Materials and methods

CHAPTER III

MATERIALS AND METHODS

This chapter explains the materials and approved methodologies followed for the standardisation and evaluation of thermal and non-thermal processing of ripe jackfruit bulb (RJB) and ripe jackfruit pulp (RJP). The session comprises the detailed procedures followed for the thermal process standardisation of ripe jackfruit by retort pouch processing, non-thermal standardisation via HPP and PL technology. For a better understanding and elucidation, this session is subdivided as:-

Experiment I: Thermal process standardisation of RJB and RJP utilizing retort pouch processing

Experiment II: Standardisation of HPP parameters for RJB and RJP

Experiment III: Standardisation of PL for RJP

3.1 Raw material collection and sample preparation

Jackfruit (variety: *Varikka*) was sourced from the Fruits and Vegetables Research Station at Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. The external impurities over the jackfruits were removed by washing them properly in tap water and surface sanitisation was carried out by dipping washed jackfruits in 1% (120 ppm) sodium hypochlorite solution for 10 min. (Saranya *et al*., 2024). The surface-sanitised jackfruits were cut into four pieces vertically, and the central core was removed to separate the RJB (Plate 3.1). The jackfruit bulbs, thus separated and de-seeded were used for further processing. RJP for thermal and non-thermal processing was prepared with the aid of an industrial mixer (Plate 3.2) (Make: Sarahas Techno, Kerala).

Plate.3.1 Cutting and deseeding and packing of RJB

Plate. 3.2 Jackfruit pulping using industrial mixer

EXPERIMENT I:

3.2 THERMAL PROCESS STANDARDISATION OF RJB AND RJP UTILIZING RETORT POUCH PROCESSING

Thermal process standardisation of ripe jackfruit was carried out in a steam-air retort (Plate 3.3c) following the method given by Gobikrishnan *et al*. (2019). The retort was equipped with a high-pressure water circulation pump for cooling and compressed air for overriding pressure. Retort pouches of 250 g capacity were selected as the packaging material during the experimental trials. Treatment samples were prepared by filling 200 g bulbs in each packet with 35˚Brix sugar syrup as a filling solution (Fig.3.1). Concomitantly, the jackfruit pulp in each packet was 150 g. Excess air inside the packets was exhausted via, high-pressure steam from a steamer (Plate 3.3 a) and immediately packed in a pneumatic sealer (Make: Sevana, India; Model: QS300PNI) prior to processing. The sealed jackfruit samples were placed in the retort trays and loaded inside the machine for processing. The thermal processing was initiated after achieving a steam boiler pressure of 2 bars and closing the retort door and pressure valves in the process chamber, so that the internal pressure can be maintained within the chamber. Immediately after thermal processing, the cooling process was carried out two times by passing normal water to the process chamber along with a blast of compressed air to

avoid the rapid pressure difference. Upon the completion of the cooling cycle, processed samples were unloaded and preserved for storage. The retort pouch pasteurisation and sterilisation conditions applied for the safe preservation of RJB and RJP are detailed in Table. 3.1

Table 3.1 Thermal processing of ripe jackfruit samples

Sl. No.	Retort pouch Processing	Process variables	Range
1.	Pasteurisation	Pasteurisation Temperature	75-95ºC
		Pasteurisation Time	5-15 min
2.	Sterilisation	Sterilisation Temperature	105-120ºC
		Sterilisation Time	5-15 min

(a) **(b)** **(c)**

Plate 3.3 Air exhausting, thermocouple insertion and retorting in steam air retort

Copper-constantan thermocouples were used for monitoring the internal temperature and external temperature of the pouch during heat processing Plate 3.3 b). Thermal process conditions of ripe jackfruit were fixed by conducting preliminary study. The processed samples were analysed for their quality characteristics and shelf life for standardisation and better preservation of ripe jackfruit samples. The detailed flow chart for retort pouch processing of ripe jackfruit is given below in Fig.3.1

