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Effect of Thermal Processing on Physicochemical Properties, Bioactive Compounds and Microbial Safety of Dragon Fruit (*Hylocereus polyrhizus*) Based Beverage

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A B S T R A C T

The present investigation was carried out at the College of Food Processing Technology & Bioenergy, Anand. The present study was planned to observe the impact of thermal processing (70-90^{0}C, 1-5 min) on physicochemical, bioactive compound and microbial safety of dragon fruit RTS (Ready to Serve) beverage. Thermal treatment at 90^{0}C, for 1 min resulted in better retention of nutritional properties as compared to other treatments. Thermally processed beverage retained ascorbic acid 56.83% whereas antioxidant activity was observed at 25.78% DPPH inhibition. The betacyanin content in thermally treated beverage was found1.80 mg/100mlwhich was higher than other treatment. Thermal treatment attained the goal of microbial reduction in dragon fruit RTS beverage and it was below the requirement given by Food Safety and Standard authority of India. The nutritional properties of prepared beverage were preserved during 90 days of storage at refrigerated conditions.

Introduction

Dragon fruit is the species of cactus, which is originally grown in southern Mexico and South and Central America (Britton and Rose, 1963; Morton, 1987; Mizrahi *et al.,* 1997). In early 19th century, the French brought it to Southeast Asia. More than 93% of the world's dragon fruit production is produced by three major countries: Vietnam, China, and Indonesia. Over an area of 55,419 ha, Vietnam accounts for more than half (51.1%) of global output with an average productivity of 22-35 MT per ha per year. More than 1 MMT of dragon fruit are produced in Vietnam, valued US$ 895.70 million (Chen and Paull, 2018).

Dragon fruit belongs to order of Caryophyllales (Rebecca *et al.,* 2010). The fruits of the red dragon (*Hylocereus polyrhizus*) have a huge rectangular form, a dark red peel with enormous scales and a crimson pulp with small black seeds inside. This fruit has thrived in arid tropical climates and can resist temperatures of up to 40°C (Ortiz and Car, 2012). The previous studies revealed that dragon fruit is rich in nutrients such as vitamin C, B1, B2, B3, high fiber content, minerals such as Ca, Fe, P; while consists of low levels of carbohydrates and zero fat. The seeds are rich in essential fatty acids namely, linoleic acid and linolenic acid which are necessary in human metabolism as cannot be synthesized from other food components by human body (Madhuri, 2017). A variety of processed foods like RTS (ready to serve) beverage, jam, jelly, juice, nectar, squash, cordial smoothies and more can be prepared by using dragon fruit. All these products have pleasing flavors and essences. Soft drinks can also be made from its rich pulp. It promotes probiotic production, which facilitates digestion. It helps those with type 2 diabetes lower their blood glucose levels (Patel and Ishnava, 2019). RTS refers to a fruit beverage that contains at least 10% fruit juice and 10% of total solids, as well as about 0.3% acid. It is named ready to serve because it is not diluted before serving (FSSAI, 2011; Ahmad *et al.,* 2015).

Preservation by high temperature is the most common and conventional method used for food preservation in food industry. Preservation by pasteurization i.e. low temperature ling time (LTLT), high temperature short time (HTST) and sterilization are one of the best methods for extension of shelf life of food products by inactivating the microorganisms. The major drawback of this process is that it has negative impact on nutrient content and color retention. Generally, colors get degraded and brown pigments are formed during heating process (Awuah *et al.,* 2007).

Dragon fruit is an interesting agricultural commodity as it is enriched with pigments like betalains group (betacyanin pigment) and total phenols which act as antioxidant. Present study was focused with objective to optimize process conditions for the preparations of dragon fruit RTS beverage by thermal processing to evaluate its quality characteristics in terms of microbiological, sensorial and physicochemical changes of the formulated RTS beverage. Then it was subjected for storage study for determination of shelf life by subjecting at room temperature and refrigerated conditions.

