.024			S	0.072	0.024			S	0.051	0.017		
T X S	0.014	0.048			T X S	0.014	0.049			T X S	0.103	0.035		

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days

Table 3. Effect of ambient storage condition on flavor, and overall acceptability score of developed lime-flavored dragon fruit RTS beverage

Flavor score					Overall acceptability score				
Treatments	Storage days			Mean T	Treatments	Storage days			Mean T
	0	3	6			0	3	6	
T_o	7.76	7.39	5.13	6.76	T_o	7.83	7.19	5.32	6.78
T_1t_1	7.49	7.24	5.23	6.65	T_1t_1	7.55	7.22	5.41	6.72
MW_1	7.59	7.33	5.26	6.73	MW_1	7.61	7.26	5.44	6.77
C_1	7.64	7.29	5.30	6.74	C_1	7.87	7.40	5.57	6.95
Mean S	7.62	7.31	5.23		Mean S	7.71	7.27	5.43	
Factor	C.D. (5%)	SEm	CV%		Factor	C.D. (5%)	SEm	CV%	
T	0.059	0.020	0.89		T	0.074	0.025	1.12	
S	0.051	0.017			S	0.064	0.022		
T X S	0.101	0.035			T X S	0.129	0.044		

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days

Table 4. Effect of refrigerated storage condition on pH, and TSS of developed lime-flavored dragon fruit RTS beverage

pH							TSS						
Treatments	Storage days					Mean T	Treatments	Storage days					Mean T
	0	15	30	45	60			0	15	30	45	60	
T_o	3.96	3.82	3.43	3.34	3.28	3.57	T_o	12.09	12.29	12.61	12.66	12.67	12.46
T_1t_1	3.94	3.85	3.56	3.45	3.41	3.64	T_1t_1	12.12	12.27	12.51	12.75	12.77	12.49
MW_1	3.94	3.78	3.46	3.34	3.31	3.57	MW_1	12.05	12.32	12.41	12.45	12.53	12.35
C_1	3.91	3.83	3.54	3.43	3.37	3.62	C_1	12.00	12.38	12.71	12.76	12.80	12.53
Mean S	3.94	3.82	3.50	3.39	3.34		Mean S	12.06	12.32	12.56	12.66	12.69	
Factor	C.D. (5%)		SEm		CV%		Factor	C.D. (5%)		SEm		CV%	
T	0.014		0.005		0.538		T	0.017		0.006		0.184	
S	0.016		0.006				S	0.019		0.007			
T X S	0.032		0.011				T X S	0.038		0.013			

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days

Table 5. Effect of refrigerated storage condition on betacyanin content (mg/L), and color score of developed lime-flavored dragon fruit RTS beverage

Betacyanin content (mg/L) Treatments	Storage days					Mean T	Color score Treatments	Storage days					Mean T
	0	15	30	45	60			0	15	30	45	60	
T_o	38.04	29.20	22.27	22.00	20.26	26.35	T_o	8.18	7.76	7.72	7.47	7.43	7.71
T_1t_1	23.80	19.17	16.58	13.93	12.48	17.19	T_1t_1	8.01	7.77	7.73	7.47	7.44	7.68
MW_1	26.69	21.74	15.08	15.48	13.64	18.53	MW_1	8.06	7.76	7.72	7.48	7.44	7.69
C_1	38.00	34.47	25.88	24.49	24.26	29.42	C_1	7.93	7.76	7.72	7.48	7.44	7.66
Mean S	31.63	26.14	19.95	18.98	17.66		Mean S	8.05	7.76	7.72	7.48	7.44	
Factor	C.D. (5%)		SEm		CV%		Factor	C.D. (5%)		SEm		CV%	
T	0.750		0.261		4.43		T	NS		0.013		0.64	
S	0.838		0.292				S	0.041		0.014			
T X S	1.677		0.584				T X S	0.081		0.028			

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days; NS: non-significant

Table 6. Effect of refrigerated storage condition taste, and body score of developed lime-flavored dragon fruit RTS beverage