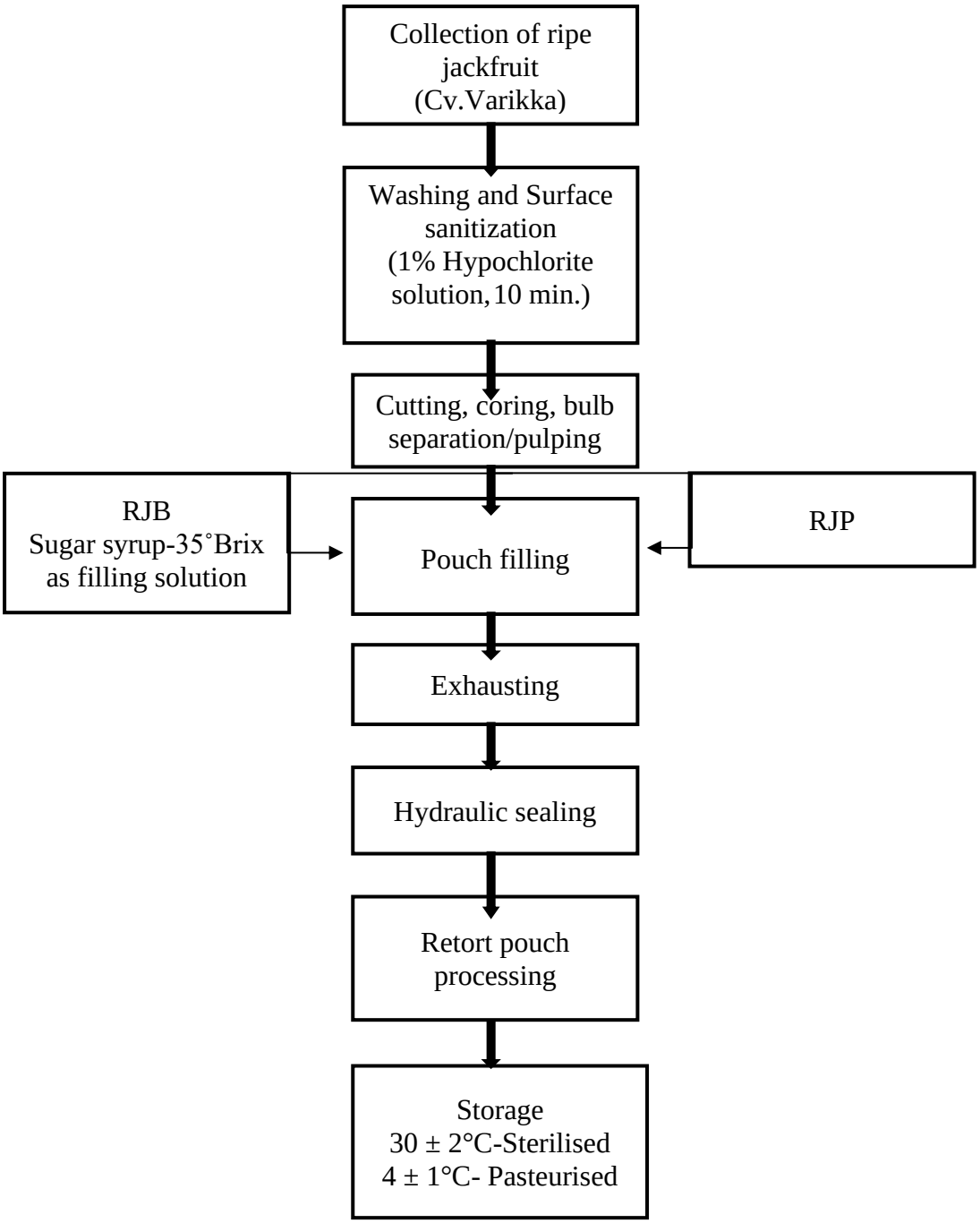


Fig.3.1 Flow chart for retort pouch processing of ripe jackfruit

3.2.1 Experiment design

In this study, the effect of time (min.) and process temperature (ºC) were investigated in relation to the quality and shelf-life extension of ripe jackfruit using a face-centered central composite response surface analysis. The Central Composite

Design (CCD) is an experimental approach used to fit a second-order response surface, drawing inspiration from the structure of balanced incomplete block designs. For the experimental plan, two independent variables: temperature and process time were encoded. Each independent parameter was set at a level based on limitations associated with the sample and the equipment. The experimental data were adjusted to fit a polynomial response surface.

Table 3.2 Coded and un-coded values of process factors in CCD design for retort pouch processed ripe jackfruit

Retort pouch processing	Independent variables	Levels in coded form				
		-1	0	+1	-1.414	+1.414
Pasteurisation	Temperature (°C)	75	85	95	70.85	99.14
	Time (min.)	5	15	25	0.86	29.14
Sterilisation	Temperature (°C)	105	112.50	120	101.89	123.19
	Time (min.)	5	10	15	2.92	17.07

3.2.2 Quality analysis of retort pouch processed ripe jackfruit

The physicochemical characteristics like pH, TSS, titrable acidity (TA), colour characteristics, Ascorbic acid content (AA), Total Phenolic compounds (TPC), Total Flavanoid Compound (TFC), DPPH radical scavenging activity, total sugar, texture, rheological characteristics, microbial activity and sensory analysis were analysed for ripe jackfruit after retort pouch processing are detailed below.

3.2.2.1 pH

A digital pH meter (Model: ECPHTUTOR-S; Make: R-Initiative Enterprises, Faridabad, Haryana) was used to determine the pH of the processed jackfruit samples (AOAC, 2000). The equipment was calibrated with distilled water and buffer solutions of pH 4, 7, and 9. The sensor probe was immersed in the samples to measure the pH values. Prior to testing, RJB samples were ground into a paste using a pestle and mortar.

Each experiment was conducted in triplicate, and the average pH along with the standard deviation value was recorded.

3.2.2.2 Titrable acidity (TA)

The total acidity of treated jackfruit samples was assessed following the AOAC (2000). To prepare the sample, 5 g of treated ripe jackfruit bulbs were ground into a fine paste and mixed with 100 mL of distilled water. After adding a few drops of phenolphthalein indicator, the mixture was shaken thoroughly. A burette was then filled with 0.1 N NaOH, and the sample solution was titrated against the NaOH until the colour changed to pale pink, which was maintained for 30 seconds. The calculation for TA is provided in Eq 3.1:

TA (% malic acid) =

$$\frac{\text{Volume of titrant (ml)x Normality of titrant} \times 0.067 \text{ x } 100}{\text{Sample weight(ml)}} \quad \ldots (3.1)$$

Where 0.067 is the milliequivalent of malic acid.

