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V Vasudeva Rao
Ph.D. Scholar, Department of Processing and Food Engineering, Dr. N.T.R. College of Agricultural Engineering, Bapatla, Andhra Pradesh, India

Venkata SP Bitra
Principal Scientist & Head, Post Harvest Technology Centre, Bapatla, Andhra Pradesh, India

DD Smith
Associate Dean, College of Food Science and Technology, Pulivendula, Andhra Pradesh, India

A Mani
Professor & Head, Department of Soil and Water Conservation Engineering, Dr N.T.R. College of Agricultural Engineering, Bapatla, Andhra Pradesh, India

Vimala Beera
Assistant Professor, Department of Food Safety and Quality Assurance, Dr. N.T.R. College of Food Science & Technology, Bapatla, Andhra Pradesh, India

Corresponding Author
V Vasudeva Rao
Ph.D. Scholar, Department of Processing and Food Engineering, Dr. N.T.R. College of Agricultural Engineering, Bapatla, Andhra Pradesh, India

Optimization of enzymatic clarification of sapodilla juice using response surface methodology

V Vasudeva Rao, Venkata SP Bitra, DD Smith, A Mani and Vimala Beera

Abstract
Most of the fruits contain a high quantity of pectin, making the juice extraction difficult by conventional methods. Clarified juices are making a remarkable place in the market. Conventional treatment of sapodilla pulp yields very viscous, turbid, and low juice recovery. To overcome this problem, a technique called enzyme-assisted extraction is used which consists of an enzymatic treatment applied during the first steps of fruit processing. This study was initiated to optimize the enzymatic clarification process of sapodilla juice using response surface methodology (RSM). Sapodilla pulp was treated with pectinase enzyme at various concentrations (0.05-0.15%), temperatures (30-50 °C), and incubation time periods (40-120 min) with uniform stirring at 1500 rpm. A Box-Behnken design was used to establish the optimum conditions for enzymatic clarification of sapodilla juice to obtain maximum juice yield. A significant quadratic regression model describing the changes in juice yield with respect to process parameters was established with the coefficient of determination, R^2=0.93. Incubation time was the most significant variable affecting the juice yield. Maximum juice yield of 93.05% was obtained at the optimum desirable process treatment conditions at the enzyme concentration of 0.1% of sapodilla pulp, incubation time of 40 min, and incubation temperature of 30 °C.

Keywords: Juice yield, box-behnken design, pectinase, incubation time

1. Introduction
Sapodilla (*Achras sapota* L.) is one of the major tropical fruit cultivated in 84,000 Ha with a production of 9,06,000 MT and an export of 1134 MT in the year 2019-20 from India. Presently, it is cultivated throughout the tropical countries across the world and the leading producing countries are India, Philippines, Sri Lanka, Malaysia, Mexico, Venezuela, and Guatemala. It has a fleshy berry, light brown, and with scurfy brown peel, oval or conical with one or two seeds. Its texture varies from gritty to smooth with a mild aroma and sweet taste (Jacob *et al*., 2008; Sin *et al*., 2006) [10, 15]. Sapodilla tree is grown in India in the coastal climate region and is extensively cultivated in Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, and West Bengal states. Presently, crop production has increased because of continuous fruiting throughout the year, hence, fruit availability in the market is increased. At present, the per unit area of production under this crop has increased, resulting in a glut in the market during peak season. Being a climacteric fruit, it undergoes rapid ripening within 5-8 days, during which physiological and chemical changes of the fruit occur, these changes increase the activities of catalase and peroxidase that, in turn, accelerates biochemical changes leading to shorter shelf-life. Even if it is stored for longer period under low temperatures, it is susceptible to chilling injury. It undergoes post-harvest losses compared to other climacteric fruits (Jadhav, 2018) [9]. To overcome these losses, it is, therefore, necessary to convert the sapodilla fruit into processed and value-added products to avoid post harvest losses.
Sapodilla juice is rich in polyphenols, vitamins, iron, calcium, phosphorous and fibers. It prevents viral infections, cavities in the tooth, constipation, weight loss and helps in the prevention of cough and cold (Chaudhary and Kumar, 2020) [5]. It does not just strengthen the intestines but also boosts immunity. It also reduces inflammation by reducing swelling and pain. Among the tropical fruits, it has the highest antioxidant activity. Now-a-days, there has been a considerable increase in the consumption of fruit beverages due to which, the fruit processing industry is growing at a faster pace, so it is necessary to develop fruit juice-based beverages with assured quality (Baskar and Hemalatha, 2021; Jadhav, 2018) [3, 9].
Unlike other tropical fruits, the presence of colloids in sapodilla makes it difficult to extract juice from pulp which leads to fouling during the filtration process (Sharma *et al.*, 2015; Jacob *et al.*, 2008; Rai *et al.*, 2004) [17, 10, 14].

