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Impact of retort thermal pasteurization on the quality and stability of red dragon fruit (*Hylocereus polyrhizus*) juice

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Abstract
Red dragon fruit (*Hylocereus polyrhizus*) is an exotic fruit with high consumer demand due to its rich content of bioactive compounds and significant antioxidant potential. Retort pasteurization is a novel thermal processing technology with high commercial importance. The retort pasteurization of red dragon fruit juice presents an opportunity for the development of various value-added products, including squash, jelly, syrup, and powder. This diversification not only enhances the economic returns for farmers but also contributes to the growth of the fruit processing industry. Despite its potential, the application of retort pasteurization technology for dragon fruit juice preservation remains largely unexplored. Investigation and optimization of process parameters for thermal pasteurization of red dragon fruit juice can open new avenues for improving product stability, shelf life, and marketability. Hence this study was undertaken to evaluate how different retort pasteurization parameters (temperature and time) affect key bioactive compounds and spoilage enzymes in red dragon fruit juice and optimized the process parameters. A central composite design was applied, with product temperatures ranges between 70°C to 90°C and processing times from 5 to 15 minutes. The analysis focused on betacyanin content,total flavonoid content (TFC), total phenolic content (TPC), and the residual activity of peroxidase (POD) and polyphenol oxidase (PPO). The numerical optimization was carried out to maximize TPC while minimizing PPO and POD activity. The optimal processing conditions were determined to be a temperature of 90°C and a processing time of 5 minutes. On validation, the juice exhibited a betacyanin content of 22.24 ± 0.15 mg/100 ml, a TPC of 64.56 ± 0.35 mg/100 ml, a TFC of 20.26 ± 0.16 mg/100 ml, a PPO activity of 5 ± 0.02 % and no peroxidase activity was detected. The microbial study confirmed that retort pasteurization maintains the juice's safety with no observed bacterial, yeast and mould growth. The findings indicated that retort pasteurization at 90°C for 5 minutes is an effective technique for the preservation of quality and safety of red dragon fruit juice.

Keywords: Bioactive compounds, Fruit juice preservation, Pasteurization, Red dragon fruit juice, Retort pasteurization, Thermal processing.

1. Introduction

Red dragon fruit (*Hylocereus polyrhizus*) or Pitaya is a precious fruit enriched with health-promoting compounds. It has anti-inflammatory, antimicrobial, anticancerous, anti-lipidemic and antidiabetic properties (Nishikito et al., 2023). Producing juice from red dragon fruit is an effective method for creating value-added products and boosting the income of farmers and economic potential of the fruit industry (Truong & Dang, 2016; Wakchaure et al., 2023). Fruit juices are traditionally preserved by thermal pasteurization at a higher temperature ranging from 60-100 ^{0}C followed by the addition of preservatives for room temperature storage (Mezgebe, 2011; Agçam et al. 2018).Thermal pasteurization reduce the pathogenic organisms and spoilage enzymes with minimal loss of food quality (Peng et al., 2017). Even though, the higher temperatures used during thermal pasteurization may deteriorate the quality of fruit juices like pitaya juice where the major antioxidant pigments called betalains are heat sensitive. Betalains begin to break down at temperatures exceeding 50 °C, which poses a major limitation for their application as food colorants (Sadowska-Bartosz & Bartosz, 2021). Moreover, the addition of sweetening agents and preservatives in excess amounts in fruit juices are harmful to the health of consumers. (Tahmassebi & Banihani, 2020). Retort pasteurization of fresh pitaya juice and refrigerated storage is a good alternative to traditional methods of juice preservation.

