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LOW-TEMPERATURE EXTRACTION OF JAMUN JUICE (INDIAN BLACK BERRY) AND OPTIMIZATION OF ENZYMATIC CLARIFICATION USING BOX-BEHNKEN DESIGN

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ABSTRACT

Extracting juice from Jamun is difficult. The fruit consists a seed inside and cell wall consists of high amount of pectin-protein bond which makes the pulp thicker. The Box-Behnken design is a type of response surface design which is as useful as the central composite designs but avoiding treatment combinations that are in extreme. The objective of the study is to develop a process for optimization of process parameter to obtain Jamun juice using pectinase enzyme from the strain *Aspergillus niger* at different temperature (30–50°C), concentration (0.01–0.1% w/v) and time (40–120 min). Physical parameters (yield, turbidity, viscosity, clarity and color) and chemical parameters (polyphenol, protein, TSS and total solid) were evaluated as response variables. Significant regression models with a coefficient of determination (R^2) was established with a value more than 0.9. The recommended enzyme for clarification of juice was 0.09% enzyme concentration at 33°C for 75 min.

PRACTICAL APPLICATIONS

The juice of Jamun as well as the concentrate has a high-therapeutic value. A fresh fruit contains all the nutrients needed for good health, but because it may not always be possible to obtain fresh food, preservation becomes necessary. The cell structure of fruit contains protein-pectin bond and high amount of tannin. Due to this nature removal of juice is difficult. Using of enzyme to clarify the juice not only increase the yield but retained the original physicochemical properties of the juice. To get the juice through out the year and to help the diabetic people this study is useful.

INTRODUCTION

Among a few tropical fruits, Jamun [Indian Black Berry] (*Syzygium cuminii*) is one of the vital indigenous minor organic product in India with high-therapeutic worth. The fruit is fleshy, ovoid in shape and dark purple colored with a hard seed inside. The duration of harvesting of Jamun is very short (30– 40 days) during monsoon months (May–July). Jamun has a higher level of antioxidant activity compared to other popular fruits like banana, sapota, guava and papaya. Antioxidant vitamins, tannin and anthocyanins are responsible for the higher antioxidant activity of the fruit. The fresh fruit is mainly consumed by people as such and also use for preparing juices, jam, squash. The seed has its own theraputeic value mainly for diarrhea and diabetics. There is a huge loss of this fruit every year due to unorganized cultivation of Jamun. Postharvest management become difficult due to the perishable nature of the fruit.

Fruit juices contain a high amount of pectin. Due to the presence of pectin, cloudiness creates a major problem in fruit juice industry. The cloudiness can be removed by enzymatic depectinization. Pretreatment with enzyme and clarification and concentration through membrane separation of jamun juice can be considered as an advanced step in the beverage industry. Several works have reported on depectinization using pectinases (strains obtained from *Aspergillus niger*) as enzymatic treatment for various fruit juices

(Chamchong and Noomhorm 1991; Yusof and Ibrahim 1994; Isabella *et al.* 1995; Grassin and Fauquembergue 1996; Alvarez *et al.* 1998; Ceci and Lozano 1998; Vaillant *et al.* 1999; Kashyap *et al.* 2001). Pectin-protein complexes are obtained by pectinase enzyme as it hydrolyses pectin. Viscosity will decrease as the juice obtained from the pectinase treatment will have a much lower amount of pectin, which can also beneficial for filtration processes. As a result, higher clarity, as well as more concentrated flavored and colored juice can be obtained as a final product (Blanco *et al.* 1999; Mutlu *et al.* 1999; Kaur *et al.* 2004; Abdullah *et al.* 2007; Ghosh *et al.* 2015).

Response surface methodology (RSM) is characterized as the factual instrument that uses quantitative information from proper exploratory configuration to decide and all the while understand multivariate mathematical statements (Giovanni 1983). Numerous trial plans are accessible to apply the RSM strategy (Cornell 1990). The principal objective for RSM is to locate the ideal reaction. At the point when there is more than one reaction, then it is critical to discover the trade off ideal that does not improve stand out reaction (Oehlert 2000). Box-Behnken configuration is a three-level factorial course of action. All element levels must be balanced just at three levels with similarly divided interims between these levels (Bezerra *et al.* 2008). RSM was utilized for synchronous investigation of the impacts and streamlining of enzymatic treatment states of brooding time, hatching temperature and chemical fixation on physical attributes. RSM has been utilized broadly to optimize forms as a part of the tropical natural product juice generation (Yusof *et al.* 1988; Wong *et al.* 2003; Lee *et al.* 2006; Sin *et al.* 2006).

The objectives of the present study were first to use the commercial pectinase (*A. niger*) for clarification and juice extraction from Jamun fruit with a better yield and use the RSM to determine the optimal conditions for incubation time, temperature and concentration. Also the change in physical properties like viscosity, turbidity, yield, clarity and color and chemical properties like total polyphenol, protein concentration, TSS and total solid were evaluated. This study will be useful for the food industry for obtaining a high yield of Jamun juice. This study will also explore the methods of increasing the clarity and yield with a decreasing haze formation (cloud stability) of the juice.

MATERIALS AND METHODS

Sample

Fresh, mature and ripen Jamun (Ram *Jamun*) were obtained from the local area of Rourkela, Odisha, India (located at 84.54°E longitude and 22.12°N latitude). Due to the perishable nature of the fruits, the samples were cleaned and washed manually and packed in perforated polythene bags and stored at −20°C for further use. Before experiments, samples are taken out from deep freezer and thawed for 3 h at 25°C.

Enzyme and Other Chemicals

Pectinase (EC 232-885-6, activity 8000–12000 U/g) from *A. niger*, potassium sodium tartrate, hydrazine sulfate and hexamine LR grade were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India, and used for enzymatic treatment and for preparing the stock solution of turbidity meter. For protein estimation Folin–Ciocalteu reagent was purchased from Sigma-Aldrich, Bangalore, India. Anhydrous sodium carbonate from Sisco Research Pvt. Ltd., Mumbai, India, and copper (II) sulfate pentahydrate were procured from Merck Specialists Pvt. Ltd., Mumbai, India. For calibration of protein estimation, Bovine serum albumin (BSA) was purchased from Otto Chemie Pvt. Ltd., Mumbai, India. For calibrating polyphenol content Gallic acid standard also obtained from Otto Chemie Pvt. Ltd., Mumbai, India. The commercial grade filter papers (Whatman filter paper no. 1) were procured from Whatman, GE Healthcare UK Ltd., UK, and the glassware used for the experiment was obtained from Borosil Glass Works Ltd., Mumbai, India. All the chemicals listed above were of analytical grade.