Obtaining clear juice is a very important process as it enhances the acceptability (palatability). Enzymatic and non-enzymatic clarifications are the two methods for clarification of juices. Now-a-days enzymatic clarification is most common. Many researchers carried out enzymatic clarification of sapodilla pulp (Sin *et al.*, 2006; Jacob *et al*., 2008; Jadhav, 2018) [15, 10, 9]. Pectinase enzyme hydrolyzes the pectin and causes pectin-protein complexes to flocculate. The resulting juice had a lower amount of pectin and viscosity which increased the filtration process to aid in higher juice yield. In addition to enzyme incorporation, fining agent helped to increase the yield of clarified juice for the concentration process in guava juice (Brasil, 1995) [4]. Fruit juices contain colloids that may lead to fouling problems during the filtration process, and these colloids are basically polysaccharides such as pectin and starch.

Pectinases play a key role in the fruit juice industry as they can degrade pectin and can cause pectin protein complexes to flocculate. Pretreatment of juices with pectinases is performed to lower the amount of pectin present and to decrease the viscosity of the juice, which in turn accelerates the subsequent filtration process. Enzymatic degradation of pectin substances depends on several physic-chemical factors such as treatment time, enzyme concentration, and incubation temperature and so it is necessary to optimize the levels of these parameters.

Most of the studies on enzymatic clarification of sapodilla juice process carried out with the addition of water along with enzyme, which will increase the cost of evaporation process during concentration of juice. The juice, thus, obtained was cloud juice but not clarified juice. To obtain clarified juice fining agent should be added, also to get uniform distribution of enzyme concentration continuous stirring is required and also to maintain constant temperature throughout the process is required. Hence, present study is taken up by taking care of addition of fining agent, continuous stirring and maintenance of constant process temperature, which was not reported by previous researchers. The objective of the present investigation is to optimize the process parameters (temperature, incubation time, enzyme concentration) for enzymatic clarification of sapodilla juice by addition of fining agent and without the addition of water.

2. Materials and Methods

sapodilla (Variety: *Pala*) fruits were procured from the farmer of Duggirala village of Guntur district, Andhra Pradesh, India. The good, healthy and matured fruits harvested a day before were selected for the study. The fruits were cleaned with water to remove all dirt adhering to it and then shade dried at room temperature to remove adhered moisture. Then, the fruits were peeled and sent through a 1/32 inch sieve fitted pulper (Make: M/s Naresh and Co., Vijayawada) and the obtained pulp was collected. The pH of the pulp was measured using a digital pH meter (Model: ECPHTUTOR, Eutech Instrument, Singapore) and resulted value was in the range of3.44-4.23. Then the pulp was treated with Pectinase enzyme (activity of 1200 PGU/g (Biolax corporation, India) source organism, *Aspergillus niger*) with different concentrations of 0.05%, 0.1%, and 0.15% weight basis. The enzyme-treated sapodilla pulp was incubated at different temperatures of 30, 40, and 50 ℃ on a hot plate (Make: JSW, India) and at different time periods of 40, 80, and 120 min. During which, pulp was uniformly stirred at 1500 rpm using a stirrer (Make: Remi, India, Model: RQ-121/D) then the pulp was heated at a temperature of 90 ℃ for 3 min to inactivate the enzyme (Deshmukh *et al.*, 2015) [6] and immediately cooled to room temperature using an ice pack. The sapodilla pulp was then filtered using a fourfold muslin cloth and obtained cloud juice. Further, cloud juice was treated with fining agent gelatin 300 ppm at 40 ℃ for 30 min for obtaining clarified juice. Sodium benzoate @ 0.1% was added as a preservative to the clarified juice to increase the shelf-life. Figure 1 shows the treatment process for clarification and extraction of sapodilla juice.