The retort pouch was invented in the 1950s by the United States and is a multiple layer flexible packaging material made of polyester, aluminum foil, and polypropylene (Fung

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et al., 2018; Byun et al., 2010). It combines the advantages of both metal and plastic packaging.Retort pasteurization is a moist heat sterilization method that involve stages like filling, steam exhausting, sealing, traying, and retorting (Ahmed & Eun, 2018). Most often retort pasteurization is used for sterilization of foods. Since fruit juices are highly sensitive to heat, pasteurization and refrigerated storage are favored over sterilization to maintain their volatile aroma and bioactive compounds. Rajan et al.(2024) recently optimized retort pasteurization parameters and evaluated the shelf life of coconut inflorescence sap (neera) packaged in retort pouches. The retort parameters were optimized at 82 °C for 19 minutes, and the samples processed under these conditions exhibited a shelf life of 25 days. Avila Gaxiola et al. (2016) studied the effect of Variable retort temperature (VRT) processes for retention of nutritive quality of papaya puree. The study found better retention of nutrients by VRT compared to continuous retort temperature (CRT) processing.

Retort pouch packaging for pasteurizing red dragon fruit juice is a relatively new approach aimed at enhancing the juice's shelf life. The review of the literature reveals a noticeable research gap regarding the effect of retort pasteurization on the biologically active components in pitaya juice, spoilage enzymes, and shelf life evaluation. Based on this gap, the current work was conducted to evaluate the impact of retort pasteurization on key bioactive compounds and the inactivation of spoilage enzymes in red dragon fruit juice. In addition, the work aims to explore the linear, interactive, and quadratic effects of process parameters on juice quality and to optimize these parameters.

2. Materials and methods

2.1. Juice extraction

The ripe red dragon fruits were sourced from the plantation nursery in Thrissur (10.5452 ^{0}N, 76.274 ^{0}E), Kerala, India. They were washed, peeled, and chopped into pieces before undergoing ultrasonic pre-treatment at a 40% amplitude for 10 minutes.The surface sterilization of ultrasonic probe system was performed with a tissue soaked in 70% alcohol atleast 2 hours before treatment. The ultrasonic pretreatment at these specific conditions was performed to enhance juice yield and bioactive compounds in the juice based on the preliminary studies. The ultrasonic device (Model: UTR 1244, Shalom Ultrasonics, Pune, India) operated at a frequency of 20 kHz, with a maximum power output of 750 W and a horn diameter of 25 mm.About 400 g fruit pulp was treated in batches with 15 minutes interval between the batches. The temperature of the juice was monitored during ultrasonic treatment. Subsequently, the juice was extracted using a cold press juicer (Model: 19003594, Pigeon, India) with a power rating of 150 W and a speed of 75 RPM. The TSS and pH of the juice were recorded as 12.18 ± 0.02 ºbrix and 4.62 ± 0.01, respectively. The juice was kept in PET bottles under refrigeration until retort pasteurization. The experiments took place at the Agribusiness Incubator, Department of Agricultural Engineering, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur.

2.2. Chemicals

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminium chloride, Folin&Ciocalteu reagent, sodium hydroxide, sodium carbonate, sodium nitrate, guaiacol, catechol, and buffer solution (pH 7) were sourced from Sisco Research Laboratories Pvt. Ltd, Maharashtra, India. Hydrogen peroxide and sodium hydroxide were procured from Merck Life Science Pvt. Ltd, Mumbai, India. Ethanol was supplied by Gemini Associates, Goa, India. All reagents and chemicals used were of analytical grade.