Materials and Methods

Collection of Materials

The raw material i.e. dragon fruits (*Hylocereus polyrhizus*) used in the present research were uniform, firm, matured and ripened. These fruits were procured from R K farm and Nursery, Nakhatrana, Dist. Kutch-Bhuj Gujarat. Sugar and citric acid were procured from local market of Anand.

Packaging materials Polyethylene terephthalate (PET) bottles of 200 ml capacity were purchased from Axar blow plast, GIDC, Anand, Gujarat. Media for microbial analysis nutrient agar, potato dextrose agar and MacConkey agar were procured from M/s Himedia Laboratories Ltd., Mumbai, Maharashtra.

Dragon fruit Processing

The selected and sorted dragon fruits were washed with tap water to remove soil and dust particles. The fruits were cut into half, peel was removed manually and small pieces were made by using stainless steel Knife. After cutting operation thick mass of pulp was passed through stainless steel sieve to extract the juice and seeds were removed. Extracted juice was filtered through muslin cloth. The dragon fruit RTS beverage was prepared and optimized parameters for formulation were fruit juice 14% citric acid 0.38 % and sugar 12.75%. It was exposed to thermal processing. Data analysis and interpretation was carried out by response surface methodology (RSM) by design expert (version 13.0).

Samples of dragon fruit based RTS beverage were exposed high temperature 70-90°C for 1-3 min and then bottled in 200 ml PET bottles, cooled and stored at ambient temperature and 4°C. Interactions between independent variables at actual levels and its responses were studied using response surface methodology with face centered design (RSM).

ANOVA was conducted on the experimental data and the optimization of thermally treated dragon fruit RTS beverage was done based on three parameters i.e. reduction in microbial count log (cfu/ml), retention of ascorbic acid, maximum antioxidant capacity.

Ascorbic acid

Ascorbic acid of the samples was estimated using 2, 6-dichlorophenol-indophenol by titration method (Rangana, 1986).

$$\text{Ascorbic acid (mg/ 100 g)} = \frac{T \times D \times V \times 100}{A \times V}$$

Where,

T = Titre value

D = Dye factor

V = Volume made up

A = Aliquot of extract taken for estimation

W= Weight or volume of sample taken for estimation

Antioxidant Activity

Antioxidant activity of dragon fruit RTS beverage was determined using the DPPH scavenging effect method as described (Joshi *et al.,* 2019). Hundred milligrams of sample were prepared by adding 1 g of sample in 10 ml of methanol.

Hundred milligrams of the aliquot of extract was withdrawn in test tube and then 2.9 ml of DPPH (0.1 mM) solution was added. After vertexing the mixture incubated in dark for 30 min. Absorbance was measured against blank at 517 nm in UV visible Spectrophotometer. DDPH solution was used as blank (control). The antioxidant activity of dragon fruit pulp was calculated by the following formula:

$$\text{Antioxidant activity (\%)} = \frac{AB - AA}{AA} \times 100$$

Where,

AB = Absorbance of blank

AA= Absorbance of sample

Aerobic plate count

One milliliter of sample was pipetted in sterilized petri plate under sterile environment and thereafter 15 ml of cooled molten nutrient agar was poured into plates. The plates were gently rotated in circular motion and media was allowed to solidify. The solidified plates were inverted and incubated in an incubator (make: Khera Instruments Pvt. Ltd., New Delhi) at 37 ± 0.5°C for 48 h and number of colony forming units (cfu/ml) was recorded. The data is presented in log number of bacterial count (cfu/ml).