Taste score Treatments	Storage days					Mean T	Body score Treatments	Storage days					Mean T
	0	15	30	45	60			0	15	30	45	60	
T_o	7.88	7.58	7.33	6.36	5.34	6.90	T_o	7.86	7.67	7.65	7.48	7.45	7.62
T_1t_1	7.70	7.60	7.40	6.32	5.49	6.90	T_1t_1	7.54	7.68	7.67	7.49	7.47	7.57
MW_1	7.78	7.63	7.48	6.25	5.44	6.91	MW_1	7.49	7.66	7.64	7.50	7.47	7.55
C_1	7.82	7.72	7.58	6.57	5.67	7.07	C_1	7.87	7.68	7.65	7.51	7.48	7.64
Mean S	7.79	7.63	7.44	6.38	5.48		Mean S	7.69	7.67	7.65	7.49	7.47	
Factor	C.D. (5%)		SEm		CV%		Factor	C.D. (5%)		SEm		CV%	
T	0.057		0.020		1.11		T	0.040		0.014		0.71	
S	0.064		0.022				S	0.045		0.016			
T X S	0.128		0.045				T X S	0.089		0.031			

T*: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days*

Table 7. Effect of refrigerated storage condition flavor, and overall acceptability score of developed lime-flavored dragon fruit RTS beverage

Flavor score							**Overall acceptability score**						
Treatments	**Storage days**					**Mean T**	**Treatments**	**Storage days**					**Mean T**
	0	**15**	**30**	**45**	**60**			**0**	**15**	**30**	**45**	**60**	
T_o	7.76	7.63	7.09	6.35	5.30	6.83	T_o	7.83	7.76	7.21	6.36	5.32	6.89
T_1t_1	7.49	7.50	7.03	6.31	5.52	6.77	T_1t_1	7.55	7.58	7.22	6.31	5.50	6.83
MW_1	7.59	7.50	7.06	6.23	5.47	6.77	MW_1	7.61	7.57	7.27	6.24	5.46	6.83
C_1	7.64	7.68	7.20	6.56	5.69	6.95	C_1	7.87	7.79	7.39	6.56	5.68	7.06
Mean S	7.62	7.57	7.10	6.36	5.50		Mean S	7.71	7.67	7.27	6.37	5.49	
Factor	C.D. (5%)		SEm		CV%		Factor	C.D. (5%)		SEm		CV%	
T	0.062		0.022		1.23		T	0.060		0.021		1.18	
S	0.069		0.024				S	0.067		0.023			
T X S	0.139		0.048				T X S	0.134		0.047			

T: treatment; S: storage days; T_o: control Sample (standardized sample); T_1t_1: optimized thermal treatment (70°C for 5 min); MW_1: optimized microwave treatment (30 sec); C_1: optimized chemical treatment (500 ppm); T X S: interaction of treatment with storage days