2.3. Retort pasteurization

Initially, the retort pouches were filled with 100 ml red dragon

Table 1. Central composite design matrix and response values of retort treatment

Exp. No.	X_1 (^{0}C)	X_2 (min)	Betacyanin content (mg BCE/100 ml)		Total phenolic content (mg GAE/ 100 ml)		Total flavonoid content (mg QE/ 100 ml)		PPO residual activity (%)		POD residual activity (%)	
			Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	80	10	22.98 ± 0.15	22.16	58.87± 0.34	55.72	20.16 ± 0.14	18.77	15 ± 0.03	13.2	1.11 ± 0.01	0.91
2	80	3	23.04 ± 0.13	23.36	56.17± 0.42	57.10	22.36 ± 0.15	21.65	17 ± 0.04	16.83	1.47 ± 0.01	1.14
3	80	10	22.52 ± 0.14	22.16	56.43± 0.32	55.72	19.06 ± 0.13	18.77	14.5 ± 0.05	13.2	1.13 ± 0.01	0.91
4	66	10	24.25 ± 0.21	23.86	46.54± 0.25	46.70	36.54 ± 0.25	38.95	37.5 ± 0.24	40.04	2.59 ± 0.01	2.51
5	90	5	22.15 ± 0.15	21.74	64.35± 0.35	64.48	19.46 ± 0.12	21.33	5.0 ± 0.02	5.17	0.02 ± 0.01	0.26
6	90	15	19.53 ± 0.12	19.68	40.27± 0.16	42.67	13.67 ± 0.07	14.10	2.5 ± 0.01	0.05	0.00	0.15
7	80	10	22.81 ± 0.14	22.16	55.90 ± 0.23	55.72	18.67 ± 0.05	18.77	13 ± 0.04	13.2	1.15 ± 0.01	0.91
8	80	10	21.16 ± 0.20	22.16	53.79 ± 0.34	55.72	19.67± 0.06	18.77	8.5 ± 0.03	13.2	1.16 ± 0.02	0.91
9	80	17	22.75 ± 0.18	22.26	47.27 ± 0.22	44.97	16.56 ± 0.11	17.91	4.0 ± 0.02	7.67	0.00	0.21
10	70	15	23.47± 0.16	24.05	48.95 ± 0.28	50.16	35.00 ± 0.22	32.5	30 ± 0.26	26.33	1.53 ± 0.02	1.41
11	70	5	23.54 ± 0.19	23.55	46.74 ± 0.32	45.68	31.67 ± 0.23	30.61	35 ± 0.21	34.05	2.05 ± 0.05	2.31
12	94	10	19.32 ± 0.12	19.54	56.15 ± 0.27	54.62	21.33 ± 0.14	19.56	0.00	0.12	0.00	0.04
13	80	10	21.32 ± 0.15	22.16	53.58 ± 0.37	55.72	16.33 ± 0.12	18.77	15 ± 0.06	13.2	0.00	0.91

X_1 represents product temperature (^{0}C) and X_2 represents time (min)

fruit juice. Then exhausting of the pouches was performed in the steam exhausting unit (model: RSMFLS-1003/92, Revathi electronics and controls). After that, the pouches were sealed in a pneumatic sealer (Model: QS 300X 10 PNI V2, Sevana Electrical Appliances Pvt. Ltd).The sealed pouches were kept in trays and loaded into a steam-air retort (SS304:ITech equipment, Bengaluru, Karnataka). The retort pasteurization was performed at various product temperatures and times based on central composite design as shown in Table 1. The processing parameters were set within a temperature range of 70–90°C and a time range of 5–15 minutes. The pressure was maintained at 1.5 kg/cm^2 during the retort operation. Product temperature was continuously monitored during retort pasteurization using a calibrated thermocouple for precise thermal assessment.After thermal pasteurization, automatic cooling took place. Finally the trays were unloaded from the retort and the pouches were kept under refrigerated storage at 4±1^{0}C.

2.4. Design of experiments, mathematical modeling, and statistical analysis

This study utilized standard central composite design, for retort pasteurization, with the assistance of Design Expert software (Version 12). Two key independent variables were evaluated for the treatment: Product temperature (70-90^{0}C) and time (5-15 min).The work involved 13 experiments, including five central points. Central composite design(CCD) is highly preferred due to its accuracy and elimination of need for a three factorial experimental design (Bhattacharya, 2021). It helps to reduce number of experiments and by exploring points outside the factorial design (using axial/ star points), it tests the robustness of the process, ensuring stability under varying conditions (Myers et al., 2016).