Yeast and mould count

One milliliter of sample was pipetted in sterilized petri plate under sterile environment, and thereafter 15 ml of cooled molten potato dextrose agar (pH adjusted to 3.5 by adding 10% sterile tartaric) was poured into plates. Plating is done in triplicate. The plates were gently rotated in circular motion and media was allowed to solidify. The plates were incubated in an incubator maintained at 27 ± 0.5°C for 3-5 days and numbers of colony forming units (cfu/ml) were recorded. The data is presented in log (cfu/ml)

Results and Discussion

Optimization of thermal processing parameters for dragon fruit RTS beverage

Conventionally dragon fruit and its products are either pasteurized or sterilized to ensure safety of the product. High temperature provides preservative effect on extending shelf life of beverages. The optimum combinations of temperature and time provides desired results in processed food products. The experiments for standardization of thermal processing of RTS beverage prepared from dragon fruit juice was conducted as per experimental design of RSM and responses obtained were presented in Table 2

Effect of thermal processing on the ascorbic acid retention of dragon fruit RTS beverage

Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of l-ascorbic acid. Ascorbic acid is one of the most important natural antioxidants in nature and acts as biological reducing agent in hydrogen transport and can take part in various enzymatic, oxidation reduction and hydroxylation reactions (Duma *et al.,* 2015). The score for ascorbic acid retention at different combinations of temperature and time varied from 41.38% to 75.5% (Table 2). The maximum ascorbic acid retention obtained was 75.5% at temperature of 70ºCfor 1 min exposure time; whereas lowest ascorbic retention obtained was 41.38% at temperature of 90ºC and 5 min time were used (Fig 1). Temperature and time have significant effect ($p<0.05$) on ascorbic acid of dragon fruit RTS beverage. The retention of ascorbic acid was decreased with increase in temperature and exposure time. Swami *et al.,* (2016) reported that loss in ascorbic acid up to 31% for aloevera litchi mixed beverage when heated for thermal processing at 95°C for 5 min. Pandraju and Rao (2020) also observed that 25% loss in ascorbic acid of freshly extracted sugarcane juice when it was subjected to thermal treatment at 90°C for 5 min.

Effect of thermal processing on the antioxidant activity of dragon fruit RTS beverage

Antioxidant capacity of a substance is reported as its capacity to scavenge reactive oxygen species as well as electrophiles. Fruits and vegetables are rich in antioxidant compounds, such as ascorbic acid and polyphenols, and their consumption is related to decreased risk of chronic diseases. The range for antioxidant activity for different combinations of (temperature and time) varied from 22.2% to 26.40% (Table 2). The higher antioxidant activity was obtained at temperature of 70°C for 1 min exposure time; whereas, lowest antioxidant activity obtained was 22.20 at 90°C temperature for 5 min exposure time (Table 2).

Temperature and time as well as their combination effect have significant effect ($p<0.05$) on antioxidant activity of dragon fruit RTS beverage. The antioxidant activity was decreased with increase in temperature and exposure time (Fig 1). Similar observation for antioxdiant activity at milder and high heat treatment for tomato RTS beverage was reported by Karnatak *et al.,* (2022). Barba *et al.,* (2012) also reported significant reductions in the antioxidant activity (between 7.5 and 11.5%) when an orange juice-based milk beverage was thermally treated at 90 or 98°C for 21 s.

Effect of thermal processing on the aerobic plate count of dragon fruit RTS beverage

The range for reduction in aerobic plate count for different combinations of temperature and time upon heat treatment was varied from 0.04 to 2.32 log cfu/ml. The lowest reduction 0.04 was found at 70°C temperature and 1 min time whereas highest reduction 2.36 was observed at 90°C temperature and 1, 3 and 5 min time (Table 2). The observed value for reduction in aerobic plate count was below the requirement given by FSSAI i.e., it should not more than 50 cfu/ml in RTS fruit beverages. Temperature and time have significant effect ($p<0.05$) on aerobic plate count of dragon fruit RTS beverage (Fig 2). The aerobic plate count was decreased with increase in temperature and exposure time. Zhang *et al.,* (2016) also observed the effects of high temperature for short time. The HTST processing of carrot juice at 110°C for 8.6 s resulted in to significant decrease in total aerobic plate count. HTST treatment was found to reduce 4.88 log cfu/ml.