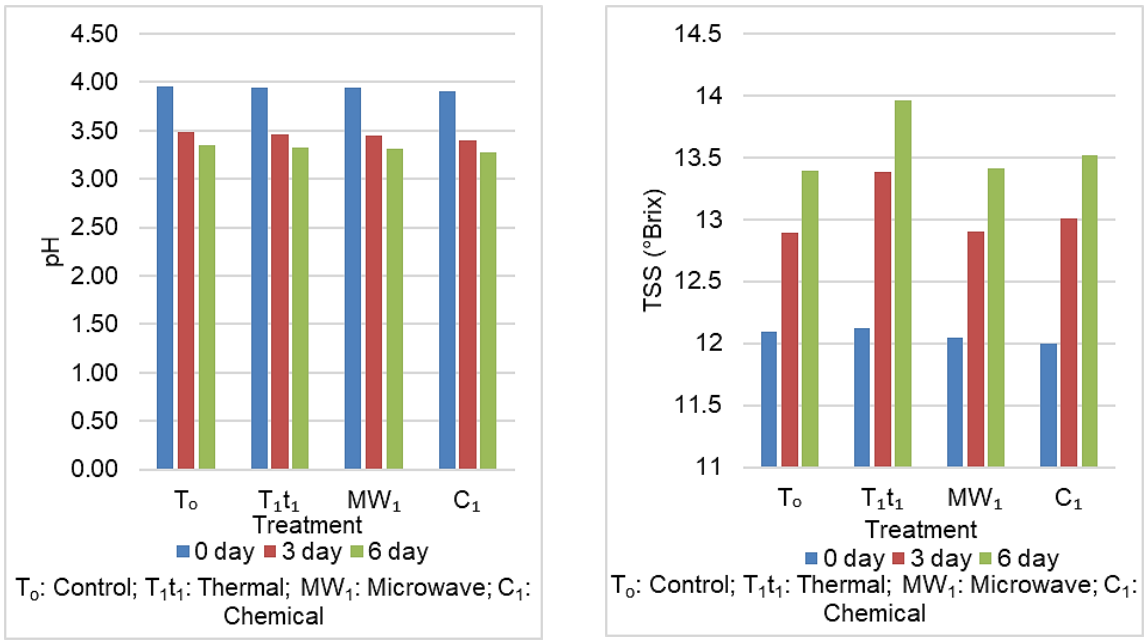

Fig. 2. Effect of ambient storage condition on pH, and of developed lime-flavored dragon fruit RTS beverage

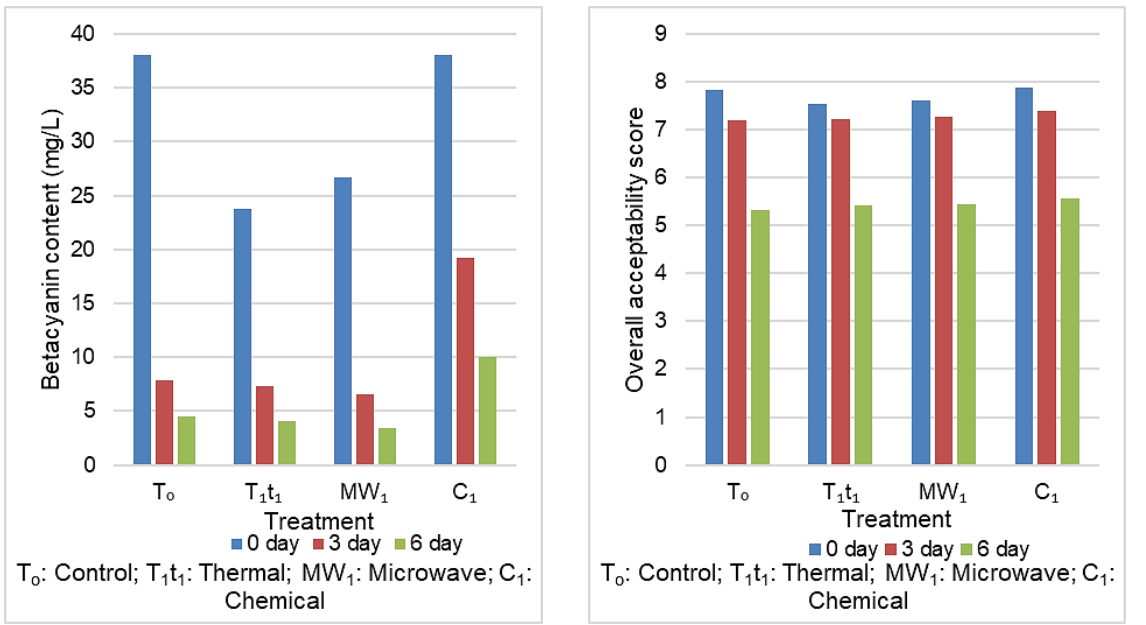

Fig. 3. Effect of ambient storage condition on betacyanin content, and overall acceptability of developed lime-flavored dragon fruit RTS beverage

3.4.3 Betacyanin content

The effect of refrigerated storage conditions on the betacyanin concentration of the developed lime-flavored dragon fruit RTS beverage is tabulated in Table 5. (Fig. 5.). The betacyanin level of developed lime-flavored dragon fruit RTS beverages was significantly affected by the individual effects of treatment, storage days, and interaction of treatment with storage days. During refrigerated storage conditions, a significant drop in the betacyanin level of RTS beverage was noted. Similar findings were reported by Liaotrakoon et al. [4], Herbach et al. [15], and Bassama et al. [10]. The degradation of betacyanin of the lime-flavored dragon fruit RTS beverage caused by exposure to temperature, light, and pH.

