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Process Variable Optimization for the Liquefaction of Guava Fruit Pulp using Soluble Enzyme by Response Surface Methodology

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Abstract

Horticultural fruits are seasonal and are highly perishable with very low-shelf life. Liquefaction of fruits is one such effective technique. In the vegetable and fruit processing industry, pectinase enzymes are used to separate and clarify the juice. Process variables namely enzyme concentration, time, temperature, and pH affect enzymatic liquefaction of guava fruit pulp. These variables can be studied independently. However, all the variables can be studied at a time and also optimized using the statistical tool Response Surface Methodology (RSM) which is adopted here. The optimum conditions for the liquefaction process using soluble enzyme by RSM were- enzyme concentration (0.121% v/v), time (95 min), Temperature (32.7 ^{0}C), and pH (2.758). At these variables, the maximum yield was 79.7% and the clarity and viscosity were 0.0475 abs and 486.66 cP respectively.

Keywords: Liquefaction, Pectinase, Optimization, Response surface methodology.

Introduction

India stands second in the production of vegetables and fruits in the world with a production of 150 million tons per annum. Various post-harvest technologies have been developed for the processing of fruits to make value-added products like jams, jellies, fruit juices, squashes, etc. A good amount of work was reported in the published literature on the liquefaction of some tropical fruits like muskmelon, ber, tamarind, etc (Suryaprakasa Rao et al., 1968; Teotia et al., 1995; Waskar and Garande. 1999; Kotecha et al., 2002). A little amount of work was done on guava fruit pulp particularly, using free enzymes (Ahmad et al., 2018; Shams et al., 2018; Sargazi and Taghian, 2020; Yasin et al., 2020). Juice yield, clarity, and stability can be improved by the addition of enzymes as they play a vital role during the processing. Various combinations of enzymes like Pectinase 62L and Macer8™ FJ that contain both pectinases and other carbohydrate degrading enzymes are useful which break open the tissues of the fruit and release more amount of juice.

In the vegetable and fruit processing industries, commercial pectinases which are generally a group of enzymes like polygalacturonase, pectin methylesterase, and pectin lyase can be used to separate the juice from the fruit cells, clarify the juice, and enhance the yield and quality of the juice by breaking the naturally occurring starches and pectin linkages that otherwise give rise to undesired viscosity, poor filtration and a cloudy appearance (Mantovani et al., 2005). They are also used in the complete liquefaction of vegetables and fruits, maceration for producing pulpy products, and nectar stabilization of apricot (Pilnik et al., 1993). The optimal conditions for using these enzymes in the fruit processing involve temperature below 50°C and pH 4 - 5 which is the natural pH of the fruit. Fruit pulps contain the fruit juice, fiber content, vitamins, minerals, and other nutrients which are all held together with water using pectin. The presence of pectin in fruits and vegetables is a challenging task to the food processor in the processing of pulps where the viscosity increases with an increase in pectin substances. To make a clear fruit juice, we need to break the pectin to release the fruit juice from the fiber content. This technique is known as liquefaction of fruit pulps. Once the clear fruit juice is available, it can be processed thermally to make it in the form of RTS beverages.

Guava is one such important fruit because of its high nutritive value, good flavor, and pleasant aroma with high consumption in the global market. To utilize the guava fruit throughout the year, guava juice preparation is considered as the most promising method (Kaur et al., 2009). Hydrolyzing the pectic substances in the presence of enzyme depends on several physical and chemical factors such as enzyme concentration, time, temperature, and pH. The general practice followed to determine the optimum process parameters is to keep all the variables constant except one variable. The major disadvantage of this method is that it does not consider the interaction effects among the process variables. As a result, it does not provide the net effects of the process variables on the rate of reaction. This problem can be solved by conducting the optimization studies using Response Surface Methodology (Sin et al., 2006).