2.1 Juice Yield

Juice yield was estimated as a percentage of the clarified juice obtained based on the initial fruit pulp (Eq. 1).

$$\text{Juice yield (\%)} = \frac{\text{Weight of clarified juice}}{\text{Weight of pulp}} \times 100 \quad (1)$$

2.2 Total soluble solid content

Total soluble solid (TSS) content of sapodilla juice was determined using a digital hand-held refractometer (Model: PAL-1, Atago co, Ltd., Tokyo, Japan) which was calibrated using distilled water and expressed as ºBrix with an accuracy of ±0.1.

2.3 pH

A digital pH meter (Model: ECPHTUTOR, Eutech Instrument, Singapore) was used to measure the pH of sapodilla juice at 25 ℃ with an accuracy of 0.01. The instrument was calibrated using pH 4 and pH 10 buffer solutions.

2.4 Moisture content

Moisture content of enzyme clarified sapodilla juice was analyzed by the vacuum oven method (AOAC, 2005) [2]. About 5 g of the sample was spread evenly over the previously dried moisture box weighed with precision balance ±0.001 (Model: PGB-200, Wensar Weighing Scales Limited, Chennai) and placed in a vacuum oven (Model: 215501 Make: Venchal Scientific, New York) maintained at 60±1°C under vacuum pressure of 100 mm-Hg (Absolute) for 24 h. After drying, cooled in a desiccator and weighed. Three replications of the sample were taken for drying so as to obtain accurate results. The moisture content was expressed as per cent on wet basis (Eq. 2).

$$\text{Moisture content (\% w.b.)} = \frac{M1-M2}{M1-M} \text{ X } 100 \quad (2)$$

where,

M = weight of empty moisture box with lid (g)

M_1 = weight of box with material before drying (g)

M_2 = weight of box with dried material (g)

2.5 Experimental Design

Response surface methodology (RSM) was chosen to optimize process conditions in the experimental design. A three-level and three-factor Box-Behnken was adopted using Design Expert 13 software. Time (40, 80 and 120 min), temperature (30, 40 and 50 ºC) and enzyme concentrations (0.05%, 0.1% and 0.15%) were three independent variables. In this experiment, pH value is kept at its natural value (4.0-5.0) due to its optimal range for exogenous pectinases (Grassin and Fauquembergue, 1995) [7]. Hence, pH was not included as an analyzing parameter in this design. The response variable, yield (y) was related to the independent

variables (temperature (x_1), incubation time (x_2) and enzyme concentration (x_3)) by a second-degree polynomial equation (Eq. 3) as given below:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (3)$$

Where, b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23} (interaction effects) and b_{11}, b_{22}, b_{33} (quadratic effects).

3. Results and Discussion

3.1 Response surface optimization of process parameters to enhance the clarified juice yield

Numerical optimization of the processing parameters, i.e., enzyme concentration (%), incubation time (min) and temperature (ºC) was carried out using Design-Expert 13 statistical software. The response, juice yield (%) was considered for optimization. The goal set up for optimization of processing parameters and response variable for enhancement of juice yield is given in Table 1. By applying the desirability function method, the solution for optimum juice yield covering the criteria were obtained as given in Table 2.