On 60th day at refrigerated storage condition, percent reduction in betacyanin content was 46.74%, 47.55%, 48.89%, and 36.15%, respectively for control sample, thermal, microwave, and chemical treated sample stored at refrigerated conditions. Control sample (To) had maximum betacyanin content (38.04 mg/L) which was then decreased to 20.26 mg/L. Percent retention of betacyanin on 60th day was 53.26%, 52.45%, 51.11%, and 63.85%, respectively for control sample, thermal, microwave, and chemical treated sample stored at refrigerated conditions. Decrease in betacyanin content was relatively lower for samples stored at refrigerated conditions than ambient conditions. So, betacyanin content in lime flavored dragon fruit RTS beverage was more stable at refrigerated storage condition than ambient storage conditions. The ascorbic acid in RTS beverage improve the significant retention of betacyanin content in lime flavored dragon fruit RTS beverages stored at refrigerated conditions.

3.4.4 Sensory quality

A panel of judges used a nine-point hedonic scale to evaluate developed lime-flavored dragon fruit RTS beverages for color, taste, body, flavor, and overall acceptability. Table 5. summarizes the effect of refrigerated storage conditions on the color score of a produced lime-flavored dragon fruit RTS beverage. On the color score of developed lime-flavored dragon fruit RTS beverages, the individual effects of storage days, and their interactions with treatment were significant. However, the influence of refrigerated storage conditions on treatment was non-significant. Due to loss of coloring pigment from the beverage, a significant fall in color score of RTS beverage was found during refrigerated storage conditions.

The developed lime-flavored dragon fruit RTS beverage's taste score was affected by the refrigerated storage condition is shown in Table 6. The developed lime-flavored dragon fruit RTS beverages were stored under refrigerated conditions, and each of the independent effects of treatment, storage days, and treatment's interaction with storage days had a significant effect on the taste score. RTS beverage taste rating significantly decreased under refrigerated storage conditions, according to reports.

The effect of refrigerated storage conditions on the body score of a developed lime-flavored dragon fruit RTS beverage is presented in Table 6. Over the course of the refrigerated storage period, the developed lime-flavored dragon fruit RTS beverages' body score was significantly affected by individual treatment, storage day, and the interaction of treatment with storage days. The body score of the RTS beverage significantly decreased under refrigerated storage conditions, according to results.