Method of Juice Extraction

Jamun fruits were stored in a deep freezer. Fruits were selected and washed properly to eliminate any microbial contaminations and to remove dust and foreign particles. Then the fruits were kept at room temperature for 3 h. Before juice extraction, seeds were removed manually from the fruits, to avoid its bitterness. Then the thick pulp of the fruit was mixed in a mixer (Bajaj Mixer, India) for 5 min at its maximum speed (2500 rpm). Afterward, the mixed pulp was transferred into small beakers, and the enzyme was added according to the experimental design with a convenient time and temperature.

Enzymatic Treatment

After collecting the homogenized pulp from the mixer, 100 g of the mixed pulp was treated with enzymatic conditions as per the experimental design shown in Table 1. The scope of the variables for enzymatic treatment conditions depended on the preparatory trials (information not specified). The independent variables were incubation temperature X_1 (30–50°C), enzyme concentration, X_2 (0.01–0.1 w/v %) and incubation time, X_3 (40–120 min). The mixture was kept in an incubated orbital shaker (REMI CIS24 PLUS) at 120 rpm to adjust the particular temperature with a vigorous mixing throughout the incubation period. The initial pH remained constant as it is considered as exo-pectinase (Kashyap *et al.* 2001). The method of heat treatment for inactivation of enzyme was excluded as it can change the juice flavor, color and nutritional properties. So at the end of the treatment pectinase was inactivated by keeping the suspension at −2°C for 5 min (Molinari and Silva 1997; Sandri *et al.* 2011). The mixture was cooled and filtered through a muslin cloth to obtain the juice. The filtrate was collected to analyze various physical and chemical property. Control samples, where there is no enzymatic treatment, were made.

Analysis of Juice

Physical properties (viscosity, turbidity, yield, clarity and color) and chemical properties (protein concentration, polyphenol, TSS and total solid) were response variables that were subjected to optimize.

Viscosity. The viscosity of juice was dictated by a U-Tube slim viscometer (Zenith Glassware, Kolkata, India) at room temperature (28 ± 10°C). The unit of measured viscosity was represent as mPa.s (Sagu *et al.* 2014).

Turbidity. Digital turbidity-meter was utilized to measure turbiduty (Model 335, Deluxe Company, India) and the outcomes were accounted for as Nephelometric Turbidity Units (NTU) (Sin *et al.* 2006).

Yield. A known weight of the Jamun was minced altogether in a blender, and the blender was keep running till blending of entire mash was accomplished. The ground material was sifted through muslin cloth with numerous folds to particular the mash pieces out totally (Shahnawaz and Sheikh 2011).The volume of the filtrate was measured in measuring cylinder. The rate of juice in the given example of Jamun was given as:

$$\text{Percentage Juice content} = \frac{\text{Vol of filtrate (mL)}}{\text{weight of the fruit (g)}} \times 100$$

Clarity. Clarity of the concentrate was measured by transmittance (%T) at 660 nm utilizing the spectrophotometer (Model:AU 2701, Systronics India Ltd.) (Rai *et al.* 2006).

Color. Color measurements were performed by Hunter colorimeter (Colorflex EZ). The instrument was standardized during each sample measurement with a black and a white tile, and the color values represented whiteness or brightness/darkness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*). L^*, a^*, b^* values were measured for clarified juice.

Protein. Protein concentration was determined with BSA as standard according to the dye binding method of Lowry *et al.* (1951).

TABLE 1. EXPERIMENTAL DESIGN INDICATING CODED AND ACTUAL VALUES OF INDEPENDENT VARIABLES

	Coded variables			Real variables		
Exp. No	x_1	x_2	x_3	X_1 (°C)	X_2 (%)	X_3 (min)
1	−1	−1	0	30	0.01	80
2	1	−1	0	50	0.01	80
3	−1	1	0	30	0.10	80
4	1	1	0	50	0.10	80
5	−1	0	−1	30	0.06	40
6	1	0	−1	50	0.06	40
7	−1	0	1	30	0.06	120
8	1	0	1	50	0.06	120
9	0	−1	−1	40	0.01	40
10	0	1	−1	40	0.10	40
11	0	−1	1	40	0.01	120
12	0	1	1	40	0.10	120
13	0	0	0	40	0.06	80
14	0	0	0	40	0.06	80
15	0	0	0	40	0.06	80
16	0	0	0	40	0.06	80
17	0	0	0	40	0.06	80

Polyphenol. The aggregate phenolic substance of Jamun juice was determined utilizing a spectrophotometer (Model:AU 2701, Systronics India Ltd.) at 650 nm taking after the Folin–Ciocalteu method as portrayed by Singleton *et al.* (1999). The juice test (0.5 mL) clear or standard was set in a 25 mL jar and 0.5 mL of the Folin–Ciocalteu reagent was included. After 5 min 10 mL of 7.5% (w/v) Na_2CO_3 arrangement was included with consistent mixing. Distilled water was added to make up the volume of the solution upto 25 mL and after that it was kept at room temperature for 60 min. A standard alignment bend was plotted utilizing Gallic corrosive as a part of the fixation range 1–500 mg/L. The outcomes are communicated as milligrams of Gallic acid equivalent per gram.

TSS. The total soluble sugar (TSS) in the sample were determined with the help of an Abbe-type Refractometer, and the values were expressed as degree Brix (°B).

