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Research Article

Enzymatic Extraction of Sapodilla (*Manilkara achras* L.) Juice: Process Optimization and Characterization

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Conventional treatment of sapodilla pulp yields very viscous, turbid, and low juice recovery. Sapodilla processing for juice requires liquefying enzyme that leads to rectifying flow of juice. This study was conducted to optimize the enzymatic pectolytic conditions of sapodilla fruit processing to extract maximum juice using a central composite design (CCD). The effect of processing variables on recovery of juice, total soluble solids (TSS), viscosity, clarity, and L-value along with physicochemical analysis was investigated. The optimized processing conditions were pectinase concentration (0.120%) at 42.02°C for 167.83 min resulting in juice recovery (62.08 ± 0.38%), viscosity (4.81 ± 0.02cP), TSS (21.48 ± 0.19 °Brix), clarity (0.72 ± 0.05%T), and L-value (28.79 ± 0.96). Optimized sapodilla juice showed higher filterability (24.16 ± 1.04 min^{-1}), conductivity (69.46 ± 0.30 S/m), total phenolic content (35.86 ± 0.60 mg/100 mL), ascorbic acid (6.38 ± 0.58 mg/100 mL), moisture content (84.85 ± 0.21% WB), and titratable acidity (0.143 ± 0.0% citric acid) as compared to control sample (60.5 ± 1.80 min^{-1}, 30.43 ± 0.35 S/m, 30.68 ± 0.85 mg/100 mL, 4.64 ± 0.0 mg/100 mL, 83.69 ± 0.18%, and 0.130 ± 0.0%). Optimized sapodilla juice was lower in sedimentation index (0.73 ± 0.11%), turbidity (13.73 ± 1.10 NTU), ash (0.57 ± 0.031%), and β-carotene (0.173 ± 0.008 μg/100 mL) as compared to control sample (1.07 ± 0.02%, 79 ± 0.75 NTU, 0.65 ± 0.031%, and 0.306 ± 0.007 μg/100 mL). The flow behavior index (n) was closer to 1 in both juice samples, which indicated Newtonian-like flow behavior. Conclusively, sapodilla juice extraction at optimal condition (0.120% of pectinase concentration) and 42.02°C/167.83 min would be potentiated to the beverage industry. The use of pectinase might reduce membrane fouling and facilitates processing operation efficiently.

1. Introduction

The sapodilla (*Manilkara achras* L.) is a tropical and climacteric fruit crop of the Sapotaceae family. It is grown for highly scrumptious and nutritious fruit which is valued for its mellow and sweet pulp. The fruit pulp is characterized by a granular texture and a pleasant aroma and is appreciated mostly by the consumer [1]. Due to widely accepted flavor and aroma of sapodilla, it can create place in the market, either as sapodilla juice or as ready to serve (RTS) drink with other juices. India is considered to be the largest producer of the sapodilla in the world, and sapodilla plant prefers coastal climate for growing. Hence, it is extensively, commercially, and intensively cultivated in states like Gujarat, Karnataka, Tamil Nadu, and Maharashtra in India. Today, production of this crop has increased because of continuous fruiting throughout the year, resulting in fruit availability in the market. At present, the per unit area of production under this crop has increased, resulting in glut in the market during peak season. Ripened sapodilla fruit is very soft and has a

pulpy texture. It requires cautious handling during its postharvest life [2]. Additionally, sapodilla is highly perishable, having 4–7 days' shelf life when stored at ambient temperature [3], whereas, at a low temperature for a longer period, it is susceptible to chilling injury [4], because of which fruits need to be utilized as early as possible after harvesting. It is, therefore, necessary to convert the sapodilla fruit into processed and value-added products to avoid postharvest losses. Moreover, sapodilla juice contains many phytonutrients and can be exploited as a healthy beverage due to its multifunctional activity. In recent years, the increasing health issues have emphasized the need for functional beverages that impart health benefits beyond energy and essential phytonutrients. The sapodilla fruit contains a wide range of free sugars such as fructose, glucose, and sucrose, as well as other nutrients. Also, several health-beneficial phytonutrients present a part of saturated and unsaturated fatty acids (heptadecanoic, palmitoleic, linolenic, linoleic, oleic, etc) and polyamines [5–7]. Consequently, fruit juices have become a regular part of the diet of a large population globally. There is an increase in consumers' awareness about health, eating habits, and revolution in living standards, which has shifted consumer acceptance of dietetics and healthy and disease-preventive food with health benefits compared to the beverages (tea, coffee, and carbonated drinks) containing alkaloids [8]. On the other hand, most tropical fruits that form a very healthy part of our diet are full of dietary fibers, antioxidants, vitamins, and minerals [9]. Most of the consumers consume skin intact sapodilla after washing due to its thin peel. Suhasini et al. [10] also revealed that sapodilla peel is probably edible because it is richer in nutrition than its fruit pulp. Therefore, crushing the sapodilla fruits with peels will support the food and health diversification program by improving the nutraceutical properties of extracted fresh sapodilla juice. Pectinase infusion in fruit pulp is a potential, industrially suitable macerating, and eco-friendly tool for extraction, clarification, and stabilization of fruit juice. Acidic pectinases are often utilized in fruit juice production derived from fungal sources (*Aspergillus niger*) [11]. In the fruit juice processing industry, pectinolytic enzyme preparations are a mixture of different pectinases. However, based on the type of juice, pectinolytic enzymes treatments vary accordingly. As per the directives of the European Union (Directive 95/EC), to manufacture various juices, concentrates, and their products, the enzymes with proteolytic, amylolytic, and pectinolytic nature can be used [12]. Multiple studies have been carried out for different fruit juices production, namely, bael (*Aegle marmelos* Correa) juice, banana (*Musa acuminata*) juice, and pineapple (*Ananas comosus*) juice, and found that the use of the pectinase enzyme not only increases the yield but also improves the quality of the juice in terms of clarity, viscosity, and filterability [13–15]. The limited study on manufacturing high-quality sapodilla juice using enzymatic extraction could confer high-value addition to sapodilla of low quality (granular texture).

The objective of this work was to find out the effect of enzymatic treatment on sapodilla juice (juice recovery, TSS, clarity, viscosity, and L-value) during the processing and to optimize processing conditions. Thereafter, the effect of optimized enzyme concentration on physicochemical proprieties, functional characteristics, and rheological behavior of sapodilla juice was studied.

2. Materials and Methods

2.1. Material

2.1.1. Selection and Collection of Experimental Material. Sapodilla (*Manilkara achras* L.), "Cricket ball," was collected from the local market Azadpur Mandi, New Delhi. The bruised, damaged, or spoiled fruits were removed before pulping. Moreover, healthy and uniform ripened fruits were taken for pulping at optimal maturity index (TSS 20.62 ± 1.0 °Brix). In this regard, TSS of fruit is a quality index for juice extraction [5]. Random sampling of ripened fruits was done for °Brix determination.

2.1.2. Pectinase Enzyme. Food-grade pectinase enzyme (EC 3.2.1.15) [source: metabolites from *Aspergillus niger* having the GRAS (Generally Recognized As Safe) status] [16] was obtained from HiMedia Pvt Ltd. (Mumbai, India). The activity of the pectinase enzyme was 8000–12000 U/mg. The enzyme was employed as solution form prepared by adding amount of pectinase to 5 mL distilled water. The amount of pectinase of each experiment is given in Tables 1 and 2.

2.2. Method

2.2.1. Saline and Heat Treatment of Fruits. Skin intact sapodilla fruits were immersed in salt solution of 0.85% for 30 minutes after heat treatment at 50°C for 10 minutes to facilitate the disinfection of fruit surface as well as homogeneous blending. Khaleghi et al. [17] documented that a similar treatment for tomato has been used for disinfection.