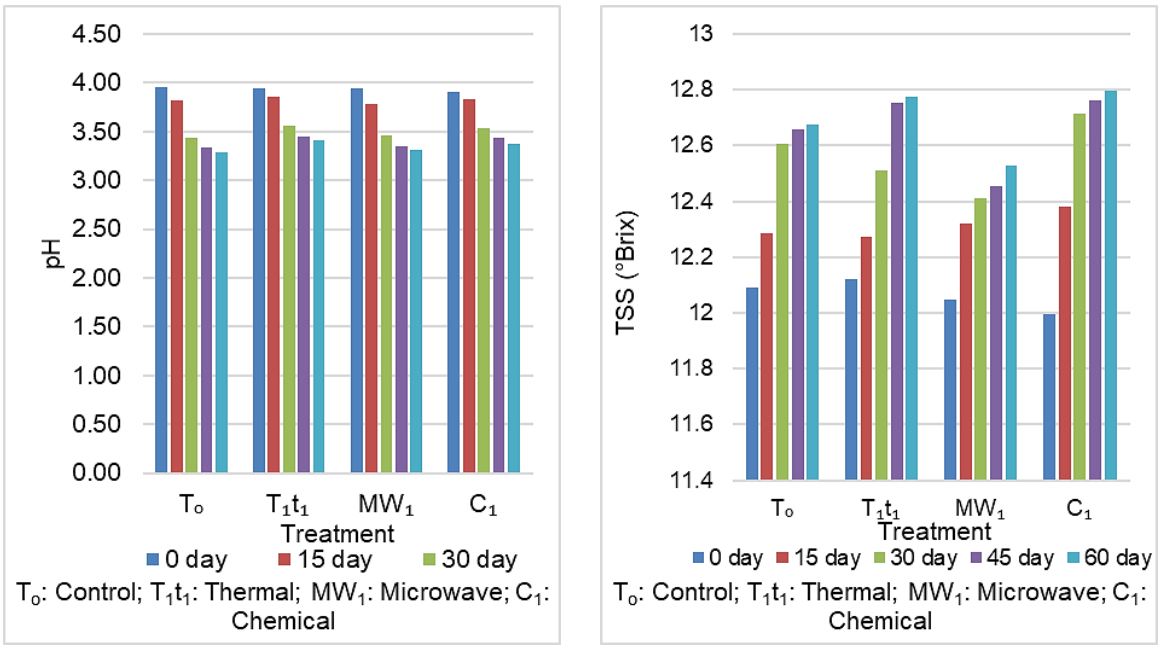

Fig. 4. Effect of refrigerated storage condition on pH, and TSS of developed lime-flavored dragon fruit RTS beverage

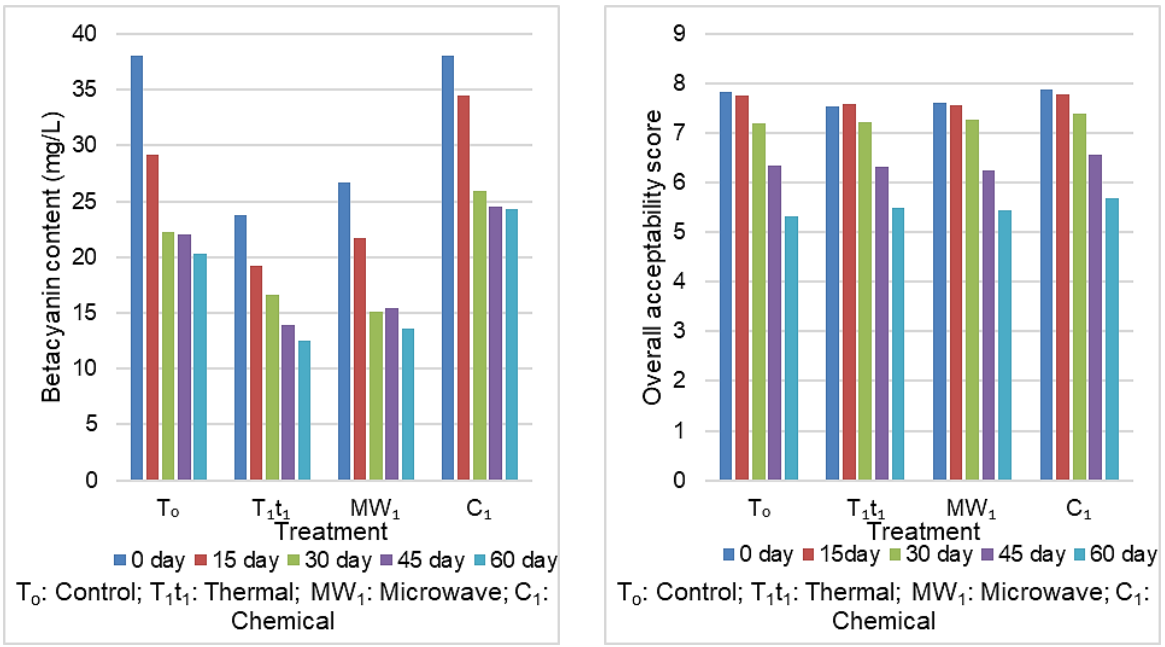

Fig. 5. Effect of refrigerated storage condition on betacyanin content, and overall acceptability of developed lime-flavored dragon fruit RTS beverage