Betacyanins, are the major antioxidant pigments present in the dragon fruit juice but are heat sensitive. Polyphenols are a larger category of bioactive compounds in fruit juices and flavonoids is a sub category of polyphenols. Polyphenoloxidase and peroxidase are the two classes of spoilage enzymes which cause discolouration and off flavour development. Thermal inactivation of these enzymes are important to ensure quality of the juice. The dependent variables evaluated in experiments included betacyanin content, total phenolic content (TPC), total flavonoid content (TFC), polyphenoloxidase (PPO) activity, and peroxidase (POD) activity. To examine the dependent variables and estimate optimal values, the empirical regression model equation (1) proposed by RSM was employed.

$$y = k_0 + \sum_{i=1}^{n} k_i x_i + \sum_{i=1}^{n} k_{ii} x_i^2 + \sum_i \sum_j k_{ij} x_i x_j \quad (1)$$

In this equation, y represents the predicted response values, where k_{ij}, k_{ii}, $k_i$signify the coefficients of regression for the interaction, quadratic, and linear terms, respectively, while x_i and $x_j$represent the independent variables.

An analysis of variance (ANOVA) was performed to improve the accuracy of the final equation's coefficients. Quadratic models were created to match the experimental data using Design Expert 12 software. The adequacy and precision of the models were evaluated using several statistical indicators as described by Vivek et al.(2019). Furthermore, response surface plots (3D) were used to explore the interactions between process variables and their effects on the responses.

The RMSE and MAE were calculated using Eqn.2 and Eqn.3.

$$RMSE = \sqrt{\frac{\sum (H_p - H_o)^2}{N}} \quad (2)$$

$$MAE = \frac{1}{N} \sum_{i=1}^{N} |H_0 - H_p| \quad (3)$$

Where H_p represents the predicted value; H_o represents the observed value; and N represents the number of observations.

Table 2. The regression coefficients for responses of retort pasteurization

Coefficients	Betacyanin content	Total phenolic content	Total flavonoid content	PPO residual activity	POD residual activity
$\hat{a}_0$	22.16	55.72	18.77	13.17	0.91
X_1	-1.54***	2.83**	-6.92***	-13.89***	-0.90***
X_2	-0.39	-4.33**	-1.34	-3.24*	-0.33
X_1X_2	-0.64	-6.57***	-2.28	0.63	0.13
X_1^2	-0.23	-2.58*	5.35***	3.73*	0.16
X_2^2	-0.33	-2.39*	0.51	-0.48	-0.12
R^2	0.85	0.93	0.95	0.96	0.85
Adj R^2	0.74	0.87	0.92	0.93	0.74
Pred R^2	0.55	0.69	0.73	0.81	0.56
Adeq precision	8.79	14.22	17.36	18.49	8.85
SD	0.75	2.26	2.11	3.18	0.44
CV	3.4	4.28	9.43	21.01	47.05
RMSE	0.55	1.65	1.54	2.33	0.32
MAE	0.48	1.37	1.32	1.87	0.25
LoF	N.S	N.S	N.S	N.S	N.S

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***significant at $p < 0.001$

The optimization of retort parameters was executed using the numerical optimization methodology refer to Candioti et al. (2014), with minor amendments. The individual desirability functions for each response were integrated into one expression, expressed as the geometric mean of those separate functions. A higher desirability score indicates a better match for the system. The predicted values of the response variables were validated by conducting retort pasteurization under optimized conditions and repeating biochemical analysis for the sample.

2.5. Quality analysis

The betacyanin content was measured using the UV-visible spectrophotometric technique at 538 nm, following Nurul & Asmah (2014) with slight modifications, and expressed as mg of betacyanin equivalent (BCE) per 100 ml. The total phenolic content (TPC) in the fruit juice was determined based on Palety et al. (2020) with slight modifications and expressed in mg of Gallic acid equivalent (GAE) per 100 ml. The total flavonoid content (TFC) was analyzed according to Zhishen et al. (1999) and expressed in mg of Quercetin equivalent (QE) per 100 ml. Polyphenol oxidase (PPO) residual activity was calculated following Liao et al. (2020), while peroxidase (POD) activity was evaluated using a modified protocol from Zhu et al. (2021), both expressed as a percentage. The total plate count and total yeast and mould count were determined using the standard pour plate method and expressed in CFU/ml.