3.2.2.3 TSS

The TSS content of ripe jackfruit samples was measured using a digital handheld refractometer (model: BX-1, KEM, Japan), with results expressed in ^{o}Brix at room temperature, following the Abrol and Joshi (2011). To conduct the TSS measurement, jackfruit pulp was placed in the measuring port of the refractometer, and the displayed value was recorded, as referenced by Saranya *et al*. (2024). For improved accuracy, the readings were taken three times.

3.2.2.4 Texture

Two-cycle texture profile analysis (TPA) tests were performed using the EZ-SX500N model from Stable Micro Systems Ltd., UK on jackfruit bulbs that had undergone thermal and non-thermal processing. In this analysis, the firmness of the RJB was measured at a constant speed of 0.5 mm/s, utilizing a 60 mm cutting probe, as outlined by Wu *et al*. (2021). During the compression process, the maximum force exerted (Newtons) was recorded, which served as an indicator of the firmness of the

samples. This method provides a quantitative assessment of the textural properties of jackfruit, allowing for a better understanding of how retort pouch processing conditions affect the firmness and overall texture of the fruit.

3.2.2.5 Colour characteristics

Visual colour characteristics were measured using a Hunter lab colour flux meter (MiniScan EZ 4510 LAV, Hunter Associates Laboratory, USA) which provides colour values in the terms of L*, a*, and b* values, where L* indicates whiteness to darkness, a* (+) redness, a* (-) greenness, b* (+) yellowness and b* (-) blueness. The working principle of the instrument is to focus the light on the samples and measure the energy reflected from the samples across the entire spectrum. The instrument was initially calibrated and the ripe jackfruit samples were placed in the transparent cup with as minimum void space as possible. Based on the colour co-ordinates, the Yellowness Index (YI) which indicates the degree of yellowness of the sample was calculated (Pathare *et al.*, 2013).

The colour of the RJB and RJP may vary from its fresh colour after processing due to the enzymatic or non-enzymatic process. According to Eq. (3.2), the deviation of colour from the fresh control sample to the processed jackfruit was analysed and indicated as the total colour difference (ΔE) of the samples.

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad \text{... (3.2)}$$

where, L^*, a^* and b^* represents the colour value of the analysed sample and L_0, a_0, and b_0 indicates the colour value of fresh RJB/RJP

The yellowness index (YI) represents the variation of colour to yellow and is given by the Eq. (3.3) (Kaushik *et al.*, 2014)

$$YI = 142.86\ b^*/L^* \quad \text{... (3.3)}$$

The Browning index (BI) of the jackfruit was studied and calculated as per Eq. (3.4) given below (Sreedevi *et al.*, 2021).

$$BI = \frac{180.232(a^* + 1.75L)}{(5.645L^* + a^* + 3.012b^*)} \quad \text{… (3.4)}$$

3.2.2.6 Ascorbic acid content (AA)

The AA of the sample was measured using a titration of the 2,6-dichlorophenolindophenol dye with AA according to its ability to reduce the dye to colourless leuco-base using the titration method described earlier by Lu *et al*. (2018) and AOAC (1990).

Treated ripe jackfruit bulbs were ground to fine pulp and 10 mL of the homogenized pulp was made up to 100 mL with 4% oxalic acid solution. After 15 min., the extract was filtered out using a Whatman filter paper No.1 for further studies.

The dye solution used for titration was a mixture of sodium bicarbonate and 2, 6 dichlorophenol indophenols. During the analysis, 42 mg of sodium bicarbonate and 52 mg of 2, 6 dichlorophenol indophenols were diluted with distilled water and made up to 200 mL. A stock standard solution is prepared by dissolving 100 mg of ascorbic acid in 100 mL of a 4% oxalic acid solution. The 10 mL of the standard solution was taken in the standard flask and made up to 100 mL using 4% oxalic acid to make a working standard solution. The 10 mL of working standard solution was pipetted out into a 50 mL conical flask and 10 mL of 4% oxalic acid was added to it and titrated against the dye, to find out the dye factor. The endpoint is the appearance of pink colour which lasts for a few mins. The titration is repeated to obtain concordant values. The amount of AA present in the working standard solution is given by the amount of dye consumed (V_1). The 5 mL of extracted jackfruit sample was taken in a standard flask along with 10 ml of 4% oxalic acid and titrated against dye. Take 5 mL of this sample and add 10 ml of 4% oxalic acid to it. Titrate this against dye to find out the AA content in the sample. The following Eq 3.5 is used for calculation.

$$\text{AA content (mg/100 ml)} = \frac{0.5\text{ mg}}{\text{V1 mL}} \text{ x } \frac{\text{V2}}{5\text{ mL}} \text{ x } \frac{100\text{ mL}}{\text{Volume of sample}} \text{ x100} \qquad \ldots (3.5)$$

V1 - Amount of dye consumed by AA in the working standard ml.