The effect of refrigerated storage conditions on the flavor rating of a developed lime-flavored dragon fruit RTS beverage is tabulated in Table 7. The flavor score of developed lime-flavored dragon fruit RTS beverages was significantly influenced by the individual effects of treatment, storage days, and combination of treatment with storage days. With longer periods of refrigerated storage, a significant decline in flavor score of RTS beverage was noted.

The effect of refrigerated storage conditions on the developed lime-flavored dragon fruit RTS beverage's overall acceptability score is presented in Table 7. (Fig. 5.). The developed lime-flavored dragon fruit RTS beverages' overall acceptability score was significantly influenced by the individual effects of treatment, storage days, and treatment's interaction with storage days. With longer periods of refrigerated storage, the RTS beverage's overall acceptability score drastically declined.

3.4.5 Microbial quality

As per the FSSAI regulations, TPC and yeast & mold count for RTS beverage should not more than 50, and 2 cfu/ml, respectively, and coliform should not present in 100ml of sample. Control, thermal, microwave, and chemically treated lime-flavored dragon fruit RTS beverages evaluated for TPC, coliform and yeast & mold count during refrigerated storage conditions. Total plate count, and yeast & mold count of lime-flavored dragon fruit RTS beverages were below the permissible limit given by FSSAI, and also, coliform was absent in control, thermal, microwave, and chemically treated lime-flavored dragon fruit RTS beverages. So, microbial study found that the lime-flavored dragon fruit RTS beverages were microbiologically safe to consume upto 60th day of refrigerated storage conditions.

The optimized lime-flavored dragon fruit RTS beverage incorporated with 500 ppm ascorbic acid had higher betacyanin content (24.26 mg/L), and overall acceptability score (5.68) on 60th day than control, thermal, and microwave treatment stored at refrigerated storage conditions.

The optimized lime-flavored dragon fruit RTS beverage with 500 ppm ascorbic acid incorporation stored at refrigerated, and ambient conditions has better retention of betacyanin content, and overall acceptability score as compared to other treatment stored at refrigerated, and ambient conditions.

4. CONCLUSION

The significant ($p<0.05$) reduction was observed in pH of lime-flavored dragon fruit RTS beverage stored at ambient well as refrigerated temperature. The pH was slowly decreased at

refrigeration than ambient storage. The storage days, treatment, and interaction of storage days with treatment had Significant ($p<0.05$) impact on TSS of the RTS beverage. A TSS was slowly increased at refrigerated storage than ambient storage. The betacyanin content of RTS beverage was decreased faster for samples stored at ambient temperature than stored at refrigerated temperature. A 3.37 pH, 12.80ºBrix TSS, 500 ppm addition of ascorbic acid and refrigeration temperature may lead towards minimum reduction in betacyanin content for lime-flavored dragon fruit RTS beverage. The lime-flavored dragon fruit RTS beverage incorporated with 500 ppm ascorbic acid was organoleptically acceptable upto 6 days at ambient storage temperature, and having betacyanin content of 10.04 mg/L; whereas for refrigeration temperature, it was sensorially acceptable upto 60 days with retained betacyanin content of 24.26 mg/L from initial betacyanin content (38 mg/L). After the shelf-life study, it can be concluded that 500 ppm ascorbic acid incorporation in lime-flavored dragon fruit RTS beverage, and refrigeration storage conditions can increase the stability of betacyanin content for prolonged time than thermal, and microwave treatment stored at refrigeration as well as ambient storage conditions. Also, it has no direct correlation for retention of betacyanin with pH, and TSS of RTS beverage at both storage conditions. So, the best way to increase shelf-life of lime-flavored dragon fruit RTS beverage is to incorporated 500 ppm ascorbic acid, and store it at refrigeration storage conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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