3. Results and discussion

3.1. Model data fitting

Table 1 presents the central composite design matrix along with the response values, including both experimental results and software-predicted values for retort pasteurization. Table 2 provides the regression coefficients for the responses associated with retort pasteurization. All regression models for the response variables were statistically significant ($p < 0.05$). The ANOVA results for each response variable showed an R^2 value exceeding 0.8, indicating high model accuracy. The lack of fit (LoF) for all models was non-significant, and the low coefficient of variation (CV) demonstrated minimal relative standard deviation. Furthermore, the reduced RMSE and MAE confirmed a low error rate in the dataset.

3.2. Effect of retort process parameters on betacyanin content

The regression model illustrating the influence of retort pasteurization parameters on betacyanin content, incorporating only the statistically significant factors, is represented in Equation No. 4.

$$\text{Betacyanin content (mg BCE/100 ml)} = 22.16\text{-}1.54X_1 \quad (4)$$

A significant decline in betacyanin concentration was observed withan increase in processing temperature. Fig.1 (a) shows the 3D response diagram of the effect of retort process parameters on betacyanin content. The maximum betacyanin retention (24.25 ± 0.21 mg BCE/100 ml) in the juice was recorded in the juice treated at 66° C for a 10-min. Conversely, the lowest betacyanin levels (19.32 ±0.12 mg BCE/100 ml) were observed at 94° C for 10 minutes. The decrease in betacyanin content can be attributed to the thermal degradation of the pigment. The breakdown of betacyanin at elevated temperatures occurs because betanin, its primary component, undergoes processes such as cleavage, decarboxylation, or isomerization ensuing in a steady fading of its red color. At these temperatures, two unstable compounds, betalamic acid and cyclodopa-5-O-glycoside, are produced (Reshmi et al., 2012).The results corroborate with the findings of Mikoajczyk-Bator & Pawla.(2016), who reported that a treatment at 90°C for 30 minutes resulted in a significant degradation of betacyanins, with a 13-15% reduction in betacyanin content and a 7% decrease in antioxidant capacity.

3.3. Effect of retort process parameters on TPC

The regression model for the effect of retort process parameters on TPC with significant factors is given in Eqn.5.

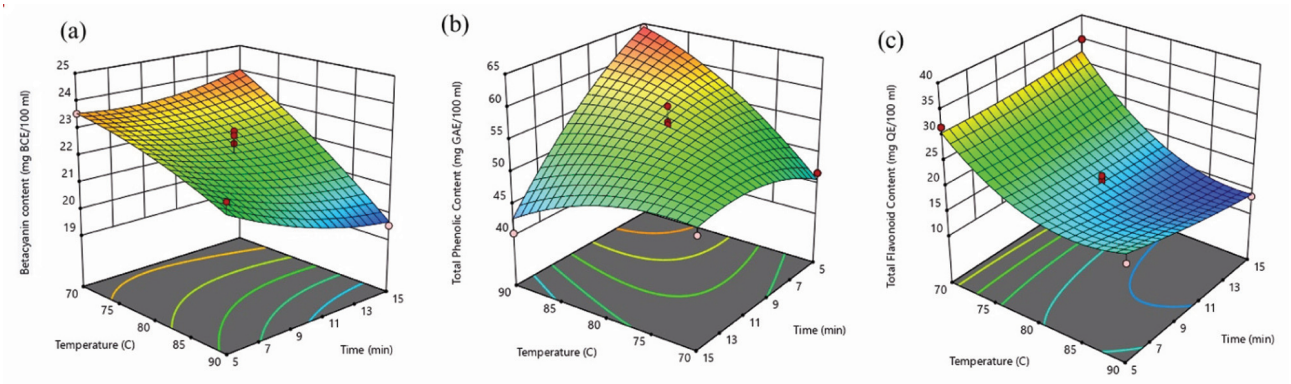

Figure 1. Effect of retort process parameters on (a) betacyanin content (b) total phenolic content and (c) total flavonoid content

All the terms showed a significant effect on TPC.