Total Solid. In the case of total solid the weight of an empty petri dish (Wp) was taken. Five millilters of sample was taken in the petri dish and measured the weight (Ws). Then the sample was kept in hot air oven at 100°C for 24 h. The weight of the dried sample along with the Petri dish (W_d) was recorded (Sagu *et al.* 2014).

$$\%\text{Total solid} = \frac{W_d - W_p}{W_s - W_p} \times 100$$

Experimental Design

RSM (Box-Behnken Design) was utilized to decide the ideal conditions for Jamun juice. The exploratory configuration

TABLE 2A. BOX-BEHNKEN DESIGN: REAL VARIABLES AND EXPERIMENTAL RESPONSES (PHYSICAL PARAMETERS)

Real variables			Experimental responses													
			Yield (%)		Clarity (% abs)		Turbidity (NTU)		Viscosity (mPa·S)		L^* value		a^* value		b^* value	
X_1 (°C)	X_2 (%)	X_3 (min)	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre
30	0.01	80	78.50	77.25	0.144	0.14	40.00	44.17	1.167	1.15	0.93	1.04	1.40	2.10	0.04	−0.39
50	0.01	80	70.15	68.25	0.295	0.26	80.00	83.07	3.214	3.23	6.39	6.40	6.46	6.00	−2.81	−2.58
30	0.10	80	76.10	77.75	0.141	0.17	75.00	71.93	1.211	1.20	1.13	1.12	2.53	2.99	−0.02	−0.25
50	0.10	80	68.25	68.75	0.344	0.35	120.00	115.82	3.489	3.50	6.89	6.78	9.69	8.99	−4.41	−3.98
30	0.06	40	80.05	78.75	0.132	0.12	90.00	88.13	1.256	1.57	1.05	0.99	2.22	1.04	−0.02	0.58
50	0.06	40	73.45	72.75	0.292	0.30	148.00	147.23	3.412	3.70	6.03	6.06	6.42	6.40	−2.65	−2.71
30	0.06	120	81.15	81.25	0.133	0.12	85.00	86.18	1.271	0.98	1.06	1.03	2.06	2.08	−0.03	0.02
50	0.06	120	68.95	69.25	0.227	0.24	108.00	109.87	3.547	3.23	6.91	6.97	5.44	6.62	−1.99	−2.59
40	0.01	40	68.45	70.00	0.221	0.24	154.00	151.70	2.248	1.95	3.22	3.18	4.53	5.02	−0.65	−0.82
40	0.10	40	72.55	71.50	0.341	0.32	182.00	186.95	2.547	2.24	3.04	3.11	6.96	7.68	−1.38	−1.75
40	0.01	120	70.45	70.50	0.225	0.23	142.00	137.05	1.241	1.55	3.42	3.35	7.09	6.37	−1.72	−1.35
40	0.10	120	72.50	70.00	0.289	0.27	160.00	162.30	1.271	1.57	3.84	3.88	8.08	7.60	−1.85	−1.68
40	0.06	80	74.45	74.80	0.178	0.18	58.00	59.20	1.304	1.31	1.26	1.25	2.74	2.73	−0.28	−0.29
40	0.06	80	75.15	74.80	0.177	0.18	59.00	59.20	1.313	1.31	1.25	1.25	2.68	2.73	−0.24	−0.29
40	0.06	80	75.50	74.80	0.180	0.18	61.00	59.20	1.315	1.31	1.24	1.25	2.70	2.73	−0.35	−0.29
40	0.06	80	75.65	74.80	0.179	0.18	60.00	59.20	1.315	1.31	1.25	1.25	2.78	2.73	−0.33	−0.29
40	0.06	80	75.50	74.80	0.176	0.18	58.00	59.20	1.312	1.31	1.24	1.25	2.73	2.73	−0.27	−0.29

and measurable investigation were completed utilizing Design Expert Software (Version 8.0.7.1).

Box-Behnken configuration was utilized to think about the joined impact of three autonomous variables, for example, incubation temperature, enzyme concentration and incubation time coded as X_1, X_2, X_3, separately, which are the key element for the enzymatic treatment of juice. The maximum and minimum values for incubation temperature was 50 and 30°C, where enzyme concentration ranges from 0.01 to 0.1% with an incubation time ranges between 40 and 120 min. The total design consists of 17 experiments with five replication at the center point. The coded and real values are mentioned in Table 1. The mathematical model indicates the effects of variables in terms of linear, quadratic and interaction terms shown below:

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j$$

where Y is the experimental responses, the levels of variables are denoted as X_i and X_j. b_0 is the constant, b_i is the linear coefficient, b_{ii} is the quadratic term and b_{ij} is the coefficient of the interaction terms.

ANOVA (analysis of variance) was used to validate the model. Keeping one variable constant at the center point and varying the other two variables within the experimental range the three-dimensional plots were drawn. Eleven responses (physical responses – viscosity, turbidity, yield, clarity and color and chemical responses – protein concentration, polyphenol, TSS and total solid) were selected.The contour plots were represented with model equations to describe the individual and cumulative effects on the responses.

All the experiments were repeated three times. Various quality parameters were measured, and the average values were reported. Validation of the model was done by the coefficient of determination (R^2) value. The heights value (near to 1.0) was considered as the proper model equation for expression of responses.

RESULTS AND DISCUSSION

Fresh Jamun juice (without any treatment) has a viscosity of 1.21 mPa·S, turbidity of 116 NTU and clarity of 0.165%abs with a yield of 65%. The color value for the raw juice is L^*(5.76), a^*(2.65) and b^*(−1.68). Nutritional composition of juice has 132.70 mg/g of protein, 89.08 mgGAE/g of polyphenol, 13°B with 12.94% of total solid. RSM (Box-Behnken design) was employed for optimizing both physical and chemical parameters of Jamun juice. Effect of three factors, incubation time, temperature and concentration on the experimental responses, i.e., physical parameters (yield, viscosity, turbidity, clarity and color) and chemical parameters (protein, polyphenol, TSS and total solid) are represented in Tables 2a and b, respectively. The regression coefficient of the variables in the models for the second order polynomial equations and results for the linear, quadratic and interaction terms and corresponding R^2 values are represented in Table 3. The ANOVA for all the responses showed that the proposed models can explain more than 90% experimental observations as a function of independent variables. The

TABLE 2B. BOX-BEHNKEN DESIGN: REAL VARIABLES AND EXPERIMENTAL RESPONSES (CHEMICAL PARAMETERS)