2.2.2. Experimental Design for Pectolysis. A central composite design (CCD) was prepared using design expert software for optimizing the extraction conditions (process variables) with the combined effect of three independent factors, pectinase concentration (X_1), incubation temperature (X_2), and incubation time (X_3), on juice yield (Y_1), viscosity (Y_2), TSS (Y_3), clarity (Y_4), and L-value (Y_5) of sapodilla juice, respectively. The hydrolysis of pectic substances is affected by the type of enzyme and its concentration, hydrolysis time, incubation temperature, and pH [18]. They are process parameters that need to be optimized for maximal juice recovery. The conditions for optimum enzyme activity are at pH 3.5–6 and temperature below 50°C [12]. Each independent variable had 5 levels which are $-\alpha$, −1, 0, +1, and $+\alpha$, shown in Table 1. A total of 20 trials, including six similar center point replicates, were experimented in random order with respect to the six response variables, as shown in Table 2.

2.2.3. Enzymatic Treatment in Sapodilla Pulp. Sapodilla fruits with peels (TSS 20.62 ± 1.0 °Brix) were cut into pieces with a knife and ground. The grinding of cut fruits was done

Table 1: Obtained levels of processing variables in design.

Independent variables	Symbols		Coded levels				
	Actual	Coded	$-\alpha$	-1	0	$+1$	$+\alpha$
Pectinase concentration (%)	$X_1 x_1$		0.1	0.12027	0.15	0.17973	0.2
Incubation temp. (°C)	$X_2 x_2$		40	42.027	45.0	47.973	50.0
Incubation time (min)	$X_3 x_3$		120	132.16	150	167.838	180

Table 2: Statistical design with experimental values of responses.

Ex. no.	X_1 Pectinase concentration (%)	X_2 Incubation temperature (°C)	X_3 Incubation time (min)	Y_1 Juice yield (%)	Y_2 Viscosity (cP)	Y_3 TSS (°Brix)	Y_4 Clarity (%T)	Y_5 L-value
1	0.15	40	150	61.00 ± 1.00	4.82 ± 0.02	21.04 ± 0.03	0.653 ± 0.00	31.01 ± 0.02
2	0.17973	42.027	167.838	66.00 ± 0.57	4.73 ± 0.00	19.62 ± 0.02	1.000 ± 0.12	27.68 ± 0.11
3	0.15	45	180	65.34 ± 0.17	4.80 ± 0.05	20.25 ± 0.03	0.550 ± 0.18	26.09 ± 0.08
4	0.15	45	150	62.11 ± 0.05	4.87 ± 0.03	20.99 ± 0.13	0.204 ± 0.00	32.20 ± 0.17
5	0.17973	47.973	167.838	66.98 ± 0.49	4.70 ± 0.20	19.84 ± 0.03	1.390 ± 0.01	30.70 ± 0.37
6	0.15	45	150	62.11 ± 0.26	4.85 ± 0.02	21.60 ± 0.26	0.140 ± 0.02	28.34 ± 0.07
7	0.15	45	150	62.40 ± 0.34	4.86 ± 0.36	20.88 ± 0.21	0.204 ± 0.00	32.20 ± 0.25
8	0.12027	47.973	167.838	58.49 ± 0.50	4.79 ± 0.02	19.65 ± 0.06	0.125 ± 0.01	26.26 ± 0.06
9	0.15	45	120	59.40 ± 0.80	4.91 ± 0.02	20.75 ± 0.03	0.179 ± 0.01	25.96 ± 0.49
10	0.15	45	150	62.00 ± 0.68	4.87 ± 0.02	20.65 ± 0.05	0.204 ± 0.01	32.20 ± 0.41
11	0.12027	42.027	167.838	61.07 ± 1.62	4.87 ± 0.03	21.51 ± 0.10	0.735 ± 0.02	28.06 ± 0.30
12	0.1	45	150	53.00 ± 1.00	4.85 ± 0.01	21.84 ± 0.02	0.482 ± 0.02	25.04 ± 0.40
13	0.15	45	150	62.48 ± 1.99	4.84 ± 0.02	20.99 ± 0.33	0.204 ± 0.03	33.38 ± 0.05
14	0.17973	47.973	132.162	64.63 ± 0.84	4.73 ± 0.04	21.17 ± 0.01	0.980 ± 0.06	30.97 ± 0.84
15	0.17973	42.027	132.162	59.18 ± 1.00	4.75 ± 0.03	20.00 ± 0.02	0.340 ± 0.02	26.96 ± 1.18
16	0.12027	47.973	132.162	55.71 ± 0.67	4.82 ± 0.02	21.68 ± 0.01	0.180 ± 0.02	25.61 ± 0.26
17	0.15	50	150	63.73 ± 1.20	4.78 ± 0.02	20.91 ± 0.01	0.480 ± 0.09	26.84 ± 0.55
18	0.15	45	150	60.00 ± 1.73	4.86 ± 0.00	20.65 ± 0.08	0.168 ± 0.01	32.93 ± 0.79
19	0.12027	42.027	132.162	58.16 ± 0.92	4.92 ± 0.00	21.57 ± 0.00	0.707 ± 0.01	27.37 ± 1.06
20	0.2	45	150	66.00 ± 1.15	4.65 ± 0.03	19.85 ± 0.05	1.318 ± 0.01	34.47 ± 1.52

Experimental values of responses are indicated as mean ± SD.

with help of electric grinder (Hl1645, Philips, India) until homogenously fine pulp was obtained. Fifteen milligrams of potassium metabisulphite (KMS) per 100 g of crushed sapodilla pulp was added while blending for controlling browning reaction and microbial growth in fruit pulp. Similarly, KMS as a food-grade GRAS chemical, employed within permissible limits, was explored to prevent microbial spoilage and litchi pericarp browning by Kumar et al. [19]. 300 g of pulp was incubated with different pectinase (mix in 5 mL distilled water) concentrations at different incubation temperature for each experiment as given in Table 2. The applied temperature during pectolysis was controlled using a water bath (Kerone, Sanco, India). Maceration of sapodilla pulp was carried out with pectinase at its natural pH between 5 and 6 and temperature not beyond 50°C. The major steps of sapodilla juice production are represented in Figure 1.

2.2.4. Sapodilla Juice Extraction. At the end of pectinase-infused fruit pulp incubation, the enzyme treated pulp was pressed to separate the juice with two-layered muslin cloth [20]. The extracted juice from each experimental trial was subjected to 65°C/5 min for thermal inactivation of the infused enzyme using a water bath (Sanco Company, India). Thereafter, each sample was bottled and maintained at 7°C. Furthermore, experimentally derived samples were tested for the response parameters.

2.2.5. Estimation of Response Parameters. (1) Juice Yield. The volume of total juice (V_{total}) of each experimental juice sample before bottling was measured, and the volume of water added (5 mL) with the enzyme was subtracted. The juice recovery was calculated using the following formula:

$$\text{juice recovery}\,(\%) = \frac{(V_{\text{total}} - 5\,\text{ml})}{300\,\text{g of fruit pulp}} \times 100. \quad (1)$$

(2) Clarity and TSS. TSS and clarity of each juice sample without centrifugation were determined according to the methods reported by Singh et al. [20].

(3) Viscosity and Instrumental Color. The viscosity and color (L-value) of each experimental juice sample were analyzed by the Brookfield viscometer (DV-II + Pro, Brookfield Engineering Laboratory) and portable chroma meter (CR-400, Konica Minolta, Japan) illustrated by Singh et al. [20].