V2 - Amount of dye used up by the jackfruit sample, ml.

3.2.2.7 Total sugar

The total sugar content of processed RJB and RJP was quantified using the method outlined by Ranganna (1986). To begin, a 5 g portion of the processed jackfruit

sample, encompassing both the RJB and RJP, was measured and transferred into a 250 mL standard flask. The sample was then diluted to the 250 ml mark by adding 45% neutral lead acetate and 22% potassium oxalate (2 mL of each). After allowing the mixture to stand for 10 min. to facilitate the precipitation of impurities, the solution was filtered to obtain a clear filtrate. Next, 50 ml of the filtrate was combined with a solution of 5 g of citric acid dissolved in 50 ml of water and brought to a boil. Once the boiling was complete, the mixture was cooled, and a drop of phenolphthalein indicator was added. The solution was then neutralized with 1 N sodium hydroxide until a light pink colour appeared, and the volume was adjusted to 250 mL using distilled water, resulting in the prepared titration solution. To determine the total sugar content, the prepared solution was transferred to a burette, and 5 ml each of Fehling's solution A and Fehling's solution B were pipetted into a conical flask. The burette solution was titrated against Fehling's solution in the flask, using methylene blue as an indicator, until a brick red colour persisted. The total sugar content was then calculated and expressed as a percentage of the original sample weight from Eq 3.6.

$$\text{Total sugar (\%)} = \frac{\text{Fehling's factor x 250 x dilution x 100}}{\text{Titer value x 50 x weight of the sample}} \quad \ldots (3.6)$$

3.2.2.8 Total Phenolic Compounds (TPC) and Total Flavonoid Compounds (TFC)

The treated jackfruit samples were tested for TPC with the Folin-Ciocalteu reagent (FCR) proposed by Kaushik *et al*. (2014). In 1.5 mL microcentrifuge tubes, 100 µL of methanolic extract from ripe jackfruit, 100 µL of MeOH, 100 µL of Folin-Ciocalteu reagent (FC), and 700 µL of Na_2CO_3 were mixed together and vortexed. The tubes were then kept in the dark for 20 min. at room temperature. Following this, the samples were centrifuged at 13,000 rpm for 3 min. using an Eppendorf Centrifuge 5417R (Germany). The absorbance was measured at 760 nm, with aqueous gallic acid (10–400 mg/L) used as a standard reference. The results were reported as mg of gallic acid equivalents per 100 g of dry sample weight, determined by constructing a gallic acid calibration curve.

A colourimetric assay method similar to that described by Saranya *et al*. (2024) was used to estimate flavonoids in retort pouch processed jackfruit samples. As per the procedure, sodium nitrate solution (0.3 mL) was added to the crude extract of retort pouch processed ripe jackfruit samples (10 mL) and allowed to stand for 5 min. Aluminium chloride solution (0.3 mL) was added to this mixture and it was then left for six mins before adding sodium hydroxide (2 mL) to it. The solution thus formed was made up to 10 ml with distilled water and was used to measure the absorbance at 510 nm. The TFC of processed ripe jackfruit samples thus obtained was expressed in mg rutin equivalents/g of fresh sample.

3.2.2.9 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant capacity of processed ripe jackfruits was assessed using the DPPH assay in terms of DPPH radical scavenging activity. To assess the DPPH radical scavenging activity of retort-pouch-processed ripe jackfruit samples, a 0.1 mM DPPH solution was created using methanol, and an extract of the ripe jackfruit pulp was prepared by homogenizing the pulp in methanol at a concentration of 10 mg/mL, followed by filtration to remove any solid particles. The resulting extract was then diluted to various concentrations ranging from 1 to 10 mg/mL. For the assay, 1 mL of each dilution was combined with 1 mL of the DPPH solution, alongside control samples that contained only DPPH and a known antioxidant for reference. The mixtures were allowed to incubate in the dark at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a spectrophotometer (Jayachandran *et al*., 2015). All measurements were conducted in triplicate to ensure accuracy. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = (A_{control} - A_{sample})/ A_{control} \times 100 \quad \ldots (3.7)$$

where $A_{control}$ is the absorbance of the control (DPPH solution without extract) and A_{sample} is the absorbance of the sample mixture.

3.2.2.10 Rheological properties

Rheological properties of fresh, retort-processed, ripe jackfruit samples were evaluated using an MCR 72 rheometer (Anton Paar GmbH, Graz, Austria) with a concentric cylinder system (CC39). The bob had a length of 60.010 mm, a diameter of

38.722 mm, and a cup diameter was 42 mm. Measurements were conducted at a constant temperature of 30°C (±0.1°C). Shear stress was recorded at increasing shear rates from 0.1 to 400 s^{-1}, collecting data points that were analyzed for viscosity using Rheoplus software. All measurements were performed in triplicate (Maria *et al*., 2015.) The viscosity-shear rate relationship can be modeled using the Ostwald-de Waele power-law equation:

$$\eta = k\dot{\gamma}^{(n-1)} \quad \text{……. (3.8)}$$

where:

- η = viscosity (Pa.s)
- $\dot{\gamma}$ = shear rate (1/s)
- k = consistency coefficient
- n = flow behavior index (n < 1 indicates shear-thinning behavior)

3.2.2.11 Microbial analysis

The microbial quality analysis of thermally processed ripe jackfruit was estimated based on the procedure followed by Pritty and Sudheer (2020). The total aerobic mesophiles (TAM) and total yeast and mold populations in the processed samples were analysed by standard procedures. Initially, all glassware and media were sterilized in an autoclave at 121°C for 15 min. to eliminate any microbial contamination. Nutrient Agar was prepared for the TAM, while Potato Dextrose Agar (PDA) was formulated for yeast and mold count by mixing 200 grams of potato infusion, 20 g of dextrose, and 20 g of agar with distilled water to a total volume of one liter. The thermally processed jackfruit samples were then serially diluted in sterile saline or distilled water up to 10^{-8} dilutions. Following this, 1 mL from each dilution was inoculated into sterile Petri dishes containing the prepared media in a sterile environment. The plates for TAM were incubated at 35°C for 24 to 48 hours, whereas the PDA plates for yeast and mould were incubated at 25°C for three to five days. After incubation, colonies were counted, and the number of colony-forming units (CFU) per g of sample (Ns) was calculated using the following formula:

$$Ns = \frac{Ncfu \times DF}{Ws} \quad \ldots (3.9)$$

Where,

Ws: weight of the sample; DF: dilution factor; $N_{cfu:}$ number of colony-forming units

After incubation, colonies were enumerated, and the microbial reduction in log CFU/g was calculated using the following formula:

$$\text{Log reduction} = \log N_0 - \log N_t \quad \ldots. (3.10)$$

where:

- N_0 = Initial microbial count before processing (CFU/g)
- N_t = Microbial count after retort pouch processing (CFU/g)

The results were expressed as a total reduction in log CFU/g, indicating the effectiveness of retort pouch processing in microbial inactivation

3.2.2.12 Sensory evaluation

Sensory evaluation of the retort pouch processed ripe jackfruit samples was conducted to assess organoleptic characteristics such as colour, flavour, appearance, texture, and overall acceptability, as outlined by Ranganna (1986). A semi-trained panel consisting of 21 members, including faculty and research scholars from the Department of Agricultural Engineering at the College of Agriculture, Vellanikkara, carried out the evaluation. The panel used a nine-point Hedonic scale for the sensory assessment, with the scorecard model provided in Appendix B1. Fresh RJB and RJP were included as the control during sensory analysis for comparative study. The mean scores from the scorecards were analyzed to determine the most acceptable product.The sensory score cared used for the analysis was given in Appendix

3.2.3 Modelling and optimisation

The optimisation of process parameters was done using Design Expert Software version 12. CCD-based RSM and regression analysis were done to optimize the parameters to achieve desired goals in retort processing (Chhabra and Deswal, 2020). The effect of the process parameters on the various quality attributes was analysed. The responses obtained from the experimental runs of CCD were modelled by a second-order polynomial equation, as follows.

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} b_{ij} X_i X_j \quad \ldots (3.11)$$

Where, *Y*: The predicted response variable, b_0: The intercept term, b_i: The coefficients for the linear terms, representing the effect of each independent variable on the response, b_{ii}: The coefficients for the quadratic terms, indicating how the response changes with the square of each independent variable, b_{ij}: The coefficients for the interaction terms, showing how the effect of one independent variable on the response depends on another variable

3.2.4 Statistical Analysis

The statistical data was analysed for ANOVA using Design Expert Software version 12. The p-values were used as a tool to check the significance of each of the coefficients, which, in turn, were necessary to understand the pattern of the mutual interactions between the test variables.

After conducting an ANOVA test to determine the statistical significance of each term in the polynomial model, the non-significant terms were deleted from the model and a new ANOVA test was conducted with a Design expert. It would allow for a more accurate determination of coefficients in the final equation. The data analysis of non-significant terms was performed using IBM SPSS Statistics© v.23.0. In the present study, optimisation was performed with significant terms to obtain the best treatment with superlative physicochemical properties. Afterwards, a one-way ANOVA of control to optimised values was performed. Ducan's test was applied to ascertain the range of values in which the differences were located.

3.2.5 Cost estimation

The total cost involved in the production of retort pouch processed ripe jackfruit was estimated using a standard procedure with suitable assumptions (Appendix G1 and G2).