Real variables			Experimental responses							
			Protein (mg/g)		Polyphenol (mg GAE/g)		TSS (°B)		Total solid (%)	
X_1 (°C)	X_2 (%)	X_3 (min)	Act	Pre	Act	Pre	Act	Pre	Act	Pre
30	0.01	80	322.48	271.94	114.27	105.74	13.10	13.29	15.4	15.49
50	0.01	80	153.55	155.22	101.57	98.69	13.80	13.84	13.69	13.20
30	0.10	80	333.25	331.58	58.98	61.86	13.60	13.56	13.05	13.54
50	0.10	80	102.00	152.24	112.86	121.40	14.60	14.41	15.38	15.29
30	0.06	40	250.34	272.35	58.37	66.67	13.40	13.34	16.81	16.03
50	0.06	40	112.70	82.49	75.06	77.71	13.90	13.99	13.69	13.50
30	0.06	120	157.17	187.38	57.10	54.45	13.80	13.71	13.21	13.40
50	0.06	120	103.48	81.48	104.20	95.90	14.40	14.46	14.62	15.40
40	0.01	40	158.87	187.40	103.09	103.32	13.00	12.87	13.10	13.78
40	0.10	40	286.53	266.20	96.31	85.13	13.20	13.30	11.82	12.11
40	0.01	120	174.40	194.73	87.51	98.69	13.40	13.30	11.96	11.67
40	0.10	120	201.42	172.89	95.96	95.73	13.60	13.73	14.17	13.49
40	0.06	80	225.61	224.96	99.70	98.28	13.80	13.76	14.38	14.11
40	0.06	80	226.10	224.96	97.22	98.28	14.00	13.76	14.43	14.11
40	0.06	80	223.48	224.96	98.21	98.28	13.80	13.76	14.21	14.11
40	0.06	80	224.15	224.96	99.04	98.28	13.60	13.76	13.98	14.11
40	0.06	80	225.97	224.96	97.21	98.28	13.60	13.76	14.28	14.11

proposed model was adequate with satisfactory values of R^2. The R^2 value for the physical parameters yield, viscosity, turbidity, clarity and color were 0.92, 0.94, 0.99, 0.95 and 0.99, respectively. For chemical parameters protein, polyphenol, TSS and total solid the R^2 values were 0.90, 0.91, 0.90 and 0.92, respectively. The values of the R^2 closer to the unity shows the better fit for the empirical models with the actual data.

Effects of Variables on Yield

As mentioned in Table 3, temperature (A) ($P < 0.0001$) and time (C) ($P < 0.001$) had a linear negative effect on the yield of the juice and quadratic parameters, concentration (B) and time (C) also possess negative effect with a significant value ($P < 0.01$). The regression model representing the effect of temperature, concentration and time on the yield of the Jamun juice, in terms of their real level is given as:

$$\text{Yield (\%)} = 74.80 - 4.50\text{A} + 0.25\text{B} - 0.25\text{C} + 1.60\text{A}^2 - 3.40\text{B}^2 - 0.90\text{C}^2 + 0.01\text{AB} - 1.50\text{AC} - 0.50\text{BC}$$

The value of coefficient of determination R^2 for the above equation is 0.92. This value indicates that the regression model is able to explain 92% of variability of the data. Figure 1a shows that as the enzyme concentration increases yield also increases. Yield is proportionally increases with time but when there is an interaction effect of time and temperature, with increase in temperature, yield decreases (Fig. 1b). Whereas time and concentration has the maximum effect on the yield of the juice. At a concentration of 0.06% with

TABLE 3. REGRESSION COEFFICIENTS AND R^2 VALUE FOR ALL DEPENDENT VARIABLES FOR ENZYMATIC CLARIFIED JAMUN JUICE

Regression coefficient	Yield (%)	Viscosity (mPa·S)	Turbidity (NTU)	Clarity (%abs)	L	a^*	b^*	Protein (mg/g)	Polyphenol (mgGAE/g)	TSS (°B)	Total solid (%)
b_0	74.80	1.31	59.20	0.18	1.25	2.73	−0.29	224.96	98.28	13.76	14.11
A (Temperature)	−4.50	1.09	20.70	0.076	2.76	2.48	−1.48	−73.94	13.12	0.35	−0.14
B (Concentration)	0.25	0.081	15.13	0.030	0.12	0.97	−0.32	14.24	−5.29	0.21	0.034
C (Time)	−0.25	−0.27	−9.82	−0.015	0.24	0.32	−0.11	−21.80	1.49	0.21	−0.18
A^2	1.60	0.75	−16.05	$-8.75 * 10^{-3}$	1.48	−0.17	−0.64	−23.26	−11.70	0.29	1.05
B^2	−3.40	0.21	35.60	0.062	1.10	2.46	−0.87	26.12	10.34	−0.28	−0.77
C^2	−0.90	0.31	64.70	0.027	1.03	1.48	−0.24	−45.78	−12.90	−0.18	−0.57
AB	0.01	0.058	1.25	0.013	0.075	0.52	−0.39	−15.58	16.65	0.075	1.01
AC	−1.50	0.030	−8.85	−0.017	0.22	−0.02	0.17	20.99	7.60	0.025	1.13
BC	−0.50	−0.067	−2.50	−0.012	0.15	−0.36	0.15	−25.16	3.81	0.003	0.87
R^2	0.922	0.947	0.995	0.950	0.999	0.943	0.940	0.901	0.912	0.906	0.926