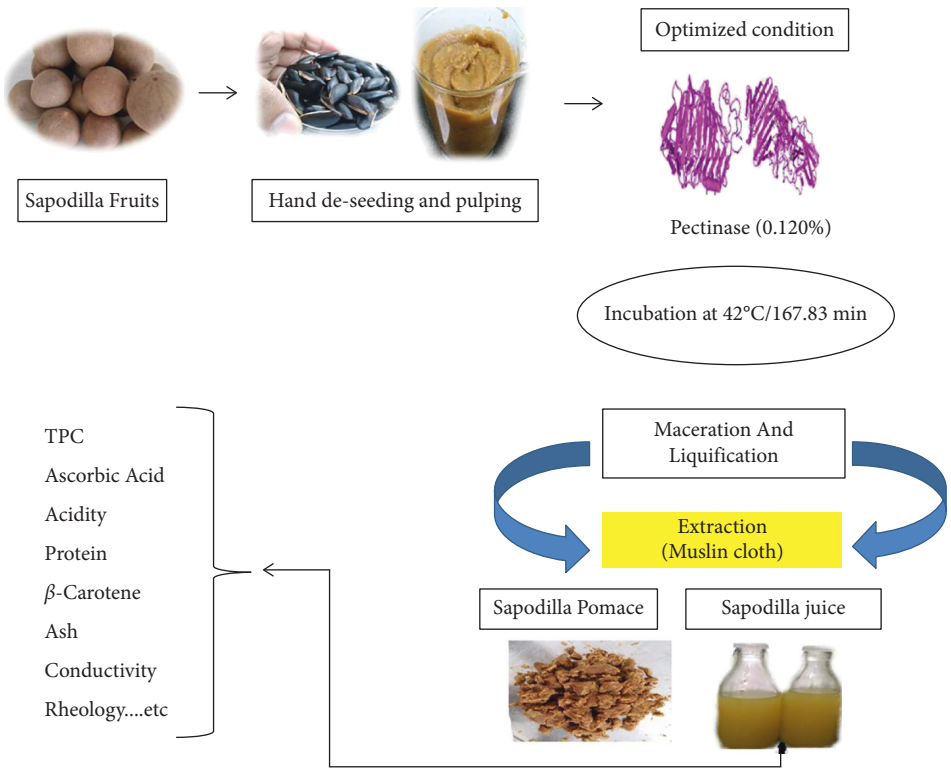


Figure 1: Experimental flow diagram adapted for the processing of sapodilla juice.

2.2.6. Statistical Analysis, Optimization, and Validation. All responses suggested a second-order polynomial model. They were expressed as a function of independent factors. The effects (linear, interactive, and quadratic) of independent factors on selected responses are in terms of the following equation as a polynomial model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2, \quad (2)$$

where Y is the response variable. The regression coefficients of polynomial terms were represented by β_0 (intercept); β_1, β_2, and β_3 (linear effects); β_{11}, β_{22}, and β_{33} (quadratic effects); and β_{12}, β_{13}, and β_{23} (interaction effects) [20, 21], and X_1, X_2, X_3 are the independent factors. The analysis of variance (ANOVA) generated by the software was used for the statistical difference of all polynomial models and their terms. The optimal formulation of sapodilla juice was achieved by setting up the goals for all response parameters. For the validation of the model, an experiment was repeated at optimum values of processing conditions, followed by obtained responses that were then compared with responses of predicted values. In the present study, a statistical analysis of triplicate observation was carried out using CCD. Significant differences among predicted, experimental, and control values and physicochemical characteristics of optimized juice and control juice were evaluated by the Duncan test (IBM SPSS Statistics 20).

2.2.7. Characterization of Optimized Juice and Control Juice

(1) Rheology. The rheological analysis was carried out using an MCR 52 controlled stress rheometer (Anton Paar, straBe 208054 Graz, Austria) comprised of coaxial cylinders (CC 27) with a diameter of 52 mm. The shear stress (Pascal) was measured with a linearly increasing shear rate (r) in the range of 1.0–1,000 s^{-1} for 10 min duration with 30 data points according to Newton's equation. The rheological measurements of both samples (optimized and control juice) were carried out at temperatures of 25 ± 1°C in triplicates. Additionally, the calculation of flow behavior index (n) and consistency index (k) was performed by experimental shear stress-shear rate data using the following power law model.

$$\tau = k.r^n, \quad (3)$$

where τ is the shear stress (in Pa), r is the shear rate (in s^{-1}) and the consistency and flow behavior index are k and n, respectively.

(2) Water Activity. The water activity of each sample was analyzed by precalibrated dew point water activity meter (Model-4 TE, Aqua Lab) at 25°C.

(3) Sedimentation Index (S.I.). The sedimentation index of samples was measured by centrifuging at 8000 rpm for 10 min (Model 3-18KS, Sigma, Germany). Centrifuge tube

with the suspended matter was placed in a hot air oven for drying at 40°C for 24 h. The dried tube was weighed using a digital balance (BSA224S-CW, Sartorius Company, Germany) at the end of drying. The S.I. was then calculated using the following equation:

$$\text{S.I.}\,(\%) = \frac{\text{total weight of dried suspended matter with tube} - \text{blank tube}}{\text{25 ml of juice sample}} \times 100. \quad (4)$$

(4) Turbidity Measurement. Turbidity of samples was measured using a microprocessor turbidimeter (S-966, Systonic Company, India), and reading was reported as Nephelometric Turbidity Units (NTU) with an accuracy of ±3%. The instrument was calibrated by mixture solution (5 mL and 5 mL) in 100 mL volumetric flask prepared from 1.0 g hydrazine sulphate and 10 g hexamethylenetetramine diluted in 100 mL volumetric flask separately.

(5) Filterability Measurement. The optimized sapodilla juice was filtered using a filter paper (Whatman No. 4, grade 4, pore size: 20–25 μm) using a filtration pump with a vacuum aspirator jar. Filterability (mL/min) was estimated from the time taken for filtering 300 mL extracted juice using vacuum filtration.

(6) Conductivity Measurement. The conductivity (electrolytic or specific conductance) of a solution estimates its ability to conduct electricity that depends upon the electrolyte concentration of the juice sample. The conductivity of juice was measured using a precalibrated water analyzer kit (kit-371, Systonic, India), and reading was reported as siemens per meter (S/m) with an accuracy of ±0.01.

(7) pH Measurement. pH values of both samples were estimated by using a digital pH meter (pH tutor, Eutech Instruments, CyberScan, India) at 25°C with an accuracy of 0.01. The instrument was calibrated using standard buffers solution provided by the company.

(8) Determination of Protein, Moisture, and Ash Content. The micro-Kjeldahl method was used to determine the protein percent of optimized and control sapodilla juice as per AOAC [22]. Both juice samples' moisture content was measured gravimetrically by drying the juice at 70°C for 20 h in a hot air oven [23]. The ash contents of both juice samples were analyzed gravimetrically by drying the juice in a hot air oven in a silica crucible, ignited in a hotplate and placed in muffle furnace at 550°C for 16 h [23].

(9) Determination of Ascorbic Acid (AA) Content. The ascorbic acid content in sapodilla juice was determined with 2,6-dichlorophenolindophenol (DCPIP) visual titration method reported by Ranganna [23].

(10) Determination of Titratable Acidity. The total titratable acidity of enzymatically extracted sapodilla juice was determined by AOAC method [24] and expressed as % citric acid of juice which was calculated using the following equation:

$$\text{total titratable acidity (as \% citric acid)} = \frac{\text{consumed volume of NaOH (titre value)} \times 0.1 \times 64 \times 100}{\text{volume of juice sample} \times 1000}. \quad (5)$$

(11) Determination of β-Carotene Content. The β-carotene content of sapodilla juice (10 ml sample) was determined using the spectrophotometric method with slight modification [25].

(12) Total Phenolic Content (TPC). The TPC of sapodilla juice was determined using the Folin-Ciocalteu (FC) reagent method, as reported by Moukette et al. [26].

3. Result and Discussion

3.1. Statistical Analysis and Model Fitting. After statistical analysis of experimental data (mean ± SD), the results were illustrated using one-way ANOVA for juice yield, TSS, clarity, viscosity, and L-value under different processing conditions which are presented in Table 3. The models (quadratic) established for each response were statistically significant ($p < 0.05$) with nonsignificant ($p < 0.05$) lack of fit. All models were judged using F-statistic value at a probability (P) of 0.001, 0.002, and 0.04. The coefficients of determination (R^2) of the responses are closer to 1.0 and their sum of square (SS), mean square (MS), and standard error (SE) are presented in Supplementary Materials file 1.