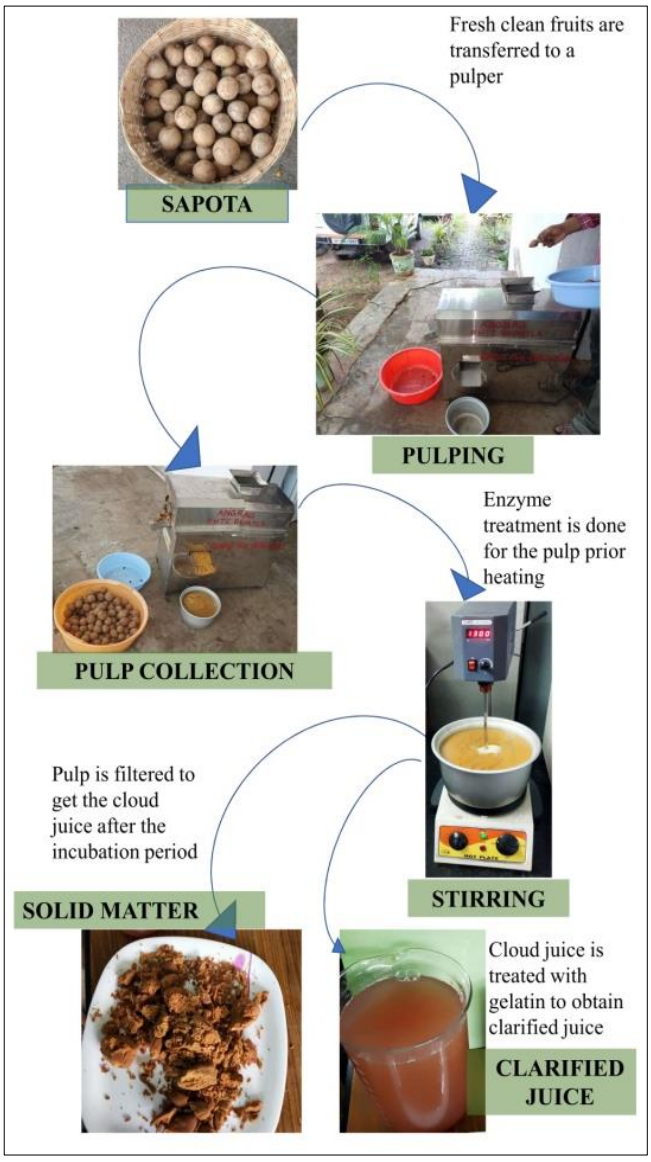

Fig 1: Diagrammatic representation of extraction and clarification of sapodilla fruits.

Table 1: Constraints for optimization of processing parameters and response variable

Parameter	Goal	Lower limit	Upper limit
Enzyme concentration (%)	In range	0.05	0.15
Incubation time (min)	In range	40	120
Temperature (ºC)	In range	30	50
Juice yield (%)	Maximum	57	92.85

Table 2 Optimum solutions for processing parameters of sapodillajuice yield

Parameter	Optimum value
Enzyme concentration (%)	0.1
Incubation time (min)	40
Temperature (ºC)	30
Juice yield (%)	96.901

The significance of the independent parameters (enzyme concentration, incubation time, and temperature) for enhancing sapodilla juice yield was carried out by analyzing the experimental data. The model for sapodilla juice yield was found highly significant (p<0.01) from the ANOVA (Table 3). It can be also seen from the table that at a linear level, incubation time affected the juice yield at 1% level of significance (p<0.01). Moreover, it can be also observed that no significant (p>0.05) interaction effect of enzyme concentration and temperature, temperature and incubation time and incubation time and enzyme concentration on juice yield was observed. Whereas, the statistical analysis indicated that there are many insignificant model terms, hence, model reduction may improve the model. Furthermore, regression analysis was performed to fit the response function, that is, juice yield. Coefficient of determination, R^2, is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon, 1977) [8]. When R^2 approaches unity, the better the empirical model fits the actual data. The R^2 value for response variable yield was 0.93, which was higher than 0.8, indicating that the regression model explained the reaction well. Developed quadratic model equation in actual terms is shown in Eq. 4.

Table 3: Analysis of variance for quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1901.41	9	211.27	11.58	0.002	significant
A-Temperature	13.06	1	13.06	0.7157	0.4255	
B-Incubation time	747.1	1	747.1	40.95	0.0004	significant
C-Enzyme concentration	6.79	1	6.79	0.3722	0.5611	
AB	77.18	1	77.18	4.23	0.0787	
AC	87.14	1	87.14	4.78	0.0651	
BC	3.65	1	3.65	0.2	0.6682	
A^2	0.3325	1	0.3325	0.0182	0.8964	
B^2	54.31	1	54.31	2.98	0.1281	
C^2	884.23	1	884.23	48.47	0.0002	significant
Residual	127.7	7	18.24			
Lack of Fit	123.08	3	41.03	35.53	0.0024	significant
Pure Error	4.62	4	1.15			
Cor Total	2029.11	16				