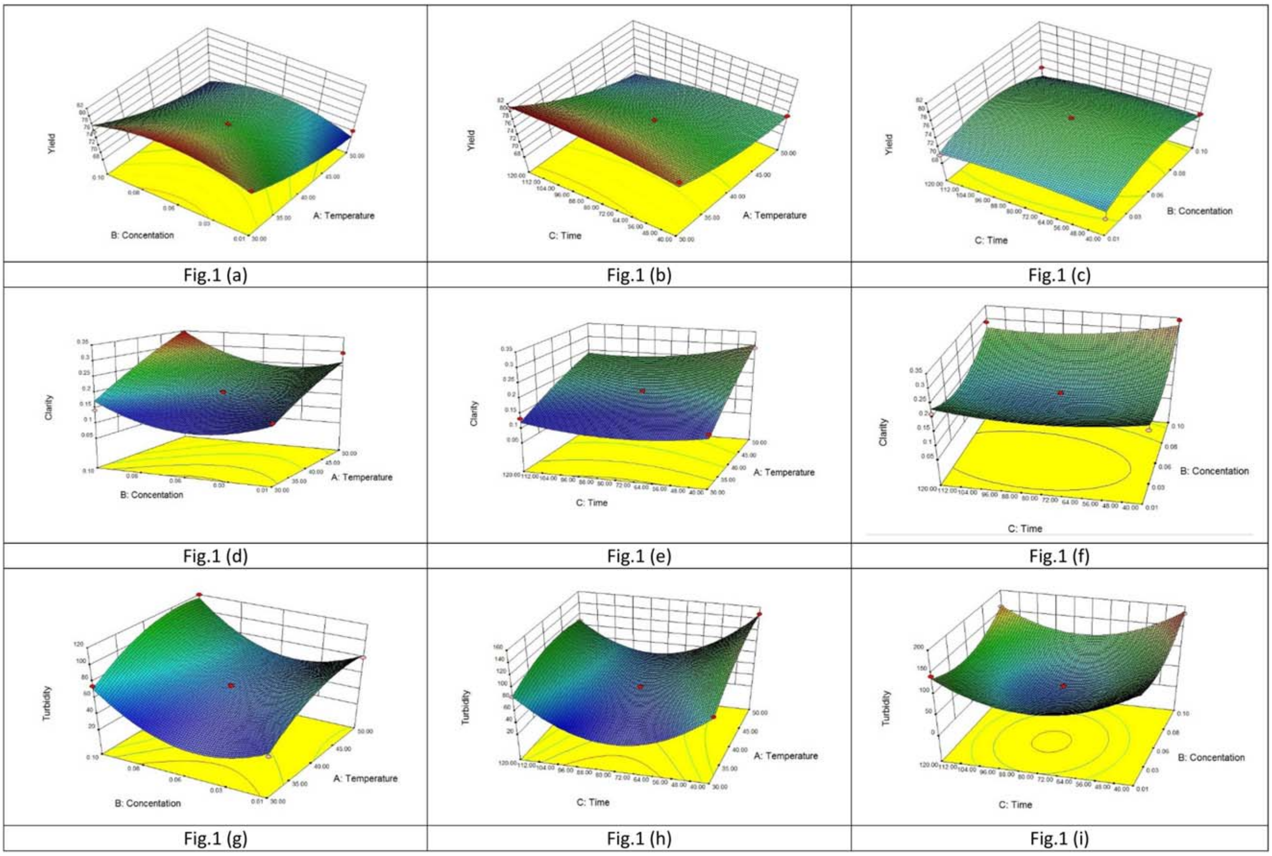

FIG. 1. RESPONSE SURFACES FOR YIELD, CLARITY AND TURBIDITY AS A FUNCTION OF (a, d, g) TEMPERATURE AND ENZYME CONCENTRATION, (b, e, h) TEMPERATURE AND TIME AND (c, f, i) ENZYME CONCENTRATION AND TIME

120 min time, yield was maximum. As the pectin and protein content breakdown with the help of the enzyme with a certain concentration and time-temperature combination, so the juice yield increases. The yield increases up-to a level and then again decreases. Similar type of result in case of eldberry juice was obtained by Landbo *et al.* (2007).

Effects of Variables on Clarity

One of the most important index of clarified juice is clarity. From the Table 3, it is observed that temperature (A) ($P < 0.0001$) and concentration (B) ($P < 0.001$) has a positive effect whereas time (C) has a negative effect. Concentration has most significant effect on clarity as the quadratic value also shows positive effect ($P < 0.0001$). The regression model for clarity representing the effect of temperature, time and concentration on the Jamun juice extracts in terms of their real value is given as:

$$\text{Clarity (\% Abs)} = 0.18 + 0.076A + 0.030B - 0.015C - 8.75 * 10^{-3}A^2 + 0.062B^2 + 0.027C^2 + 0.013AB - 0.016AC - 0.012BC$$

The coefficient of determination (R^2) for the equation is 0.95 which indicates that the regression model is able to explain 95% of variability of data. Figure 1d shows that as the temperature increases clarity also increases. In case of enzyme concentration, first the clarity goes down and then again it increases. From Fig. 1e, combination of temperature and time has a negative slope on clarity. From Fig. 1f, it can be concluded that time has no significant effect on clarity. Lower absorbance values indicate clear juice. Surajbhan *et al.* (2012), Sagu *et al.* (2014) and Sin *et al.* (2006) had obtained similar type of result for guava juice, banana juice and sapodilla juice clarification, respectively.

Effects of Variables on Turbidity

Effect of temperature, concentration and time on turbidity is mentioned in Fig. 3. As mentioned in Table 3, among three factors temperature ($P < 0.0001$) and concentration ($P < 0.0001$) has a positive linear effect and time ($P < 0.001$) has a negative linear effect. Quadratic responses show the positive effect where concentration and time has significant value ($P < 0.0001$). The regression model for turbidity with the coded value can be represented as:

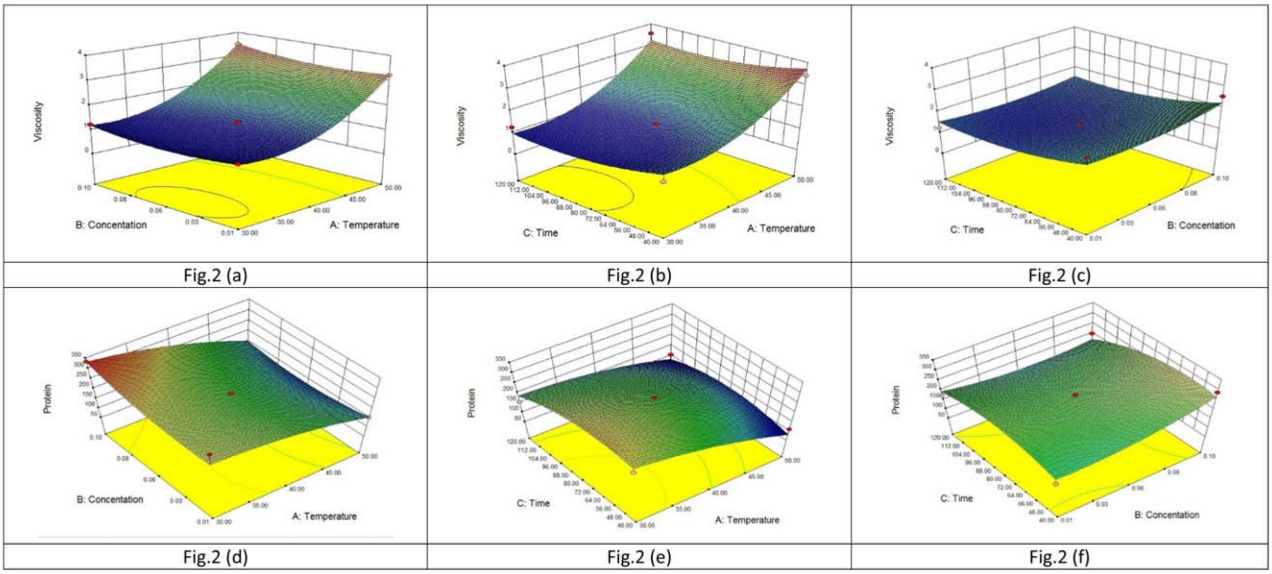

FIG. 2. RESPONSE SURFACES FOR VISCOSITY AND PROTEIN AS A FUNCTION OF (a, d) TEMPERATURE AND ENZYME CONCENTRATION, (b, e) TEMPERATURE AND TIME AND (c, f) ENZYME CONCENTRATION AND TIME

$$\text{Turbidity (\% NTU)} = 59.20 + 20.70A + 15.130B - 9.82C - 16.05A^2 + 35.60B^2 + 64.70C^2 + 1.25AB - 8.85AC - 2.50BC$$

The coefficient of determination (R^2) for the above equation is 0.99. Thus, it can explain 99% of the total variability of data. From Fig. 1g, effect of temperature and concentration changes in turbidty was ontained. As the concentration of enzyme increases turbidity value decreases up to a certain level 0.06% and then again it increases but with decrease in temperature turbidity also decreases. In Fig. 1h, it is observed that the turbidity is minimum at 80 min with a temperature at its minimum range. Finally in Fig. 1i, it is clearly visible that turbidity is less at the center point. As the clarification took place the amount of pectin decreases which causes the decreasing trend in turbidity. The interaction between concentration and time has a significant role in the turbidity. In case of guava juice clarification similar type of result has been found in previous works (Surajbhan *et al.* 2012). Enzyme concentration at its particular value (0.06%) with 80 min time shows the minimum turbidity value.

Effects of Variables on Viscosity

From Table 3, it is observed that the viscosity was affected by all the three factors such as temperature (A) ($P < 0.0001$), concentration (B) ($P < 0.001$) and time (C) ($P < 0.001$). Viscosity depends on the time as its linear effect is negative and quadratic effect is positive. The interaction terms as well as quadratic terms are also significant. The regression model for viscosity with the coded value can be represented as:

$$\text{Viscosity (mPa} \cdot \text{S)} = 1.31 + 1.09A + 0.081B - 0.27C + 0.75A^2 + 0.21B^2 + 0.31C^2 + 0.058AB + 0.030AC - 0.067BC$$

The coefficient of determination (R^2) for the equation is 0.95 which indicates that the regression model is able to explain 95% of variability for viscosity. Figure 2a shows that viscosity decreases with temperature and became constant at its lower level from 35 to 30°C. But viscosity decreases with an increasing trend in concentration up to 0.06% and then again increases. Again from Fig. 2b, it is observed that as the time increases viscosity decreases at its lowest level. This results give similar view with the literature that shows the effectiveness of pectinase enzyme decreases with an increase in temperature (Kittur *et al.* 2003). In case of high temperature pectin forms gel structure which increases the viscosity where at optimum temperature (30–40°C) pectin reduce the water holding capacity and therefore free water was released which reduce the viscosity. The viscosity was maximum at heighest level of enzyme concentration and time (Fig. 2c).

Effects of Variables on Color

Color is one of the major sensory parameter for juice. L^* value should be as high as possible for clarified juice. From Table 3, it can be observed that temperature (A) ($P < 0.0001$) and concentration (B) ($P < 0.0001$) has a positive linear effect with a positive quadratic effect ($P < 0.001$). a^* value is the representation of green and red color. The more the positive value indicated the redness of the juice. So Table 3 represents that except interaction effect linear and quadratic terms possess positive effect where temperature and enzyme concentration has the maximum effect on the