3.2.6 Storage studies

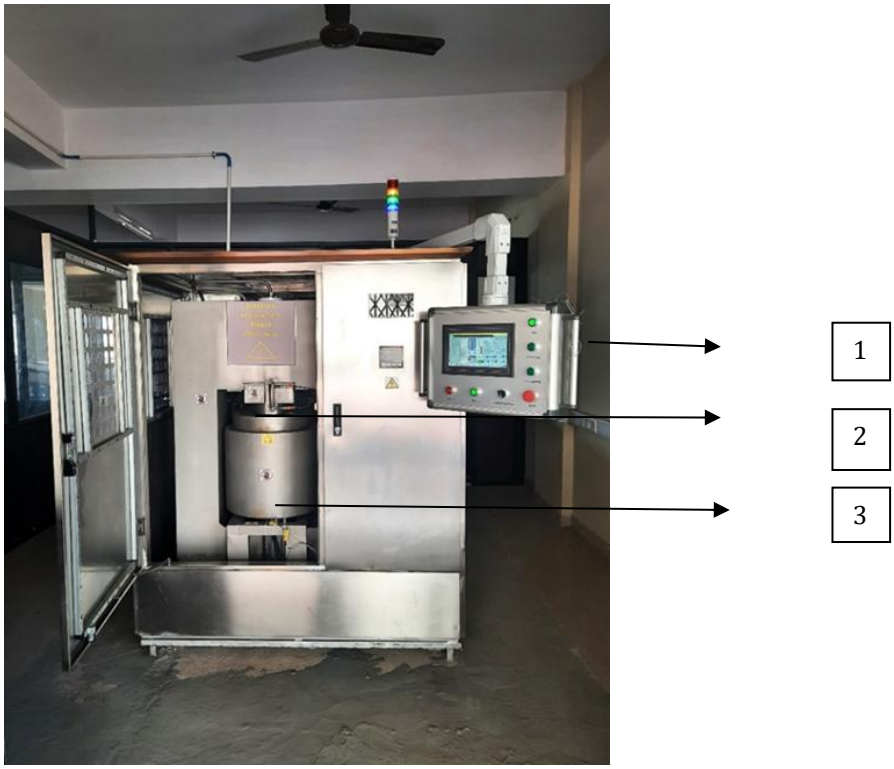
The shelf-life stability of the retort pasteurised ripe jackfruit samples was analysed at refrigerated storage conditions and retort sterilised samples were stored under ambient conditions. The best and most optimized treatment samples based on the sensory and quality evaluation were stored for shelf-life study (Chandan *et al.*, 2021). The changes in the samples' physicochemical attributes such as pH, TA, TSS, colour deviation, texture, AA content, TPC, total sugar and microbial analysis were analysed during the storage period at regular intervals. All the experiments were performed in triplicate and the mean values were taken for analysis.

EXPERIMENT II:

3.3 STANDARDISATION OF HPP PARAMETERS FOR RJB AND RJP

3.3.1 HPP system

High pressure was achieved with a batch-type HPP system (Make: KK Life science, India; Model: HPP-TE) available at College of Food Processing Technology & Bio-Energy, Anand Agricultural University, Gujarat.



1. PLC panel 2. Hydraulic piston 3. Pressure Vessel

Plate.3.4 Batch type HPP system

The pre-packaged fruit samples were treated in a chamber (Plate 3.3) surrounded by water or pressure-transmitting fluid. The main components of the system comprise a 3 L capacity pressure vessel, hydraulic piston, water storage tank, pressure valves, and a Programmable Logic Controller (PLC) unit. The equipment is fully automatic and operates within a temperature range of 30 to 80°C and maximum pressure of 600 MPa. The PLC system is the main control unit of the equipment where process inputs are entered and displayed during HPP. Perforated baskets aid in handling pre-packaged food in pressure vessel and allows filtering and reuse of compression fluid.

3.3.2 High pressure processing of RJB and RJP

Preliminary trials on HPP of ripe jackfruit were conducted prior to research trials. After fixing the treatments the vacuum-sealed jackfruit bulbs and pulp were subjected to high pressure treatment (300-600 MPa) for 10-15 min. at ambient temperature. The RJB samples were vacuum packed using vacuum packaging machine (Model:SC2, Make: Indvac ltd, Gujarat, India) and RJP samples were tightly packed or air-tight packaging was done in LPP plain transparent laminated stand pouches with overall migration of less than 10 mg/L. Followed by packing, samples were loaded into the perforated baskets inside the pressure vessel. After ensuring that the samples were closely packed process factors were keyed and the start command was given in the PLC display. Subsequently, the vessel was sealed, and pressure-transmitting fluid was pumped into the pressure vessel to displace the trapped air. Once filled, the pressure relief valve was closed, and the hydraulic piston moved downwards to pressurise the samples. Concurrently water continued to be pumped until reaching the desired process pressure.

The pressure relief valve was opened after the processing time, allowing the compression water to expand and return to atmospheric pressure. The hydraulic piston moves upwards and the pressure transmitting fluid re-enters the storage tank. During the experimental run, the compression led to an average temperature rise of 3 ± 0.5°C per 100 MPa increase in pressure, owing to adiabatic heating (Elamin *et al*., 2015). Upon completion of the HPP treatment, the samples were immediately refrigerated for further analysis. All samples were processed and analysed in triplicate for accuracy.

3.3.3 Experimental Design

The CCD of the Design Expert software was used for deciding the number of experiments and the combinations of independent variables. The experimental plan consisted of three levels of two independent variables, pressure (P) and holding time (Ht), which were encoded for detailed statistical analysis (Table 3.3). The levels of pressure for applying HPP to ripe jackfruit were set within the high pressure system's allowable limit, and based on previous studies. The regression analysis predicted by the Design Expert gave a model equation of the interaction of independent variables in the process.