3.2. Effect of Processing Conditions on Responses

3.2.1. Effect on Juice Yield. In the fruit juice industry, juice production using enzymes is imperative due to the improved press ability and yield of extraction [12]. Both pectinase concentration (X_1) and incubation time (X_3) were observed significantly ($p < 0.05$) affecting juice yield, shown in Figure 2. Moreover, juice yield expressed a positive relationship with all processing factors of the experiment, such as pectinase concentration, incubation temperature, and incubation time. The findings are in accordance with Gummadi and Kumar [27] who reported that pectinase activity is affected by the physical and chemical parameters that are vital factors

Table 3: Regression coefficient for each response under suggested models.

Regression coefficient	Juice yield (Y_1)	Viscosity (Y_2)	TSS (Y_3)	Clarity (Y_4)	L-value (Y_5)
Intercept (β_0)	61.85*	4.86*	20.96*	0.19*	30.90*
Linear term					
β_1	3.31*	−0.061*	−0.52	0.25*	1.63*
β_2	0.44	−0.022*	−0.042	−0.029*	0.68
β_3	1.82*	−0.023*	−0.34	0.12*	0.15
Interaction term					
$\beta_1\beta_2$	1.43*	0.016*	0.39*	0.27*	1.32
$\beta_1\beta_3$	0.44	0.003750	0.048	0.14*	−0.11
$\beta_2\beta_3$	−0.57	0.001250	0.36*	−0.042	−0.13
Quadratic term					
β_1^2	−0.85*	−0.040*	−0.069	0.26*	−0.92
β_2^2	0.16	−0.023*	−0.023	0.14*	0.41
β_3^2	0.16	−0.003.179	−0.19	0.072*	−1.96*

*Level of significance at $p < 0.05$ (β_1: regression coefficient of X_1; β_2: regression coefficient of X_2; β_3: regression coefficient of X_3).

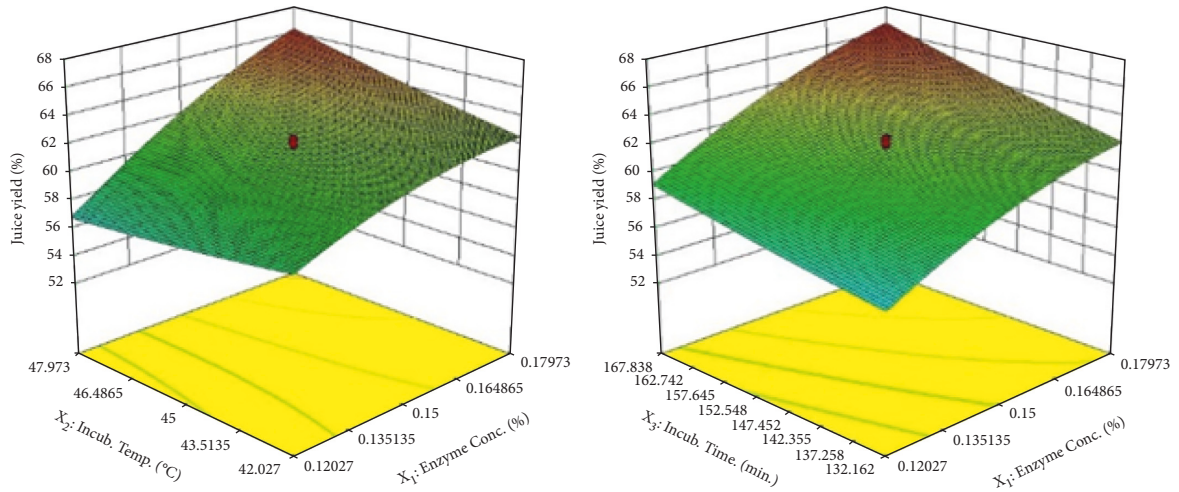


Figure 2: 3D graph plots displaying the linear and interactive effect of factors on juice yield.

for the increase in the juice yield. The recovery of juice yield was related to the positive ($p < 0.05$) interactive effect of pectinase concentration and incubation temperature, which contributes to liquefying the fruit pulp (Table 3). As pectinase hydrolyzes the cell wall of the fruits and vegetables, it facilitates higher juice yield and shortens the processing time [28]. The recovery of sapodilla juice ranged from 53 ± 1.0 to 66.98 ± 0.49%, depending upon the level of processing conditions used (Table 2). Consequently, the minimum juice yield was found at 0.1% pectinase concentration at 45°C for 150 min, whereas the maximum juice yield was found at 0.179% pectinase concentration at 47.97°C for 167.83 min. Various works have been published for different fruit juices production, namely, bael (*Aegle marmelos* Correa) juice and pineapple (*Ananas comosus*) juice. The findings were consistent with the application of pectinase enzyme for increasing the yield and improving the quality of the juice [14, 15]. The quadratic model suggested for juice yield was significant ($p < 0.001$, R^2 0.95) with linear effect (X_1, X_3), interactive effect (X_1X_2), and quadratic effect (X_1^2) as represented by significant model terms (Table 3 and Supplementary Materials file 1).

3.2.2. Effect on Viscosity. Viscosity is an important sensory parameter as low viscosity of juice might affect the mouthful of the consumer, affecting its acceptability [29]. The viscosity of juice varied from 4.65 ± 0.03 to 4.92 ± 0.0 cP depending upon the level of processing variables used (Table 2). The minimum viscosity was observed for 0.2% pectinase concentration, 45°C, and 150 min and the maximum viscosity was observed for 0.120% pectinase concentration, 42°C, and 132 min. Various studies have been reported about fruit juices, namely, bael juice extraction and banana (*Musa sapientum* cv. Berangan) juice clarification. Similar results suggest that increased pectinase concentration decreases the viscosity at a particular temperature and time application [21, 30]. The model generated for viscosity was considerable ($p < 0.001$, R^2 0.96) with linear effect (X_1X_2, X_3), interactive effect (X_1X_2), and quadratic effect (X_1^2, X_2^2) as significant model terms (Table 3 and Supplementary Materials file 1). 3D graphs display the impacts of independent factors on viscosity of juice in Figure 3. Pectinolytic enzymes increase yield and improve filterability and drop-down viscosity of the processed juice [12]. On the other hand, fluid viscosity is affected by the solute nature, solute-solvent interaction, and

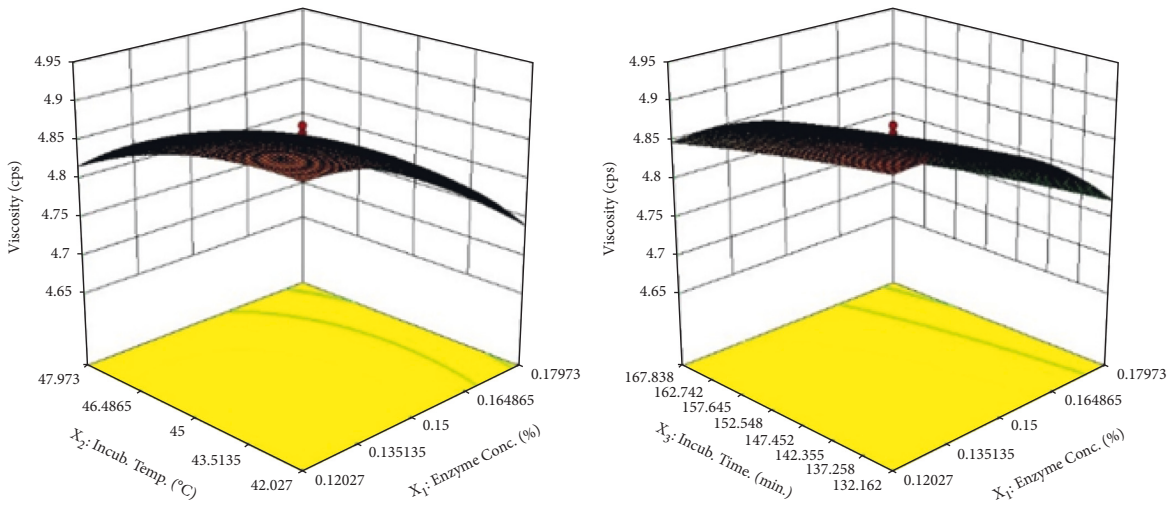


Figure 3: 3D graph plots displaying the interactive effect of factors on viscosity.

hydration state [31]. All linear terms (enzyme concentration, X_1; incubation temperature, X_2; and incubation time, X_3) affected viscosity negatively ($p < 0.05$), and it was decreased due to the contribution of pectolytic action on the fruit pulp. The findings are in accordance with Sin et al. [8] who reported that degradation of natural pectin by infused pectinase confers the loss of water holding capacity and gel formation. Therefore, free water (change in the state of hydration) liquefied the fruit pulp and subsequently reduced the viscosity of extracted juice. Ribeiro et al. [32] had reported that this enzyme was part of maceration enzymes such as a mixture of pectinase acting on cell wall components like soluble pectin and cellulose, hydrolyzed them, and lowered the viscosity.