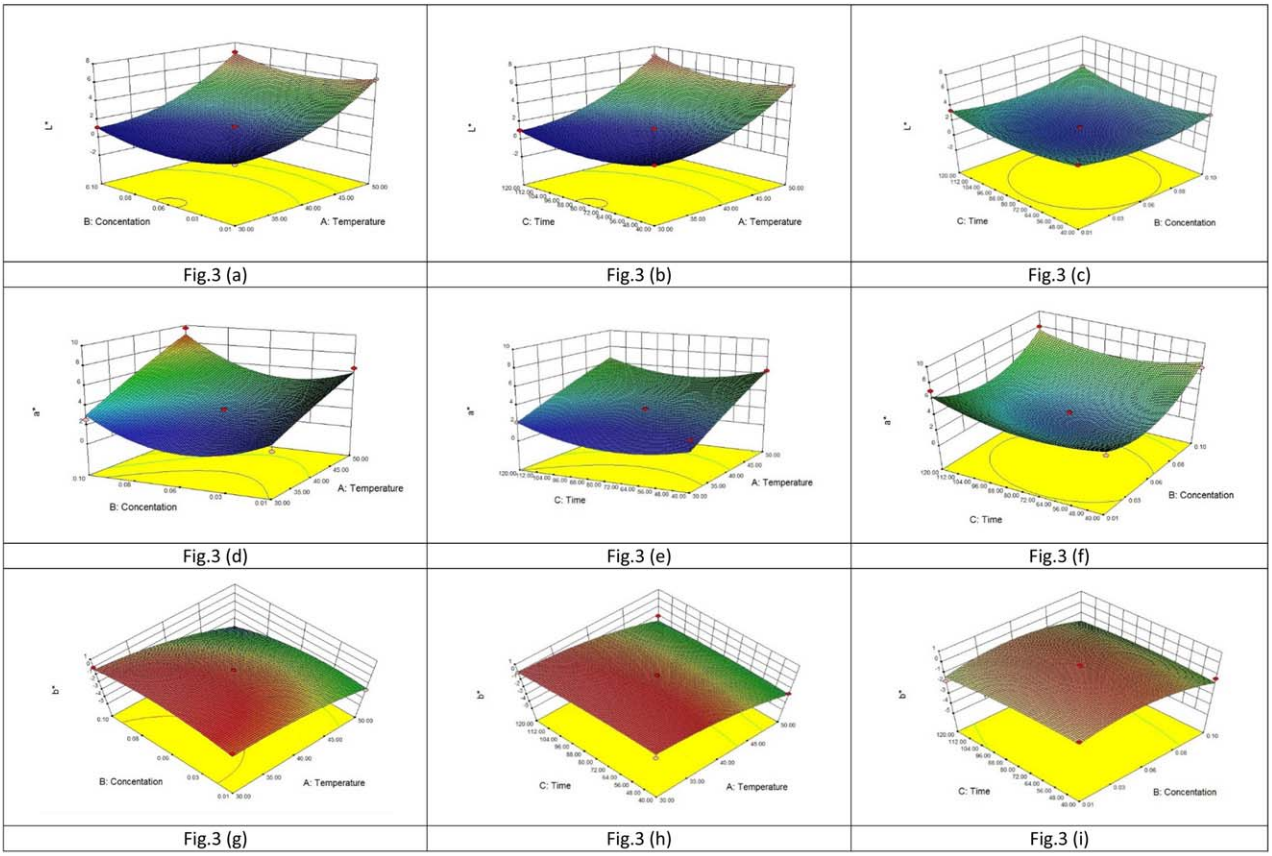

FIG. 3. RESPONSE SURFACES FOR COLOR (L^*, a^*, b^*) AS A FUNCTION OF (a, d, g) TEMPERATURE AND ENZYME CONCENTRATION, (b, e, h) TEMPERATURE AND TIME AND (c, f, i) ENZYME CONCENTRATION AND TIME

L^* and a^* value of the juice. Jamun juice is purple in nature where bluish color can be represented by the negative b^* value. Linear and quadratic effect both are negative ($P < 0.0001$) for b^* value. Here in this case also it can be observed that the value for bluish nature, responsible for the characteristics color of Jamun varies with the amount of enzyme concentration. The regression model for the color (L^*, a^*, b^*) is given as:

$$L* = 1.25+2.76A+0.12B-0.24C+1.48A^2+1.10B^2 +1.03C^2+0.075AB+0.22AC+0.15BC$$

$$a* = 2.73+2.48A+0.97B-0.32C-0.17A^2+2.46B^2 +1.48C^2+0.52AB-0.20AC-0.36BC$$

$$b* = -0.29-1.48A-0.32B-0.11C-0.64A^2-0.87B^2 -0.24C^2-0.39AB+0.17AC+0.15BC$$

The coefficient of determination for L^*, a^* and b^* equations is 0.99, 0.94, 0.94, respectively, which indicates that the model is able to explain 99, 94 and 94% of the variability of the data. Figure 3a shows the interaction effect of concentration and temperature on L^* value. At highest concentration and temperature L^* value is maximum. Time has no significant effect on the L^* value of the juice (Fig. 3b). Sin *et al.* (2006) observed similar trend in case of L^* value for the enzymatic clarification of sapodilla juice. The degradation of pectin is maximum at a high concentration of enzyme and settlement of the fine particles with time which has an effect on the whole color value. The same trend is visible for a^* value (Fig. 3d). It is clearly observed that time and concentration of enzyme has a significant effect on a^* value (Fig. 3f). But in case of b^* value from Fig. 3g a parabolic curve obtained which can conclude that concentration of enzyme and temperature has a negative slope with a maximum value of b^* with -3.97. In case of interaction between time and concentration of enzyme the minimum value obtained is -1.75 as shown in Fig. 3i.

Effects of Variables on Protein

Protein is one of the major nutritional property of juice which is generally affected by temperature. The effects of temperature, enzyme concentration and time were also