TPC(mg GAE/ 100 ml) = 55.72+ 2.83-4.33-6.57-2.58-2.39 (5)

Fig. 1(b) shows the effect of retort process parameters on TPC. The TPC in dragon fruit juice exhibited a significant linear increase with temperature and decline with increasing treatment time. A notable reduction in TPC was also observed due to the interaction between temperature and time. Both temperature and treatment duration resulted in a significant quadratic decrease in TPC. The maximum TPC (64.35 ± 0.35 mg/100 mL) was recorded at 90^0 C for 5 min. The lowest phenolic content, 40.27± 0.16 mg GAE/100 g, was recorded at 90° C for 15-min. The initial increase in TPC might be attributed to the fact that high temperatures can lead to the leaching of phenolic components from cells as a result of damage to cellular components, increasing their extraction (Wazir et al., 2011). However, excessively high temperatures may degrade these phenolic compounds, as they are sensitive and prone to destruction when exposed to elevated temperatures and prolonged heating (Argo et al., 2024). These findings harmonising with Ghafoor et al.(2019)where a reduction in both antioxidant activity and total phenolic content during the heating of plum and mahaleb was observed.

3.4. Effect of retort process parameters on TFC

The regression model for the effect of retort pasteurization parameters on TFC with significant factors is given in Eqn.6.

TFC (mg QE/100 ml) =18.77-6.92+5.35 (6)

The TFC decreased in a consistent, linear manner as the retort temperature increased. However, the flavonoid content exhibited a quadratic increase with temperature. Fig. 1(c) presents the 3D response surface plot illustrating the impact of retort process parameters on TFC. The highest flavonoid concentration (36.54 ± 0.25 mg QE/100 ml) was recorded at the lowest temperature of 66°C for 10 minutes, while the least flavonoid content (13.67 ± 0.07 mg QE/100 ml) was observed at 90°C for 15 minutes. Flavonoids are the predominant group of polyphenols present in dragon fruit. The degradation of flavonoids during pasteurization is primarily caused by various reactions, including thermal degradation, depolymerization, and polymerization (Ahmed & Eun, 2018). Temperature influences the stability of flavonoids and their biological activity. Depending on their structure, flavonoids vary in their sensitivity to heat treatment (Chaaban et al., 2017).The results are consistent with those of Igual et al. (2011) who observed a notable reduction in flavonoid levels in grapefruit juice following thermal pasteurization.

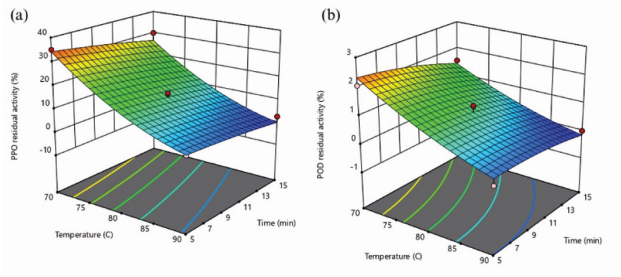

Figure 2. Effect of retort process parameters on (a) PPO residual activity and (b) Peroxidase residual activity

3.5. Effect of retort process parameters on residual PPO activity

The effect of retort process parameters on residual PPO activity, considering only significant factors, is presented in Equation 7. Additionally, Figure 2 (a) illustrates the response surface plot depicting the impact of retort pasteurization parameters on residual PPO activity.