3.2.3. Effect on TSS. In sapodilla juice, the soluble solid content mainly refers to low molecular sugars, organic acids, and marginal quantities of other macromolecules [33]. Both enzyme concentration (X_1) and incubation time (X_3) were observed to be nonsignificant for the total soluble solids (TSS) ($p < 0.05$), and incubation temperature (X_2) was also observed to be nonsignificant ($p > 0.05$). Actually, individually, each did not affect the TSS. Moreover, TSS was positively related to the interactive effects of $X_1 X_2$ and $X_2 X_3$. Soluble pectin present in ripened sapodilla fruit is considered as total soluble solid by refractometer. Enzyme-assisted extraction increases the free water content volume by releasing pectin-bound water. Meanwhile, in this phenomenon, soluble pectin conversion via deesterification takes place into its insoluble fractions like pectic acid and methanol [34] which are not considered soluble solids. Therefore, it is clear that the production of insoluble fractions in extracted juice (noncentrifuged) might be caused by decreasing the TSS. 3D graphs display the effects of pectinase concentration, temperature, and time application on °Brix in Figure 4. Thus, the TSS of extracted juice varied from 19.62 ± 0.2 to 21.84 ± 0.02 °Brix at different experimental conditions (Table 2). The quadratic model for TSS was significant ($p < 0.05$ and $p < 0.1$) with interactive effect (X_1X_2, X_1X_3) and its coefficient of determination (R^2 0.81) (Table 3 and Supplementary Materials file 1).

3.2.4. Effect on Clarity. Pectin is one of the significant substances responsible for % transmittance in most extracted fresh juices. % transmittance is directly proportional to clarity. In the case of the clarification process, enzyme concentration (X_1) and incubation time (X_3) were positively ($p < 0.05$) affecting the % transmittance of sapodilla juice because of pectic matter degradation. Reasonably, clarity was significantly related to the linear effect of enzyme and incubation time. The effect of temperature on clarity was observed to be linearly negative ($p < 0.05$). It is possibly due to lower enzyme activity. The incubation temperature, which affects the pectolytic activity of the enzyme, might be due to the slow rate of clarification. However, the treatment temperature range must not go beyond optimum pectinase activity. The results were consistent with Rajauria and Tiwari [12] who reported that the application of temperature, pH, length of reaction time, and its concentration could trigger enzyme performance. Therefore, it is clear to conduct test trials with specific enzymes under typical experimental conditions. Thus, higher incubation temperature delayed the clarification process of juice. The results are also consistent with Sin et al. [8, 21] who reported that, in general, the time taken for clarification of juice is inversely proportional to the dose of enzyme used at a constant temperature. As the results suggested, the degree of clarity index in juice is an imperative factor of consumer acceptability. Consequently, clarity of extracted juice ranging from 0.125 ± 0.01 to 1.390 ± 0.01%T depending upon the level of processing conditions was maintained (Table 2). Suggested model for clarity (%T) was significant ($p < 0.001$, R^2 0.99) with linear effect, interactive effect, and quadratic effect as significant regression terms (Table 3 and Supplementary Materials file 1) (Figure 5).

3.2.5. Effect on L-Value. An important aspect of enzymation is to lower the impacts of plant polymer leading to viscosity

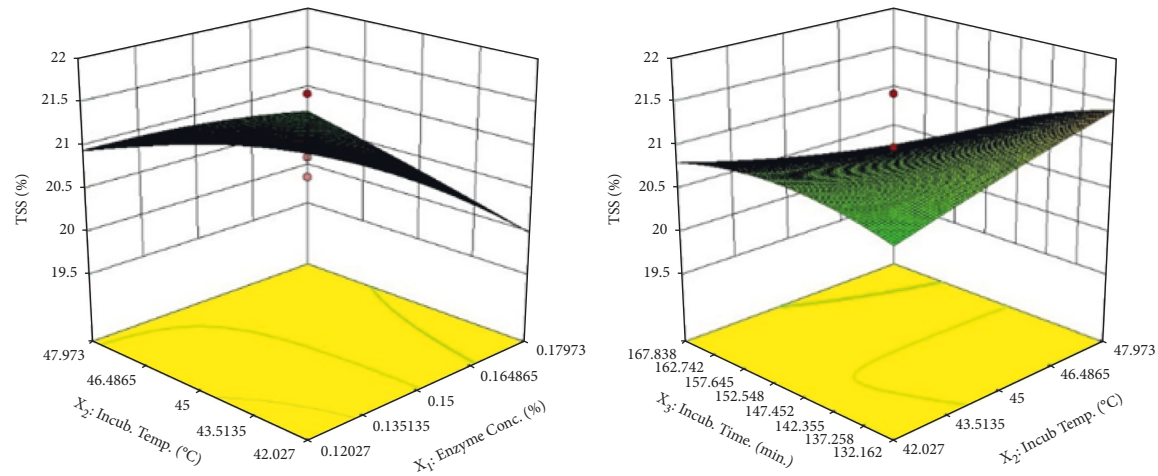


Figure 4: 3D graph plots displaying the linear and interactive effect of factors on TSS.

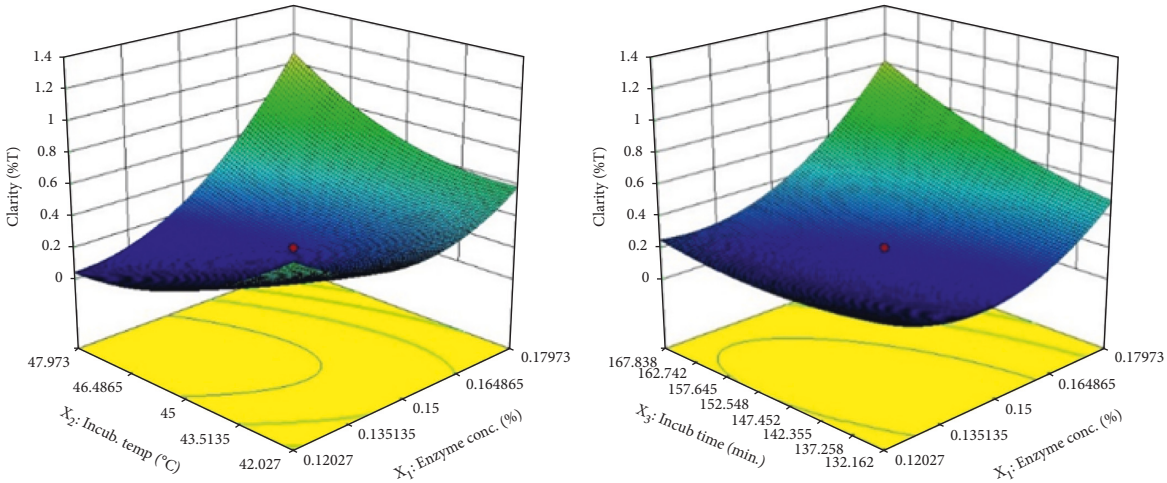


Figure 5: 3D graph plots displaying the interactive effect of factors on clarity (%T) of sapodilla juice.