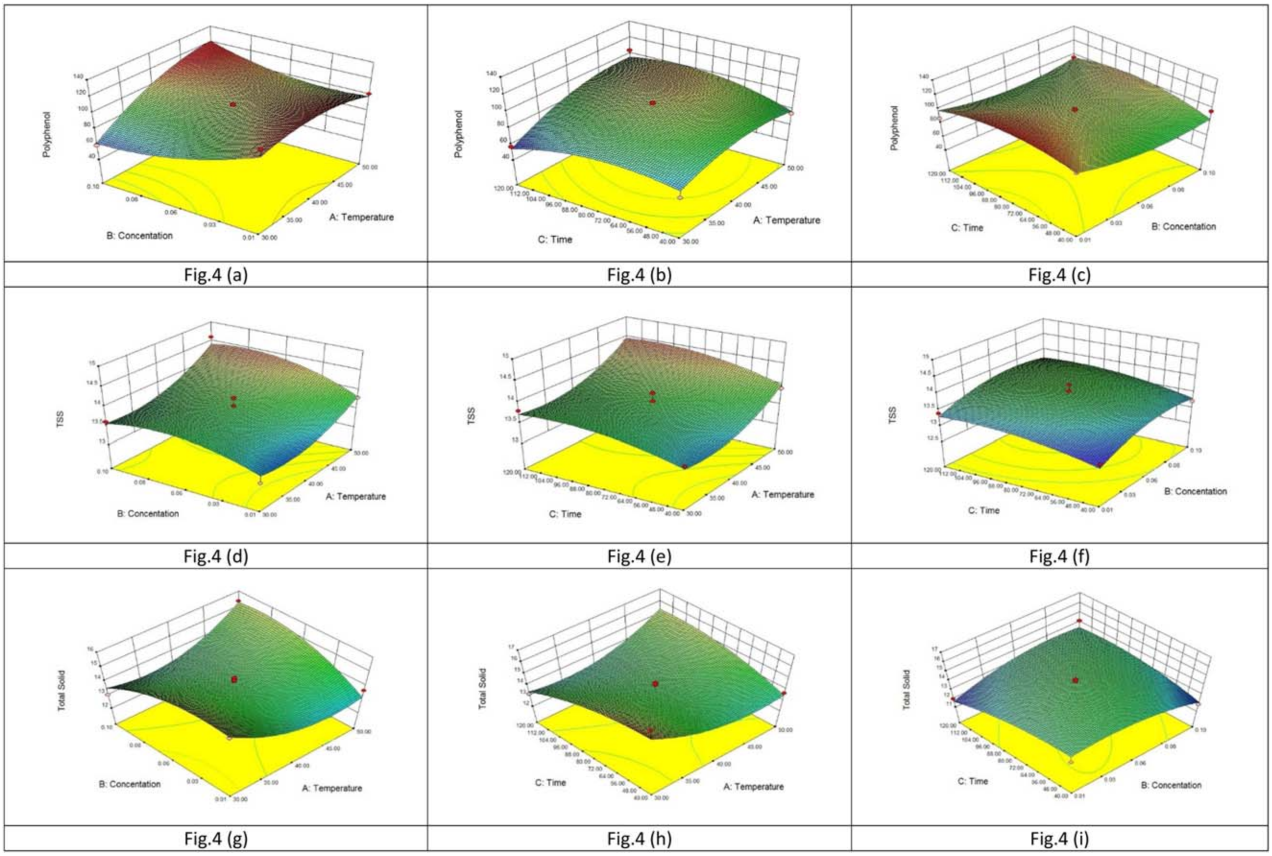

FIG. 4. RESPONSE SURFACES FOR POLYPHENOL, TSS AND TOTAL SOLID AS A FUNCTION OF (a, d, g) TEMPERATURE AND ENZYME CONCENTRATION, (b, e, h) TEMPERATURE AND TIME AND (c, f, i) ENZYME CONCENTRATION AND TIME

studied on protein content. Temperature (A) ($P < 0.001$) and time ($P < 0.0001$) has a significant effect on the protein content. Both the linear effect and interaction effect is negative in case of temperature and time of interaction. The regression model for protein with the coded value can be represented as:

$$\text{Protein (mg/g)} = 224.96 - 73.94A + 14.241B - 21.80C - 23.26A^2 + 26.12B^2 - 45.78C^2 - 15.58AB + 20.99AC - 25.16BC$$

The coefficient of determination (R^2) for the equation is 0.90 which indicates that the regression model is able to explain 90% of variability of the protein concentration of the Jamun juice. From the Fig. 2d, the maximum amount of protein retention is observed at 0.10% concentration with a minimum temperature (30°C). As the temperature increased the degradation of protein also increase. Figure 2e shows that negative effect is observed for time-temperature combination on protein value. From Fig. 2f, it is clearly understood that at the maximum time protein concentration is less and at the minimum timing retention of protein is maximum. Sagu *et al.* (2014) reported the same trends in protein value for banan juice clarification in their work.

Effects of Variables on Polyphenol

Jamun is rich in polyphenol content. In case of temperature (A) ($P < 0.001$) and time (C) ($P < 0.001$) linear effect is positive where as in case of concentration (B) ($P < 0.001$) it is negative. The interaction effect is positive in nature ($P < 0.05$). Concentration and temperature has a significant effect on polyphenol content. The regression model for polyphenol with the coded value can be represented as:

$$\text{Polyphenol (mg GAE/g)} = 98.28 + 13.12A - 5.291B + 1.49C - 11.70A^2 + 10.34B^2 - 12.90C^2 + 16.65AB + 7.60AC + 3.89BC$$

The value for coefficient of determination (R^2) is 0.91 which indicates that the regression model is able to explain 91% of variability of the polyphenol concentration of the Jamun juice. From Fig. 4a, it is visible that maximum amount of polyphenol will be obtained at highest concentration and temperature. Time (C) has also significant effect in polyphenol content. Degradation of polyphenol takes place from the cell wall of the Jamun fruit due to the concentration of enzyme. The effect was positive for short time (40 min) and maximum concentration (0.10%) and negative for long extraction time.

Effects of Variables on TSS

Both the linear and interaction effect is positive where quadratic effect is negative for concentration (B) ($P < 0.0001$) and time (C) ($P < 0.0001$) (Table 3). The regression model for TSS with the coded value can be represented as:

$$\text{TSS}\left(^{\circ}\text{B}\right) = 13.76 + 0.35A + 0.21B + 0.21C + 0.29A^2 - 0.28B^2 - 0.18C^2 + 0.075AB + 0.025AC + 0.003BC$$

The value for coefficient of determination (R^2) is 0.90 which indicates the model can explain 90% of variability of the TSS of the Jamun juice. From the Fig. 4d, it can be concluded that at high temperature (50°C) with a high enzymatic concentration maximum TSS value can be obtained (14.74°B). From Fig. 4f, it is observed that the TSS value is more in case of long time duration. The minimum value of TSS is observed in lowest combination of time (40 min) and concentration (0.01%). The TSS value ranges between 13.10 and 14.60°B.

Effects of Variables on Total Solid

From Table 3, it is observed that total solid was mostly affected by the interaction of time and concentration of enzyme added. The less amount of total solid is responsible for the clear juice. Temperature has no significant effect on the total solid content of the juice. Table 3 shows that for both time and concentration quadratic effect is negative and interaction effect is positive. The regression model for total solid with the coded value can be represent as:

$$\text{TSS}\left(^{\circ}\text{B}\right) = 13.76 + 0.35A + 0.21B + 0.21C + 0.29A^2 - 0.28B^2 - 0.18C^2 + 0.075AB + 0.025AC + 0.003BC$$

The coefficient of determination (R^2) is 0.92 which indicates the model can explain 92% of variability of the total solid content of the Jamun juice. Figure 4i shows that at 40 min with a concentration of 0.10% total solid content is minimum 11.82%.

CONCLUSION

RSM using Box-Behnken Design was used for systematic study to optimize the effects of incubation time, temperature and enzyme concentration on the physical and chemical properties of Jamun juice. The optimum conditions for extractions were evaluated as incubation temperature of 33.5°C with 75 min and the concentration of enzyme will be 0.09% (w/v). At these condition, the major properties were turbidity: 69.64NTU; clarity: 0.166% abs; viscosity: 1.20 mPa·S; polyphenol: 112.21 mg GAE/g; protein: 262.35 mg/g; yield: 75%; TSS: 13.11°B; pH: 3.35; total solid: 15.05%; L^*: 1.27; b^*: −0.30. The response surface and graphical 3D contour plot give better understanding for the clarification process. As a future prospect this clarified juice can be used for membrane filtration or flavor extraction. The response surface design will be helpful for the future scientic study.

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