Table 3.3 Experimental design for HPP ripe jackfruit

Factor	Independent variables	Units	Coded Low	Coded High
P	Pressure	MPa	-1 ↔ 300.00	+1 ↔ 600.00
Ht	Holding time	min	-1 ↔ 5.00	+1 ↔ 20.00

3.3.4 Quality analysis of HPP ripe jackfruit

3.3.4.1 Estimation of physicochemical characteristics

The physicochemical characteristics like pH, TSS, TA, AA content, total sugar, colour characteristics, TPC, TFC, DPPH radical scavenging activity, texture, rheological property, sensory evaluation and microbial analysis of HPP processed RJB and RJP etc were analysed for ripe jackfruit after HPP as detailed previously (section 3.12-3.14).

3.3.5 Statistical Analysis

The statistical analysis was conducted using Design Expert Software version v.7 for ANOVA to evaluate the significance of each coefficient as explained in section 3.2.4.

3.3.6 Process modelling and optimisation

Process parameter optimisation was carried out using Design expert software version v.7. A CCD approach based on RSM and regression analysis was employed to optimize the parameters for achieving desired outcomes in HPP as explained in section 3.2.3

3.3.7 Cost estimation

The total cost involved in the production of HP- processed ripe jackfruit was estimated using a standard procedure with suitable assumptions (Appendix G3 and G4).

3.3.8 Storage studies

The HPP ripe jackfruit samples were stored under refrigerated conditions, with quality analyses conducted at 10-day intervals. The samples selected for the shelf life study were those identified as the best and most optimized based on sensory and quality evaluations (Chandan *et al*., 2021). During the storage period, changes in physicochemical attributes, including pH, TA, TSS, colour deviation, texture, AA content, TPC, total sugar, and microbial load, were monitored as mentioned in section 3.2.21-3.2.2.12. All experiments were performed in triplicate, and the mean values were used for analysis.

EXPERIMENT III:

3.4 STANDARDISATION OF PL TECHNOLOGY FOR RJP

The PL treatment for RJP was carried out using a benchtop laboratory scale high-intensity PL system (Model: Xenon X-1100, Xenon, Wilmington, MA, USA). The PL system comprises a controller unit with a touchscreen operator display, a treatment chamber, and a blower. The treatments were performed batch-wise in an air-cooled treatment chamber where the lamp housing was positioned over the top of the sample tray. This PL machine offers several optional lamps (viz, UV-A, UV-B, and UV-C) mounted in air-cooled sealed housings. The triggered transformer present in the controller supplies the required energy for initiating the pulses. High-intensity noncollimated white light (240-1,100 nm) with a maximum voltage of 3 Kv could be produced by the linear xenon flash lamp (xenon flash lamp model LH-840, Ø 1.9 ×30.5 cm, UV-C, mercury-free). The lamp overheating is controlled by an air blower connected to the quartz window at the lamp housing. The distance of the sample from the lamp housing can be adjusted by shifting the sample tray inside the treatment chamber. The touchscreen-based graphical user interface (GUI) enables to input of the pulse parameters or program recipes such as voltage, pulse duration, energy, pulse number, and sequencing. The maximum and peak energies of each program recipe are

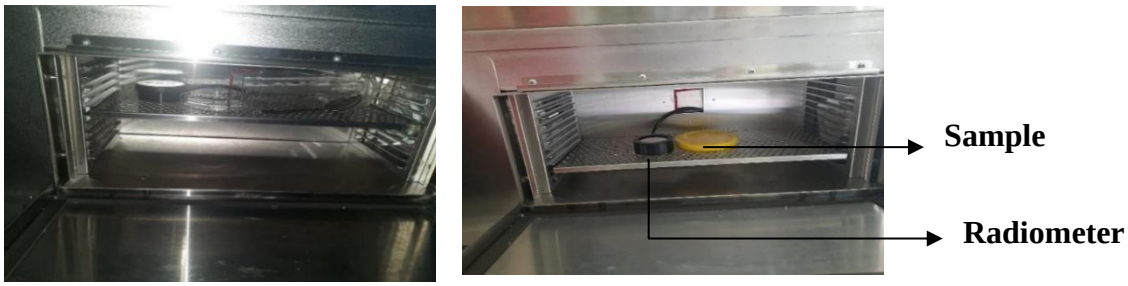
automatically calculated and displayed on the screen after treatment. The results of each input can be saved to the system for further studies (Vollmer *et al*., 2020).