and turbidity reduction and color (lightness) attributes of the final product [12]. The established model (quadratic) for L-value was significant ($p < 0.04$) as well as satisfactory coefficient of determination (R^2 0.73) with linear effect (X_1), interaction effect (X_1X_2), and quadratic effect (X_2^2) as model terms (Table 3 and Supplementary Materials file 1). In the case of L-value, temperature and time were observed to be nonsignificant ($p > 0.05$), whereas pectinase concentration was observed to be most significantly related to the L-value at linear level ($p < 0.04$). Moreover, it is clear from Figure 6 that the color of juice (L-value) increased with pectinase concentration and contributed to turbidity reduction. In contrast, lightness was decreasing towards the linear effect of incubation time and temperature beyond its certain level during enzyme treatment. In this way, optimum pH (above 4.5) of sapodilla juice at a specific temperature (45°C) usually facilitated the polyphenol oxidase (PPO) reaction, which masks the lightness of juice. The results suggested that less incubation time and lower incubation temperature show higher L-value in juice and vice versa. However, significant interaction effect (X_1X_2) between pectinase concentration and the temperature was also positive on lightness of juice, meaning that the activity of pectinase was related to the incubation temperature during enzyme treatment. Therefore, increasing incubation temperature up to a certain limit for increasing enzyme activity might increase L-value. The findings are in accordance with Sin et al. [8] who reported that L-value measures color intensity and, thus, color is an essential sensory attribute. Consequently, the L-value varied from 25.04 ± 0.4 to 34.47 ± 1.52 at various experimental conditions (Table 2). The minimum color (L-value) was explored at 0.1% pectinase concentration and 45°C/150 min, and the maximum color was explored at 0.2% pectinase concentration and 45°C and 150 min.

3.3. Optimized Level of Variables and Verification of Fitted Model. Numerical optimization was performed for responses data for obtaining the optimum level of variables (processing conditions) based on better set goals of every criterion. These set goals were selected based on suitable indexing of quality characteristics of the extracted juice. The criteria of set goals for optimization are given in Table 4.

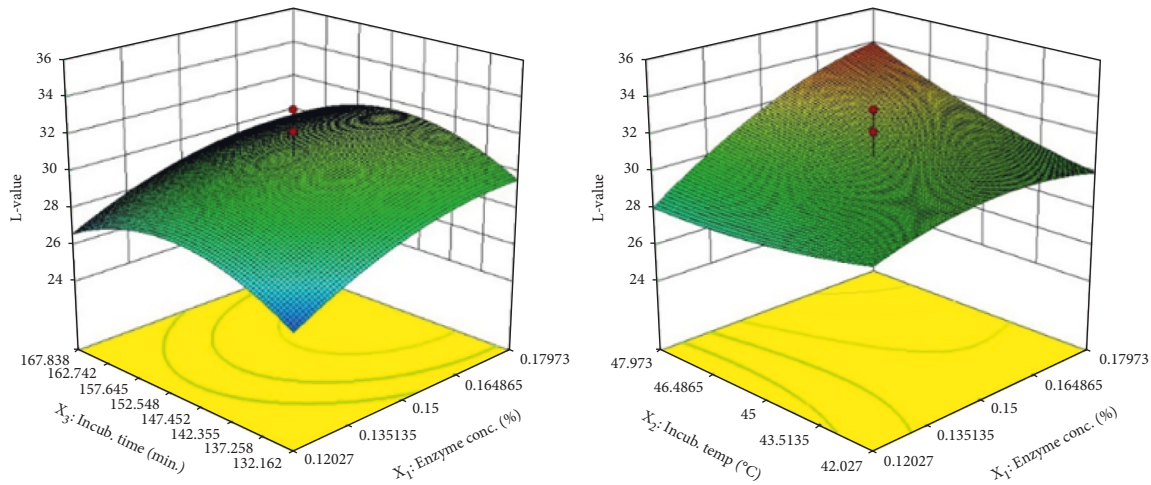


Figure 6: 3D graph plots displaying the linear effect of enzyme concentration (X_1) and quadratic effect of time (X_3) on L-value.

Numerical optimization suggested that the highest desirability (0.76) could be obtained by formulating sapodilla juice at optimum combined condition (0.120% pectinase concentration, 42.02°C incubation temperature, and 167.83 min. incubation time). At the optimal level, predicted responses, namely, juice yield, viscosity, TSS, clarity, and L-value, were 60.96%, 4.86 cP, 21.61 °Brix, 0.744%T, and 27.83, respectively.

Model validation was also done by performing three experiments at optimum levels of pectinase concentration (0.120%) at 42.00°C for 168 min and generated an optimum region in the overlay plot as depicted in Figure 7. A similar hypothesis was also reported by Chauhan et al. [35], Joshi et al. [36], and Landbo and Meyer [37]. They have explored the optimized conditions such as pectinase concentration of 0.5% at 45°C for 300 minutes, 2.5% at 40°C for 240 min, and 0.18% at 60°C for 30 minutes for extraction of maximum apricot, pear, and black currant juices, respectively. The major findings of optimized sapodilla juice were in agreement with Singh et al. [20] who also reported the optimized concentration of enzymes with respect to juice yield (58.96%), viscosity (2.99 cP), TSS (18.03 °Brix), L-value (34.08), and clarity (1.896 abs). The observed values (experimental values) were found to be in good agreement with the predicted response, thereby ensuring the selected model's adequacy (Table 5). Furthermore, experimental values showed the highest compatibility in contrast with the control sample made without any enzymation.

3.4. Physicochemical Characteristics of Optimized Juice and Control Juice

3.4.1. Rheological Characteristics. The rheograms show the relationship between shear stress and shear rate of optimized sapodilla juice and control juice at specific TSS and water activity, depicted in Figure 8. The rheograms exhibited that there was "a linear increase in shear stress with respect to increasing shear rate and one rheogram (optimized juice) passes through the origin but second rheogram (control) deviates slightly from the origin." The linearity up to an extent between shear stress and shear rate data of both juice samples indicated that the flow behavior was like Newtonian liquid, described by the following Newtonian equation:

$$\tau = \eta \cdot r, \tag{6}$$

where η is the Newtonian viscosity.

The similar findings are in accordance with Deshmukh et al. [38] who investigated the relation between rheological parameters such as shear stress and shear rate of enzyme clarified sapodilla juice at constant temperature (25 ± 1°C) and different soluble solid contents and constant soluble solid content (55.6 °Brix) with varying temperatures as rheograms demonstrated that the enzyme clarified sapodilla juice and its concentrates had a Newtonian-like behavior. Shear stress-stress rate data of both juice samples at specific TSS and water activity with respect to few major measuring points (initial, middle, and final points) are presented in Table 6.

Besides this, the effect of enzyme treatment and no treatment on the flow behavior of sapodilla juice was described by power law (equation (2)). The consistency index (k) and flow behavior index (n) of optimized juice (at 20.48 °Brix and a_w of 0.984) and control juice (at 21.88 °Brix and a_w of 0.982) are represented in Table 7. The outcomes showed that the consistency of enzyme extracted sapodilla juice was considerably ($p < 0.05$) lower than that of control juice due to the loss of pectin-water interaction (release of bound water) during enzymatic pectolysis. Deshmukh et al. [38] reported that the viscosity of liquid foods typically also depends on "intermolecular forces between molecules and water-solute (sugars and acids) interactions."

3.4.2. Sedimentation Index, Water Activity, and Turbidity Measurement. Remaining nondegraded complex substrates in enzyme extracted juice were an essential sign of turbidity [39] and were found to be settled down in the bottom of juice bottles due to unstable turbidity. This unstable turbidity (comprised of pectic materials) of optimized sapodilla juice

Table 4: Constraints fixed for processing condition and responses.