Effect of thermal processing on the yeast and mould count of dragon fruit RTS beverage

The range for reduction in yeast and mould count for different combinations of temperature and time upon heat treatment was varied from 0.04 to 2.38 log cfu/ml. The lowest reduction 0.04 was found at 70°C temperature and 1 min time whereas highest reduction 2.38 was observed at 90°C temperature and 1, 3 and 5 min time. The observed value for reduction in aerobic plate count was below the requirement given by FSSAI i.e., it should not more than 2 cfu /ml in RTS fruit beverages (Table 2). Temperature, time and their combined effect have significant effect ($p<0.05$) on yeast and mould count of dragon fruit RTS beverage. Whereas, there was non significant effect of temperature and time on yeast and mould count of beverage within the treatment. The yeast and mould count was decreased with increase in temperature and exposure time (Fig 2). Similar findings for reduction in yeast and mould count with reduction of 1 log cfu was reported using HTST processing at 110°C; 8.6 s for cucumber juice by Liu *et al.,* (2016). Bi *et al.,* (2020) also reported that when mango smoothie was subjected to of HT at 90°C for 20 min there were less significant reduction of yeast and mould count.

Physicochemical, nutritional and microbial properties of thermally optimized dragon fruit RTS beverage

Thermally optimized dragon fruit RTS beverage found to show 15.63ºBx of TSS with 56.83% ascorbic acid retention, 24.85 mg GAE/100 ml of TPC, 1.80 mg/100 ml of betacyanin, showing 25.62% antioxidant activity. The beverage after thermal processing found to have pH of 4.79 and 0.228% of titratable acidity with color (a*) of 1.14 (Table.4) Thermal treatment to dragon fruit RTS beverage resulted in decrease in ascorbic acid as it sensitive to high temperature. Thermal treatment was also resulted in reducing the betacyanin and color value of beverage. The aerobic plate count and yeast and mould count was found absent. High temperature during thermal treatment was major hurdle in enhancing growth and multiplication.

Table.1 Levels of independent variable for preparation of dragon fruit RTS beverage Code

Variable	Low	High
Temperature (°C)	70	90
Time (min)	1	5

Table.2 Standardization of thermal treatment for dragon fruit RTS beverage

Run No.	Variables		Responses			
	Temperature (˚C)	Time (min)	Ascorbic acid retention (%)	Antioxidant activity (%)	Aerobic plate count (log cfu/ml)	Yeast and mould count (log cfu/ml)
1	70	1	75.50	26.40	2.32	2.38
2	80	3	55.27	25.18	1.17	1.22
3	80	3	53.33	24.85	1.2	1.31
4	80	5	50.55	24.15	0.45	0.62
5	80	1	65.83	26.10	1.48	1.52
6	80	3	53.88	25.95	1.3	1.4
7	90	1	56.94	25.70	0	0
8	70	5	60.27	25.88	1.74	1.6
9	90	3	46.66	23.91	0	0
10	80	3	55.83	25.40	1.01	1.15
11	80	3	55	25.05	1.08	1.26
12	70	3	68.33	26.15	1.85	1.92
13	90	5	41.38	22.20	0	0

Table.3 Optimized parameters for thermal treatment of dragon fruit RTS beverage

Parameters	Optimized Value
Temperature	90˚C
Time	1 min

Table.4 Physicochemical, nutritional and microbial properties of thermally optimized dragon fruit RTS beverage

Parameters	Mean ± SD
TSS (°Bx)	15.63 ± 0.05
pH	4.79 ± 0.005
Titratable acidity (%)	0.228 ± 0.002
Ascorbic acid retention (%)	56.83 ± 0.27
Total phenols (mg GAE/100 ml)	24.85 ± 0.14
Color (a*)	1.14 ± 0.01
Betacyanin (mg/100 ml)	1.80 ± 0.01
Antioxidant activity (%)	25.62 ± 0.12
Aerobic plate count (log cfu/ml)	Absent
Yeast and mould count (log cfu/ml)	Absent