Materials and Methods

Guava fruit pulp was produced by using a blender and screened through a sieve size of 0.7mm. The same was pasteurized at 80 ^{0}C

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up to a time of 20 min as discussed by Rastogi and Rashmi. (1999) and stored in a refrigerator. When needed, it was adjusted to the desired temperature. The pH of the pulp was adjusted to the required pH with 1N NaOH and 1N HCl. The Liquefaction process of guava fruit pulp was carried out using the pectinase enzyme. The effect of the four variables was used to study three responses. Initially, the effect of enzyme concentration (0.03%, v/v-0.17%, v/v), time (5-95 min), temperature (10-55 ^{0}C), and pH (2-7) on three objectives i.e percentage yield, clarity, and viscosity was examined.

Design-Expert software was used to generate the design table. Based on the response surface methodology, in which matrix operation was the main feature used to find the coefficients of the regression equation. The resulting response surface plots denote the response function on Z-axis with X- and Y-axes representing the two independent variables while keeping the other variables constant. Analysis of Variance (ANOVA) was conducted to identify the effects of individual variables. The experiments were performed at the design conditions and the percentage yield, clarity, and viscosity was determined. The viscosity of the samples was determined using a Brookfield viscometer. A working spindle No.63 and a spindle speed of 100 rpm were chosen for all viscometric determinations. The clarity of the juice was determined by measuring the absorbance at 660 nm using UV-spectrophotometer. Distilled water was used as a reference. The lower the absorbance value, the higher is the clarity of the juice. To estimate the percentage yield, the treated sample was centrifuged at 10,000 rpm for 20 min in a cold centrifuge. The juice yield was determined as the percentage of the juice obtained based on the initial pulp.

Results and Discussion

Design Expert and Minitab software were used to generate the design table. Response Surface Methodology (RSM) is a tool of statistics that are used to conduct optimization studies. Central composite design (CCD) is an experimental design used in RSM. Response surface Methodology using CCD was employed to calculate the optimum conditions for the liquefaction of guava pulp using a soluble enzyme. The effect of enzyme concentration (0.03%, v/v-0.17%, v/v), time (5-95 min), temperature (10-55 ^{0}C) and pH (2-7) on three responses i.e percentage yield, clarity and viscosity was examined.

The four factors were enzyme concentration (a1), time (b1), Temperature (c1) and pH (d1). The actual and coded values of the process variables were shown in Table 1

Table 1 Actual and Coded levels of the independent variables for the experimental design using soluble enzyme

Independent Variables	Symbols	Code levels		
		-1	0	+1
Enzyme concentration(%w/v)	X_1	0.03	0.1	0.17
Time(min)	X_2	5	50	95
Temperature(^{0}C)	X_3	10	32.5	55
pH	X_4	2	4.5	7

The experimental designs, experimental results, and the predicted results from RSM for percentage yield, clarity, and viscosity were given in Table 2. From these results, we can understand that the percentage yield, clarity, and viscosity were dependent on the combination of enzyme concentration, time, temperature, and pH. The data were fitted to the second-order polynomial equation and the regression coefficients were calculated. The results from equation (1) to equation (3) indicate that the model fits the data appropriately.

Equations obtained from RSM using Design Expert software in Terms of Coded values

(A) Percentage yield(%) (Y1)

yield=45.6564+133.818*a1+0.223823*b1+0.510639*c1+3.81978 *d1-381.310*a1*a1-9.72060*10^{-4}*b1*b1-6.45614*10^{-3}*c1*c1-0.442947*d1*d1-0.083333*a1*b1-0.309524*a1*c1+2.92857*a1*d1-1.07407*10^{-3}*b1*c1-3.00000*10^{-3}*b1*d1-7.33333*10^{-3}*c1*d1 ---------(1)

(B) Clarity (Abs) (Y2)

Clarity = 0.081898-0.32702*a1 +1.05839*10^{-4}*b1 -9.80254*10^{-4}*c1 +1.22156*10^{-3}*d1 +2.37379*a1*a1 -1.41650*10^{-6}*b1*b1 +1.90253*10^{-5}*c1*c1 -1.38947*10^{-4}*d1*d1 -8.13492*10^{-4}*a1*b1 -1.46825*10^{-3}*a1*c1 -0.0110714*a1*d1-2.407*10^{-6}*b1*c1+2.83333*10^{-5}*b1*d1+1.6667*10^{-5}*c1*d1 ----------(2)