PPO residual activity (%) = 13.17-13.89-3.24+3.73 (7)

PPO induces the conversion of polyphenols to quinones which further polymerizes with proteins and amino acids and produce discoloration in fruit juices (Taranto et al., 2017). In the present study, PPO residual activity significantly decreased linearly with process temperature and time. At the same time, the PPO activity showed a significant quadratic increase over time with temperature. Zero residual PPO activity was observed at 94 ^{0}C and 10 minutes of treatment time while the maximum PPO activity (37.5%) was observed at 66^{0}C and 10 minutes. A similar observation was reported by Chutintrasri & Noomhorm (2006), where the residual activity of PPO in pineapple puree was only 1.2% after 5 minutes at 90 °C.The results also align with Engmann et al. (2014), who found that increasing the temperature and heating time significantly decreased residual PPO activity in mulberry juice.

3.6. Effect of retort process parameters on residual peroxidase activity

The effect of retort pasteurizations parameters on residual POD activity with only significant factors is given in Eqn.8.

POD residual activity (%) = 0.91- 0.90 (8)

The response surface plot Fig.2 (b) depicts the impact of retort pasteurization parameters on residual POD activity.Peroxidase activity decreased significantly as the temperature increased. No peroxidase activity was detected above 80°C after 10 minutes of treatment. The highest residual activity recorded was only 2.59 ± 0.01% at 66 °C after 10 minutes. These findings are consistent with those of Gonçalves et al.(2010), who reported no peroxidase activity after 2 minutes of thermal treatment at 90°C. Similarly, the results align with Imaizumi et al. (2019), who observed that mild heating at 70°C effectively inactivated POD, while

heating at 60°C was insufficient to deactivate the heat-resistant fraction of POD in carrots.POD is more heat-sensitive than PPO and several studies have indicated a synergistic relationship between the two enzymes. PPO uses the products of POD, so a decrease in POD activity results in a reduction of PPO activity (Martin-Diana et al., 2004).

3.7. Process optimization

For optimization of retort pasteurization parameters, betacyanin content and flavonoid content were kept in range, TPC was kept at a maximum and PPO and POD activity were kept at minimum. An optimum desirability value of 0.92 was achieved at 90 °C with a treatment time of 5 minutes. The predicted outcomes under these optimal conditions were validated experimentally, and a paired t-test confirmed no significant difference between the experimental and predicted results. On validation of the optimized condition, the betacyanin content, TPC, TFC, residual PPO activity, and POD activity were found to be 22.24 ± 0.15 mg/100 ml, 64.56 ± 0.35 mg/100 ml, 20.26 ± 0.16 mg/100 ml, 5 ± 0.02 % and zero, respectively.

3.8. Microbial study

After retort pasteurization at 90 °C for 5 minutes, no bacterial or fungal colonies were detected, confirming the microbiological safety of the juice. The total plate count and total yeast and mould count indicated the complete elimination of microbial contamination. In contrast, the unprocessed control juice, which did not undergo thermal treatment, had a significantly higher microbial load, with a total plate count of 21×10^5 CFU/ml and a yeast and mould count of 2×10^3 CFU/ml. These findings demonstrate that retort pasteurization effectively ensures the microbial safety of the juice, making it suitable for consumption.

4. Conclusion

In conclusion, this study highlights the critical influence of retort pasteurization parameters, particularly temperature and time, on the nutritional and enzymatic properties of red dragon fruit juice. Optimal pasteurization conditions (90°C for 5 minutes) were identified to maximize total phenolic content (TPC) while minimizing residual polyphenol oxidase (PPO) and peroxidase (POD) activities. These conditions effectively preserved the nutritional quality of the juice, as evidenced by the retention of significant levels of betacyanin, TPC, and total flavonoid content (TFC), along with negligible enzymatic activity. Additionally, microbiological analysis confirmed the safety of the juice, with no detectable microbial colonies observed. These findings provide valuable insights into the optimization of thermal pasteurization to enhance the safety, quality, and shelf life of functional fruit beverages.

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