$$\text{Yield} = 119.622 - 2.16455\ T - 0.369444\ IT + 729.295\ EC + 0.010981\ T * IT + 9.335\ T * EC + 0.4775\ IT * EC + 0.00281\ T^2 - 0.002245\ IT^2 - 5796.6\ EC^2 \quad (4)$$

where, T is temperature, ºC, IT is incubation time, min and EC is Enzyme concentration (%).

3.2 Effect of processing conditions on juice yield

To visualize the dependence of response (juice yield) with the independent variables temperature, incubation time and enzyme concentration, 3D plots were generated by keeping one variable fixed at the optimum point and varying the other two variables. Three representative plots are shown in Fig. 2. Figure 2(a) illustrates the effect of incubation time and temperature at the optimum enzyme concentration (0.1%) on juice yield. It is evident from the Fig. 2(a) that at all levels of the temperature, with increasing incubation time, the yield was found to be decreased, and also at the lower level of incubation time, with the decreasing temperature, an increase in yield was observed while, at a higher level of incubation time, with the decrease in temperature, the yield was found to be decreased.

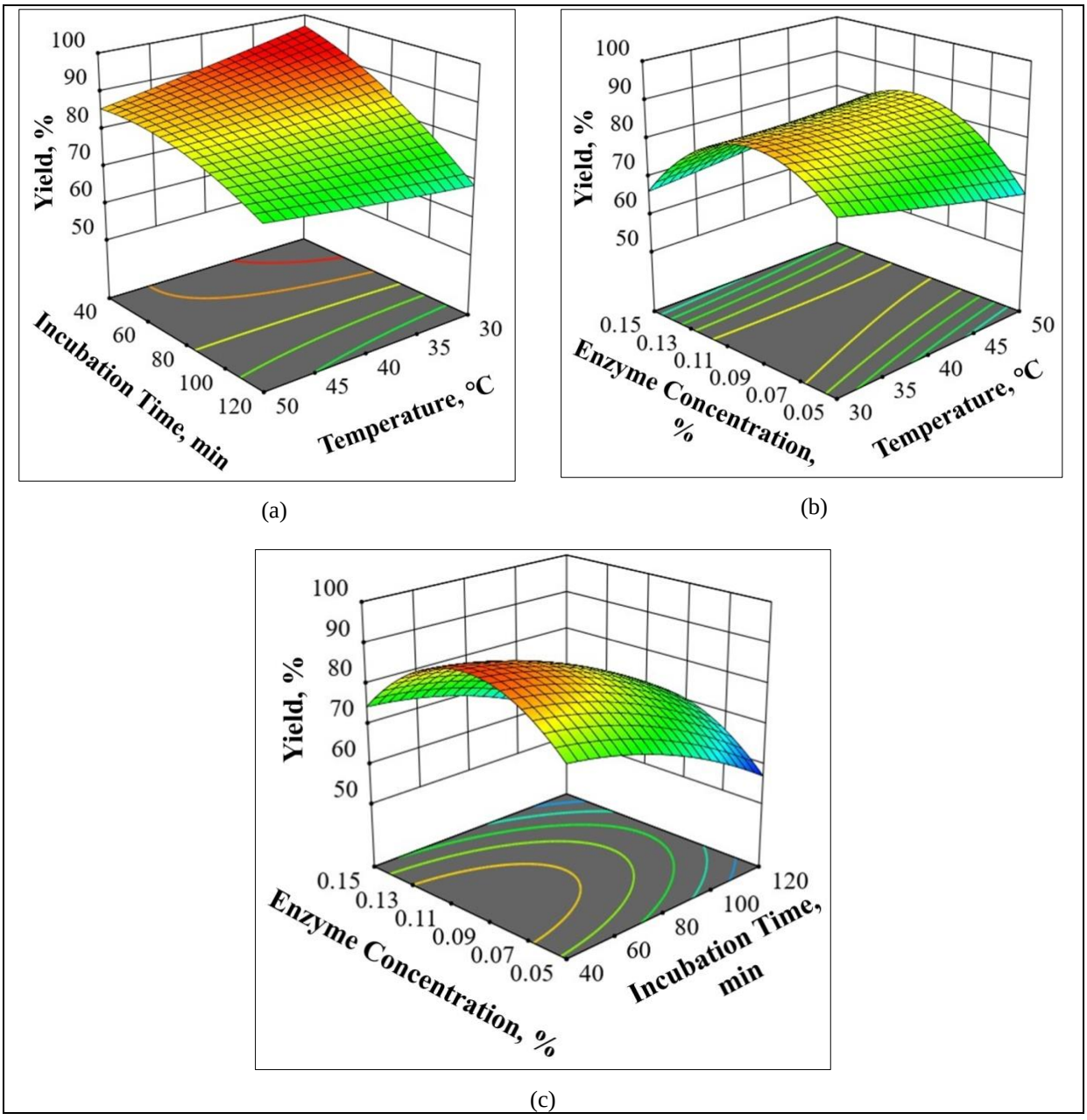

Fig 2: Individual and interaction effect of process parameters on yield during enzymatic clarification of sapodilla juice a) 0.1% enzyme concentration, b) 30 min incubation time and c) 30 ºC temperature

Figure 2(b) shows that keeping incubation time (30 min) constant, at all the levels of temperature, the yield was found to be maximum at the middle level of enzyme concentration and minimum at low and high levels of enzyme concentration. It is also evident that at all levels of enzyme concentration, with the increase in temperature, the yield was found to be slightly decreased. Figure 2(c) shows that keeping temperature (30 ºC) constant, at all the levels of incubation time, the yield was found to be maximum at the middle level of enzyme concentration and minimum at low and high levels of enzyme concentration. It is also evident that at all levels of enzyme concentration, with the increase in incubation time, the yield was found to be decreased. Kaur *et al.* (2009) [12] reported a similar effect while treating with an enzyme in the case of guava pulp. Sin *et al.* (2006) [15] using RSM, optimization of enzymatic clarification of sapodilla juice was done and found at 0.1% enzyme concentration at 40 ºC for 120 min and these results are in agreement with the present study except incubation time was reduced to 40 min, which may be due to continuous stirring during treatment and that may lead to degrade the pectic substance and increased the yield with lesser time and also temperature reduced from 40