(C) Viscosity (cP) (Y3)

Viscosity=4841.41-1623.27*a1-67.8911*b1-54.2249*c1-277.097*d1+12977.1*a1*a1+0.360784*b1*b1+0.102889*c1*c1 +12.0940*d1*d1-62.5198*a1*b1+94.7222*a1*c1-500.357*a1*d1 +0.323889*b1*c1+ 2.26611*b1*d1 +1.00111*c1*d1 ----------(3)

Where a1- Enzyme conc b1- time c1- temperature d1-pH

Table 2: Central Composite Experimental Design in terms of uncoded variables and the experimental and predicted responses for the liquefaction of guava fruit pulp using soluble pectinase enzyme

Variables					Responses					
Run No.	Enzyme concentration (%w/v)	Time (min)	Temperature (^{0}C)	pH	Yield (%)		Clarity (abs)		Viscosity (cP)	
					Experimental	predicted	Experimental	predicted	Experimental	predicted
1	0.1	50	32.5	4.5	79	78.384	0.055	0.057	625	613.289
2	0.17	95	10	7	79	77.455	0.07	0.075	550	308.529
3	0.17	95	55	7	73	72.28	0.069	0.07	398	593.96
4	0.17	50	32.5	4.5	76.8	80.471	0.083	0.069	460	583.988
5	0.1	50	32.5	4.5	79	78.384	0.045	0.057	625	613.289
6	0.1	50	32.5	2	77	76.271	0.055	0.054	650	869.988
7	0.17	95	10	2	78.6	77.591	0.071	0.07	550	448.626
8	0.17	5	10	7	70	70.191	0.073	0.075	2314	2409.07
9	0.03	5	55	7	60	60.88	0.082	0.082	1250	1051.4
10	0.03	95	55	2	69	68.68	0.069	0.066	1010	614.96
11	0.17	5	55	7	69.8	69.366	0.079	0.08	1390	1382.75
12	0.03	95	55	7	64	64.844	0.08	0.082	925	1050.36
13	0.1	5	32.5	4.5	73.4	73.271	0.064	0.057	2225	2124.21
14	0.1	50	55	4.5	77	74.516	0.073	0.068	297	274.655
15	0.03	95	10	7	68	68.069	0.083	0.078	1399	1361.68
16	0.03	5	10	7	61	59.755	0.065	0.068	2409	2674.47
17	0.1	50	32.5	4.5	79	78.384	0.045	0.057	625	613.289
18	0.03	5	10	2	60	60.591	0.07	0.068	3980	3484.07
19	0.1	50	32.5	7	73	74.96	0.067	0.058	705	507.766
20	0.03	95	10	2	70	70.255	0.063	0.065	850	1151.53
21	0.1	95	32.5	4.5	78.2	79.56	0.054	0.051	440	563.544
22	0.1	50	10	4.5	72	75.716	0.07	0.065	1011	1056.1
23	0.03	50	32.5	4.5	75	72.56	0.064	0.068	871	769.766
24	0.03	5	55	2	62	63.366	0.08	0.078	1100	1635.75
25	0.17	5	10	2	70	68.977	0.082	0.083	3400	3568.92
26	0.17	95	55	2	73	74.066	0.061	0.062	480	508.807
27	0.1	50	32.5	4.5	79	78.384	0.054	0.057	623	613.289
28	0.1	50	32.5	4.5	79	78.384	0.055	0.057	625	613.289
29	0.1	50	32.5	4.5	79	78.384	0.055	0.057	625	613.289
30	0.17	5	55	2	70	69.802	0.08	0.084	2580	2317.35

Table 3 shows the estimated regression coefficients for percentage yield, clarity, and viscosity and their effect has been determined**.** The significance of all the terms in the polynomial was judged statistically at a probability level (P) of 0.01, 0.05, or 0.25. Terms with P>0.25 were considered insignificant. The Table indicated that the response surface models developed for all the response variables were adequate. The goodness of fit of the regression equations was tested by examining the adjusted determination coefficient, R^2Adj. Adjusted determination coefficient, R^2 Adjusted was found to be 0.897, 0.577, and 0.903 respectively for percentage yield, clarity, and viscosity, and R^2 values for Yield, Clarity, and Viscosity were found to be 0.947, 0.781, and 0.949 respectively. The closer the values of R^2 to unity, the better the empirical model fits the actual data.