3.4.1 PL processing

In the present study, the PL treatment of RJP was carried out according to the methodology described by Vollmer *et al*. (2020) with slight modifications. The factors considered for PL treatment of RJP consist of input voltage, pulse number, sample depth, distance from the sample to the light source and sample concentration (%). Preliminary trials were conducted to fix the treatment trials and 100% pulp and 1 mm sample thickness were standardised for the final treatment which gave the best colour, flavour and quality retention after treatment and storage. For each treatment, the ripe jackfruit pulp (100 g) was dispensed into Petri dishes (100 mm diameter) so that the entire dish surface was covered with the sample to a pre-set depth (1-5 mm). During the study, the sample was positioned at different perpendicular distances (4-10 cm) from the lamp source. PL treatment of RJP was carried out at varying voltage levels from 1 to 2.5 kV. The wave period was 950 ms, with a frequency of 1 Hz. The average fluence per pulse was determined using a radiometer (Model: PE-50, Ophir Optronics Solutions Ltd., Israel), which was positioned alongside the sample at varying distances from the light source (Plate 3.5b) and total fluency and fluency rate were calculated from the equation (3.10 and 3.11) by considering total number of pulses as treatment time in seconds. Aqueous ethanol (80% v/v) was used to disinfect the surfaces of the PL equipment and petri dishes before each treatment. Additionally, all utensils and labware were pulsed (3 kV/20 pulses) in the system for better surface sterilisation (Plate 3.5a) and hand gloves were sprayed with ethanol (80% v/v) prior to transferring sample from Petri dishes to PET bottles to avoid cross contamination. Immediately after processing, the samples were transferred to sterilised PP bottles and stored in refrigerated condition for further shelf-life study. The process optimisation was carried out by CCD with the aid of Design expert software version-V.7.

Total fluence ($J \cdot cm^{-2}$) = average fluence per pulse × number of pulses …(3.12)

Fluence rate ($W \cdot cm^{-2}$) = total fluence/treatment time … (3.13)



(a) **(b)**

Plate 3.5 Surface sterilisation of glassware and PL processing of RJP respectively

3.4.2 Experimental design

A face-centered central composite response surface analysis was used to determine the effect of voltage (kV), pulse number, and lamp to sample distance (cm) on the quality characteristics of PL-treated jackfruit pulp. The RSM with Box-Behnken design was carried out to test several variables using a limited number of trials, revealing interactions between the variables. The levels for each independent parameter were chosen considering sample and equipment limitations. Three (maximum, minimum and central) values of each factor were considered, leading to 17 experiments (Table 3.4). The experimental design was performed twice, resulting in one block of experiments. Experimental data were fitted to a polynomial response surface.

After conducting an ANOVA test to determine the statistical significance of each term in the polynomial model, the non-significant terms were deleted from the model and a new ANOVA test was conducted with a Design expert. It would allow for a more accurate determination of coefficients in the final equation. The data analysis of non-significant terms was performed using IBM SPSS Statistics© v.23.0. In the present study, optimisation was performed with significant terms to obtain the best treatment with superlative physicochemical properties. Afterwards, a one-way ANOVA of control to optimised values was performed to identify the range of values showing the differences.

Table 3.4 Experimental design for PL processed RJP

Treatment No.	Voltage (kV)	Pulse number	Lamp to sample distance (cm)
1	1.5	50	7
2	2.5	50	7
3	1.5	200	7
4	2.5	200	7
5	1.5	125	4
6	2.5	125	4
7	1.5	125	10
8	2.5	125	10
9	2	50	4
10	2	200	4
11	2	50	10
12	2	200	10
13	2	125	7
14	2	125	7
15	2	125	7
16	2	125	7
17	2	125	7

3.4.3 Quality analysis of PL processed RJP

The physicochemical characteristics like pH, TSS, TA, AA content, total sugar, colour characteristics, TPC, TFC, DPPH scavenging activity, texture, rheological property, sensory evaluation and microbial analysis were analysed for RJP after PL processing by standard procedures discussed under the section section:3.2.2.1-3.2.2.12. The statistical data was analysed for ANOVA using Design Expert Software version 12.

3.4.4 Process modelling and optimisation

The optimisation of process parameters was done using Design Expert Software version 12. Box Behnken design-based RSM and regression analysis was done to optimize the parameters to achieve desired goals for PL processing of ripe jackfruit pulp. The effect of the process parameters on the various quality attributes was analysed. The responses obtained from the experimental runs of BBD were modelled by a second-order polynomial equation, as follows.

$$Y = b_0+b_1A+b_2B+b_3C+b_4D+b_{11}A^2+b_{22}B^2+b_{33}C^2+b_{44}D^2+ b_{12}AB+b_{13}AC+b_{14}AD+b_{23}BC+b_{24}BD+b_{34}CD \quad ...(3.14)$$

where Y is the predicted response, A, B, C and D are the coded independent variables, b_0 is the intercept term, b_1, b_2, b_3, and b_4 are the linear coefficients, b_{11}, b_{22}, b_{33}, and b_{44} are the quadratic coefficients and b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, and b_{34} are the interactive coefficients

3.4.5 Cost estimation

The total cost involved in the production of PL processed ripe jackfruit was estimated using a standard procedure with suitable assumptions (Appendix G5).

3.4.6 Storage studies

The shelf-life stability of PL ripe jackfruit samples was evaluated under refrigerated storage conditions, with assessments conducted at 10-day intervals. The samples selected for this study represented the most optimized treatments, determined through sensory and quality evaluations (Chandan *et al*., 2021). Physicochemical parameters such as pH, TA, TSS, colour deviation, texture, AA content, TPC, total sugar, microbial load and sensory analysis were monitored using standard analytical procedures (section: 3.2.2.1-3.2.2.12). All experiments were conducted in triplicate, and the mean values were used for data analysis.