to 30 ºC in present study. Kilara (1982) [13] stated that temperature may aid in the rate of enzymatic clarification and suggested moderate temperature should be used during enzymatic clarification. Similarly Kareem and Adebowale (2007) [11] also obtained optimum yield of 97% when treated with 1% pectic enzyme for clarification of orange juice. Singh *et al.* (2012) [16] reported for bael fruit, keeping incubation temperature of 35 ºC, time 210 min with enzymatic concentration of 24 mg/100g as optimized process parameters and obtained clarified juice. In another study Sreenath and Santhanam (1992) [18] obtained maximum white grape yield at optimum process parameters of 30 min incubation time, 30 ºC temperature and at enzyme concentration of 0.048%. Abdullah *et al*. (2007) [1] had obtained similar results for carambola juice at incubation time of 20 min, temperature of 30 ºC at enzyme concentration of 0.1%.

Experiment is conducted once again using the predicted optimized process treatment conditions at the enzyme concentration of 0.1% of sapodilla pulp, incubation time of 40 min, and incubation temperature of 30 °C with stirring at 1500 rpm and maintaining uniform temperature controlled with thermocouple and obtained maximum clarified sapodilla juice yield of 93.05% which was varied with 3.97% only

4. Conclusion

The present study revealed that sapodilla juice yield is a function of enzymatic hydrolysis pretreatment conditions. A significant regression model describing the variation of juice yield with respect to the independent variables (enzyme concentration, temperature, and incubation time) was established with the coefficient of determination, R^2>0.8. The enzyme concentration was the highly significant (p<0.05) variable affecting the juice yield. The recommended enzymatic treatment condition from the study was: enzyme concentration of 0.1% of sapodilla pulp, incubation time of 40 min, and incubation temperature of 30 °C.

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