Table 3: Estimated regression coefficients for the fitted second-order polynomial for percentage yield, clarity, and viscosity using soluble enzyme

Regression Coefficient	Percentage Yield (%)		Clarity(abs.)		Viscosity (cP)	
Constant	coefficient	P	coefficient	P	coefficient	P
B_0	45.6564	0.000	0.081898	0.000	4841.41	0.000
Linear Coefficient						
B_1	133.818	0.000	-0.32702	0.705	-1623.27	0.195
B_2	0.2238	0.000	1.058×10^{-4}	0.098	-67.8911	0.000
B_3	0.51064	0.221	-9.803×10^{-4}	0.417	-54.2249	0.000
B_4	3.81978	0.184	$+1.222 \times 10^{-3}$	0.253	-277.097	0.018
Non-Linear Coefficient						
B_{11}	-381.310	0.153	+2.37379	0.022	12977.1	0.729
B_{22}	-9.721×10^{-4}	0.133	-1.417×10^{-6}	0.539	+0.3608	0.001
B_{33}	-6.46×10^{-3}	0.019	$+1.903 \times 10^{-5}$	0.052	+0.1029	0.777
B_{44}	-0.443	0.041	-1.389×10^{-4}	0.851	+12.0940	0.681
Cross Coefficient						
B_{12}	-0.083333	0.606	-8.135×10^{-4}	0.183	-62.5198	0.016
B_{13}	-0.309524	0.344	-1.468×10^{-3}	0.227	+94.7222	0.058
B_{14}	+2.92857	0.321	-0.01107	0.308	-500.357	0.246
B_{23}	-1.07407×10^{-3}	0.041	$-2.407 * 10^{-6}$	0.204	+0.3239	0.000
B_{24}	-3.000×10^{-3}	0.509	$+2.83 \times 10^{-5}$	0.103	+ 2.2661	0.003
B_{34}	-7.333×10^{-3}	0.421	$+1.667 \times 10^{-5}$	0.617	+1.0011	0.450
R^2 adjusted	0.897		0.577		0.903	
R^2	0.947		0.781		0.949	
S	1.995		0.007		290.18	

Combined Effect of Variables on Yield using Soluble Enzyme:

From Table 3, it may be observed that the Juice yield depended on enzyme concentration and time as their linear effect was positive (P<0.01), and the quadratic effect was negative (P<0.25). Juice yield was also found to be a function of linear (P<0.25) and quadratic effects (P<0.05) of pH. The yield was quadratically related to temperature (P<0.05) but the linear term was not found to be significant. It was interesting to note that the interaction effect was insignificant. The only interaction effect was between time and temperature and showed a negative effect (P<0.05).

The Analysis of Variance (ANOVA) for the percentage yield was shown in Table 4. It was evident from the data that the first and second-order terms were found to be significant and lack of fit was not significant. The lack of fit measures the failure of the model to represent data in the experimental domain at the points which are not included in the regression.

Table: 4 Analysis of Variance for percentage yield using soluble enzyme

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	1064.77	1064.77	76.055	19.10	0.000
Linear	4	473.83	473.83	118.457	29.75	0.000
Square	4	558.37	558.37	139.593	35.06	0.000
Interaction	6	32.57	32.57	5.429	1.36	0.291
Residual Error	15	59.72	59.72	3.981	--	--
Lack-of-Fit	10	59.72	59.72	5.972	--	--
Pure Error	5	0.00	0.00	0.00	--	--
Total	29	1124.49	--	--	--	--

(a) Fig 1.1, 1.2, and 1.3 showed the combined effects of enzyme concentration with time, temperature, and pH on percentage yield. It was evident from the figure that the yield increased with an increase in enzyme concentration as well as time. However, yield increased with time and pH up to a certain value and decreased thereafter. The maximum yield was obtained when enzyme concentration is at 0.12%v/v, while time was 55 min, the temperature was 32.5 ^{0}C, and pH 4.5.