Variables	Goal status	Lower limit	Upper limit
Pectinase concentration (%)	Minimize	0.120	0.179
Incubation temp. (°C)	Minimize	42.02	47.97
Incubation time (min.)	Within range	132.16	167.83
Juice yield (%)	Maximize	53.00	66.98
Viscosity (cP)	Within range	4.65	4.92
TSS (°Brix)	Maximum	19.62	21.84
Clarity (%T)	Maximum	0.125	1.390
L-value	Maximum	25.04	34.47

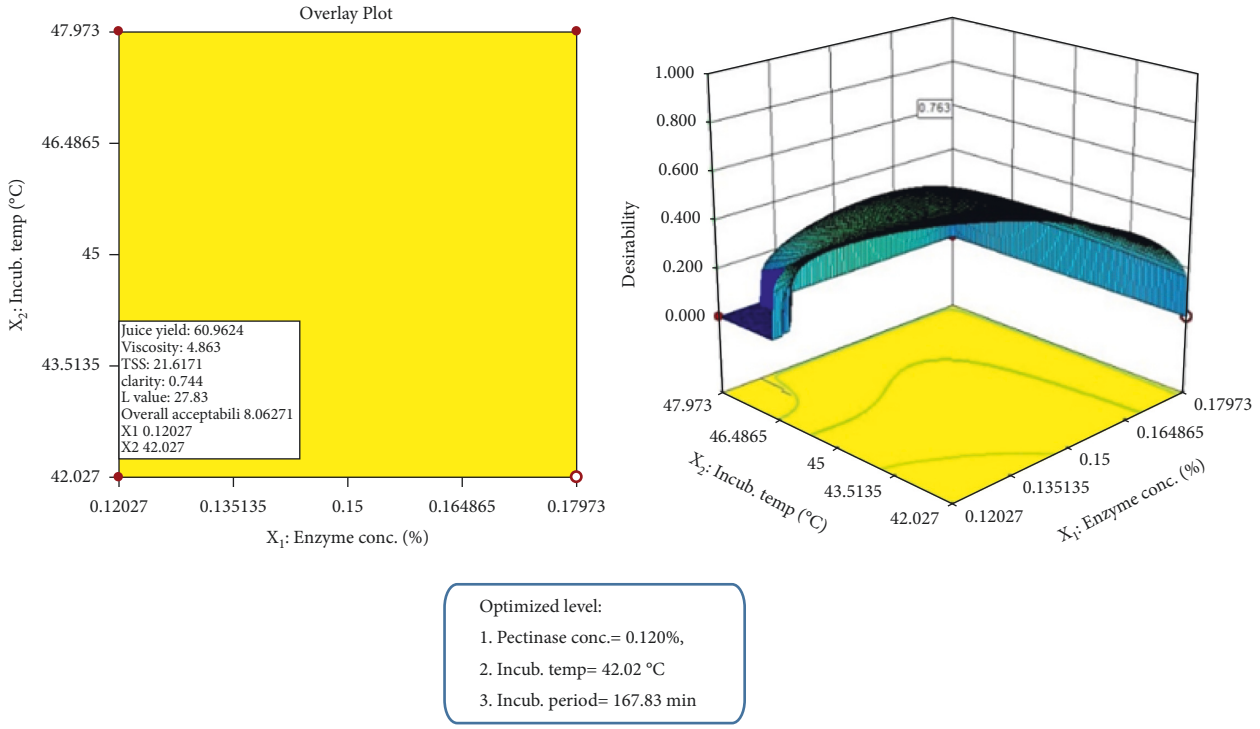


Figure 7: Overlay plot and desirability graph for optimum processing conditions as function of optimized sapodilla juice production.

Table 5: Experimental validation of predicted responses and comparison with control juice (at optimal condition).

Optimized sapodilla juice (at 0.120% of pectinase, 42.02°C/167.83 minutes)				
Response variables	Predicted values	Experimental values	Control	Difference
Juice yield (%)	60.96^{b}	62.08 ± 0.38^{b}	35 ± 2.0^{a}	27.08
Viscosity (cP)	4.86^{a}	4.81 ± 0.02^{a}	9.1 ± 0.17^{b}	4.29
TSS (°Brix)	21.61^{a}	21.48 ± 0.19^{a}	22.88 ± 0.06^{b}	1.4
Clarity (%T)	0.744^{b}	0.724 ± 0.05^{b}	0.144 ± 0.00^{a}	0.58
L-value	27.83^{b}	28.29 ± 0.96^{b}	24.85 ± 0.76^{a}	3.43

Values of experimental and control samples are indicated as mean ± SD. Control: without treated sample.

was observed as sedimentation index. The sedimentation index of juice was found to be lower (0.730 ± 0.11%) compared to control (1.07 ± 0.02%). The turbidity and water activity of optimized juice marked 13.73 ± 1.10 NTU and 0.984 ± 0.0, whereas those of control juice marked 79 ± 0.75 NTU and 0.982 ± 0.0, respectively (Table 8). Lee at el. [21] reported that turbidity is the main function associated with enzyme concentration. The results showed that magnitudes of the aforementioned physical characteristics are significantly ($p < 0.5$) related to the efficiency of pectolysis, which depends on pectolytic conditions of sapodilla fruit pulp treatment. Therefore, it is conspicuous that optimized conditions of pectolysis contributed to enhancing the elimination of solid residues of pectic nature, which facilitates reducing the sedimentation index and leaching out pectic bound water in sapodilla juice. Moreover, the

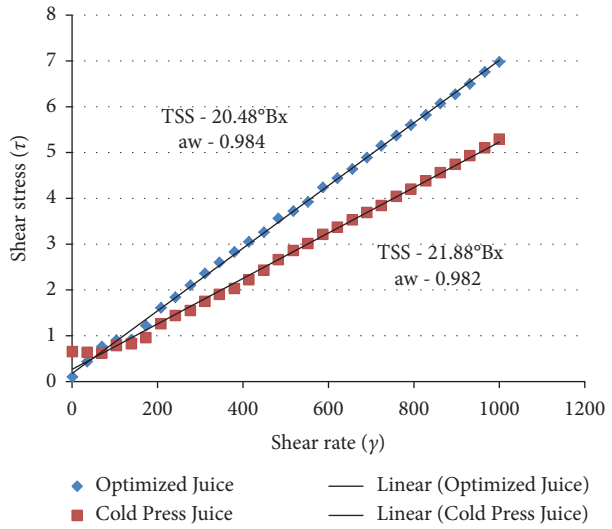


Figure 8: The rheogram of enzyme-optimized juice and control juice at specific TSS and water activity at 25°C.

Table 6: Shear stress-stress rate data of optimized juice and control juice at major points.

Optimized sapodilla juice at 20.48 °Brix and a_w of 0.984			
	Measuring points	Shear rate (r), s^{-1}	Shear stress (τ), Pa
Newtonian equation (η) = (τ/r)	Initial (1)	0.999 ± 0.00	0.101 ± 0.00
	Middle (15)	483 ± 0.00	2.440 ± 0.22
	Ending (30)	1000 ± 0.00	5.363 ± 0.80
Control sample at 21.88 °Brix and a_w of 0.982			
	Measuring points	Shear rate (r), s^{-1}	Shear stress (τ), Pa
Newtonian equation (η) = (τ/r)	Initial (1)	0.994 ± 0.00	0.651 ± 0.00
	Middle (15)	483 ± 0.00	3.580 ± 0.01
	Ending (30)	1000 ± 0.00	7.086 ± 0.09

Table 7: Consistency index (k) and flow behavior index (n) of optimized juice and control juice.

Samples	Consistency index (k)	Flow behavior index (n)
Optimized juice (20.48 °Brix and a_w of 0.984)	0.008 ± 0.00^a	0.97 ± 0.00^b
Control juice (21.88 °Brix and a_w of 0.982)	0.012 ± 0.00^b	0.87 ± 0.00^a

Table 8: Physicochemical properties of sapodilla juice (mg/100 mL or %).