Figure.1 Flow chart for thermal processing of dragon fruit RTS beverage

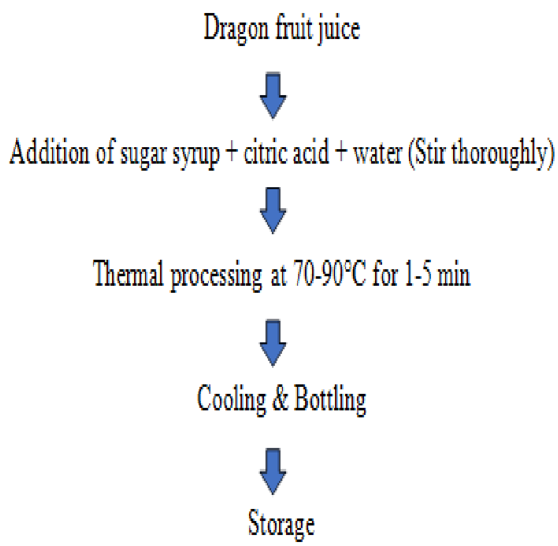


Figure.2 Effect of thermal processing on ascorbic acid and antioxidant activity of dragon fruit RTS beverage

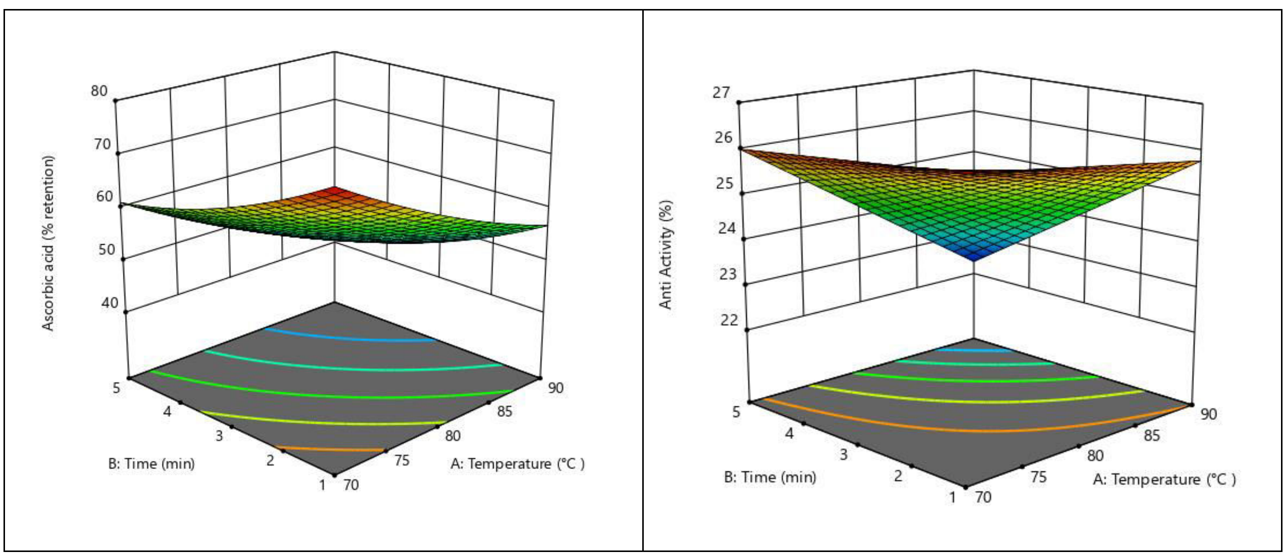
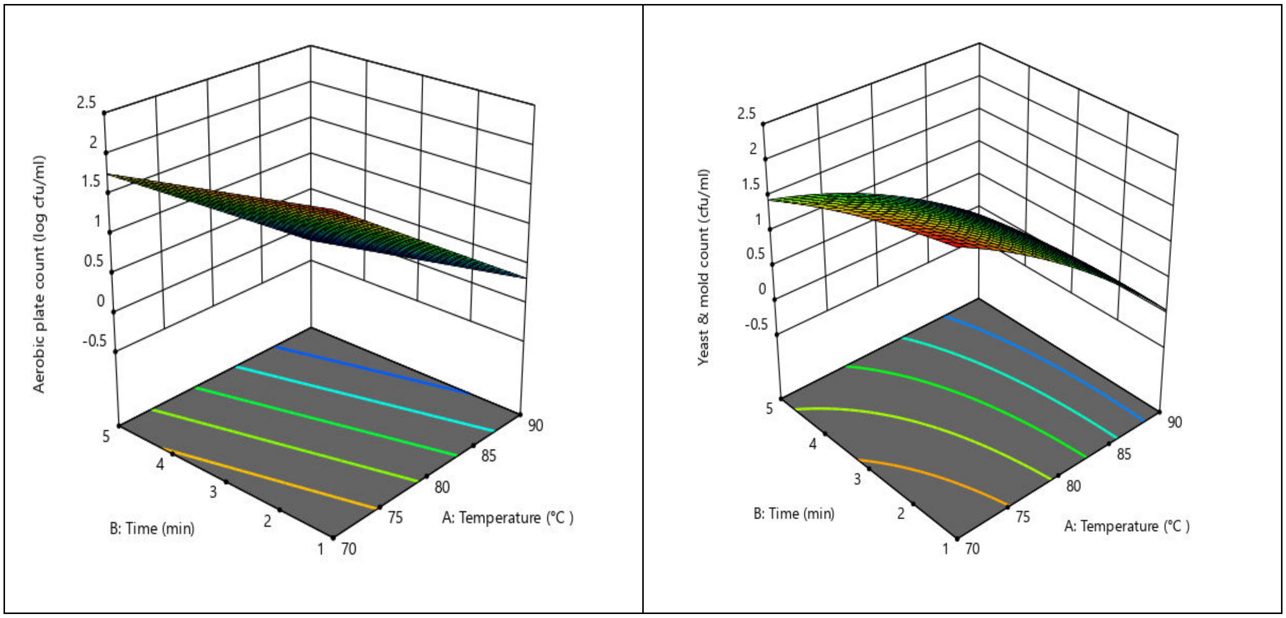