Physicochemical characteristics	Optimized juice	Control juice
Sedimentation index (%)	0.730 ± 0.11^a	1.07 ± 0.02^b
Turbidity (NTU)	13.73 ± 1.1^a	79 ± 0.75^b
Conductivity measurement (S/m)	69.46 ± 0.30^b	30.43 ± 0.35^a
Water activity	0.984 ± 0.0^a	0.982 ± 0.0^a
pH of juice	5.35 ± 0.03^a	5.78 ± 0.07^b
Filterability (m^{-1})	24.16 ± 1.04^a	60.5 ± 1.80^b
Protein (%)	0.320 ± 0.0^a	0.330 ± 0.0^a
Acidity (% citric acid)	0.143 ± 0.0^b	0.133 ± 0.0^a
Ascorbic acid (mg/100 mL)	6.38 ± 0.58^b	4.64 ± 0.0^a
TPC (mg GAE/100 mL)	35.86 ± 0.60^b	30.68 ± 0.85^a
Ash (%)	0.572 ± 0.031^a	0.654 ± 0.031^b
Moisture (%)	84.85 ± 0.021^a	83.69 ± 0.18^a
β-Carotene (μg/100 mL)	0.173 ± 0.008^a	0.306 ± 0.007^b

Each value is indicated as mean ± SD; significance level: ($p < 0.5$). NTU: Nephelometric Turbidity Unit.

sedimentation index was inversely proportional to transmittance, affecting the turbidity of sapodilla juice and vice versa. The aforesaid findings of turbidity are supported by Lee et al. [21] who reported that the optimum combinations of enzyme concentration, temperature, and time application in clarification treatment of banana juice had markedly affected the turbidity.

3.4.3. Filterability and Electrical Conductivity Measurement. Filterability of juices was affected by degradation of pectic substances of fruit pulp. Release of ions in juice due to maceration could lead to an increase in electrical conductivity (EC). Optimized sapodilla juice was observed to be significantly ($p < 0.5$) higher in filterability ($24.16 \pm 1.04\ \text{min}^{-1}$) and conductivity ($69.46 \pm 0.30$ S/m) as compared to control juice ($60.5 \pm 1.80\ \text{min}^{-1}$ and 30.43 ± 0.35 S/m) (Table 8). Deshmukh et al. [38] documented that the soluble solid content of enzyme clarified sapodilla juice mainly constitutes sugars, the marginal quantity of organic acids, and macromolecules. The results showed that the difference in the aforementioned macerating characteristics is significantly ($p < 0.5$) associated with the extent of sapodilla fruit tissues' maceration (cytosol release), which depends upon optimum pectolysis condition. Therefore, it is conspicuous that optimized conditions contributed to the loss of rigidity and liquefying the sapodilla fruit tissue, which facilitates elevating total soluble solids, electrolyte concentration, and filtration rate due to viscosity reduction in sapodilla juice. Lee et al. [21] supported the aforesaid findings of filterability. They reported that optimum combinations of enzyme concentration, temperature, and time application were taken to treat banana juice, which markedly affected the filterability.

3.4.4. Total Phenolic Content, Ascorbic Acid Content, and β-Carotene Content. Ascorbic acid is a water-soluble vitamin, which is an indicator for the quality of fruit juices. β-Carotene is present in crystalline form in the chromoplasts of fruits and vegetables. Both phytochemicals act as an antioxidant [40]. The total phenolic content of enzyme-optimized sapodilla juice was 35.86 ± 0.60 mg/100 mL, and that of control juice was 30.68 ± 0.85 mg/100 mL. A similar trend was also found in the case of the ascorbic acid content of enzyme-optimized sapodilla juice (6.38 ± 0.58 mg/100 mL) and control juice (4.64 ± 0.0 mg/100 mL) (Table 8). No doubt, the findings suggested that an optimized combination of pectolysis had marked a significant ($p < 0.5$) and positive impact on TPC and ascorbic acid contents. In this sense, this deviation in phytochemical contents may be due to enzymatic pectolysis during maceration. Similarly, the effect of optimization demonstrated that phenolic yields could be improved with an enzyme treatment and the optimal reaction conditions for obtaining the highest phenolics in elderberry juice [41]. Notwithstanding, β-carotene of enzyme-optimized sapodilla juice was lower ($0.173 \pm 0.008\ \mu$g/100 mL) than that of control juice ($0.306 \pm 0.007\ \mu$g/100 mL) (Table 8). However, the findings suggested that an optimized combination of pectolysis was not found to be significantly ($p < 0.5$) effective over β-carotene content. Conversely, β-carotene is hydrophobic, due to which this was not leached out with water in sapodilla juice during the extraction process. Similarly, Khoo et al. [42] reported that carotenes are fat-soluble pigments found in many dark-colored vegetables.

3.4.5. Protein, Acidity, Ash, Moisture Content, and pH. The chemical compositions of optimized sapodilla juice such as protein, titratable acidity, ash, and moisture content were found to be $0.320 \pm 0.0\%$, 0.143 ± 0.0, $0.572 \pm 0.031\%$, and $84.85 \pm 0.21\%$ (wet basis), respectively, while those of control were found to be $0.330 \pm 0.01\%$, 0.133 ± 0.0, $0.654 \pm 0.031\%$, and $83.69 \pm 0.18\%$ (wet basis), respectively (Table 8). The results suggested that proximate contents are not significantly ($p > 0.5$) affected by pectolysis of sapodilla fruit pulp. The obtained findings were similar to the results reported by Kulkarni et al. [5], Yahia and Gutierrez-Orozco [43], and Deshmukh et al. [38]. The pH and titratable acidity of optimized sapodilla juice were 5.45 ± 0.03 and $0.143 \pm 0.0\%$, whereas the pH and titratable acidity of the control sample were 5.78 ± 0.07 and $0.133 \pm 0.0\%$ as citric acid, respectively. Moreover, the pH of fresh sapodilla fruit pulp was similar to that of control juice (5.78 ± 0.07) (Table 8). pH was averagely observed to be 5.72 ± 0.14 in sapodilla fruits reported by Jadhav et al. [44]. The findings suggested that the change in titratable acidity was due to enzyme treatment that leads to acidity increment. Moreover, the decline in the pH value of optimized sapodilla juice was associated with pectinase application at enzyme treatment time (167.83 min). Similarly, Noor and Aziah [45] also reported that the pH for pectinase extracted durian juice also decreased considerably ($p \leq 0.05$) at 0.01% enzyme concentration after 1-, 2-, and 3-hour duration of incubation.

4. Conclusion

Plant-based foods are the healthiest diets, especially fruit juices. Enzyme depectinization plays an essential role in the food industry and strongly affects processing aids such as juice yield, clarity, and degree of consumer acceptability. The depectinization demonstrated that the juice yield, TSS, clarity, viscosity, and L-value of sapodilla juice were majorly influenced by pectinase enzyme. Most of the characteristic parameters of optimized juice were found to be affected more positively than those of control juice. The qualities in terms of filterability, clarity, and viscosity of the sapodilla juice might exert suitable flux and reduce the volume of membrane fouling during membrane-based processing. Keeping in mind the fact that the heat sensitivity of sapodilla juice causes burny flavor during heat treatment (if above 65°C for 3–5 min), at the same time, further research is necessary to substitute heat treatment with immobilization or other low-cost techniques for separating infused enzyme from sapodilla juice. This study concluded that sapodilla juice with acceptable juice yield, TSS, viscosity, clarity, L-value, and other characteristic parameters compared to the control juice could be formulated with an optimum

combination of 0.120% pectinase concentration at 42.02°C for 167.83 minutes. Conclusively, the use of pectinase for sapodilla juice production would be potentiated to the beverage industry. It can be concluded that the application of pectinase enzyme is useful in enhancing the recovery of good quality juice with higher nutritive value.

Data Availability

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

ANOVA values of quadratic models for each response. (*Supplementary Materials*)

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