Figure.3 Effect of thermal processing on aerobic plate count and yeast & mould count of dragon fruit RTS beverage



Quality of dragon fruit RTS beverage during storage

The shelflife study of thermally processed dragon fruit RTS beverage stored at ambient(37 ± 5°C) and refrigerated conditions(4 ± 2°C) were carried out.

The higher and favorable temperature during storage resulted in enchantment in microbial load. Visible sign of spoilage was observed RTS beverage samples after 15 days of storage at ambient conditions and microbial load was exceeded the critical limit set by FSSAI.

Hence all samples treated by thermal treatment were discarded. But samples stored at refrigerated condition resulted the minimum changes in nutritional properties and microbial load was below the requirement given by FSSAI

Dragon fruit RTS beverage processed at 90^0C for 1min had superior quality as compared unprocessed and other samples. The retention of ascorbic acid (56.83%) and betacyanin (1.8 mg/100 ml) was in higher proportion with microbial safety.

The results thus obtained revealed that optimizing temperature–time conditions can be considered as a factor of great importance in order to obtain a better retention of bioactive compounds and physicochemical characteristics. The result conferred that storage of beverage at refrigerated conditions was suitable as compared to ambient conditions.

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Author Contributions

Anil S Ghorband: Conceived the original idea and designed the model and wrote the manuscript and analyzed the data.; Dr. Bhavesh Joshi: Visualization, Supervision, Project Administration and Dr. Anurag Nema: Designed the model and the computational framework

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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