(b) Fig 1.4 and 1.5 show the combined effect between time and temperature and time and pH on yield respectively indicating that yield increased to a certain value and thereafter decreased. The maximum yield was obtained when time, temperature, and pH were set at 55 min, 33 ^{0}C, and 4.5 respectively.

(c) Fig 1.6 shows the combined effects of temperature and pH which indicate that when the temperature was set at 33 ^{0}C and pH 4.5, maximum yield was obtained.

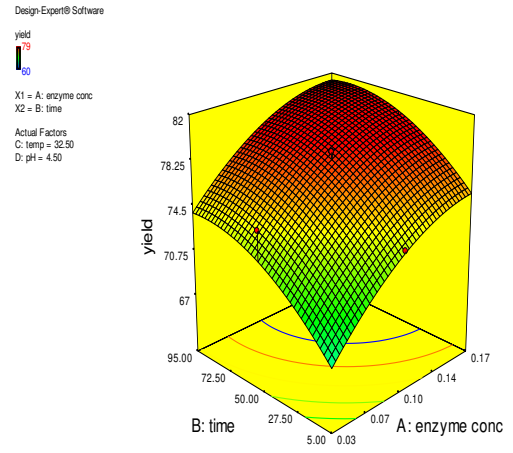

Fig: 1.1

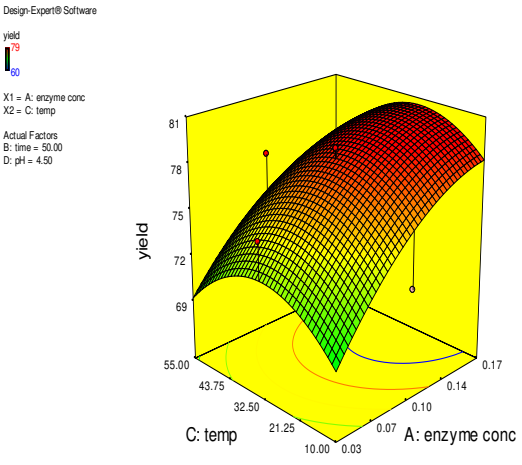

Fig: 1.2

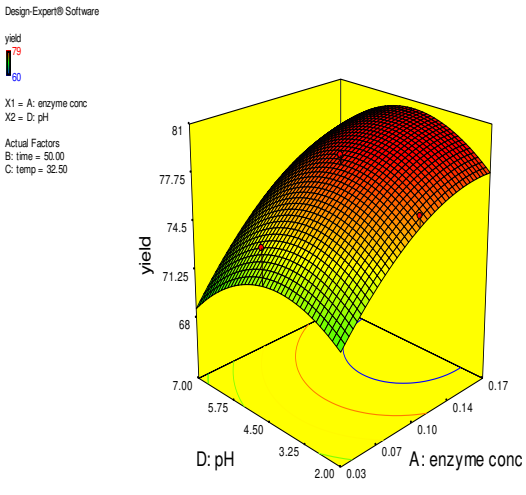

Fig: 1.3

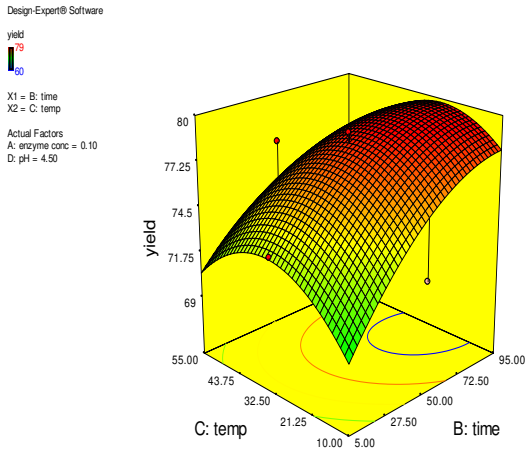

Fig: 1.4

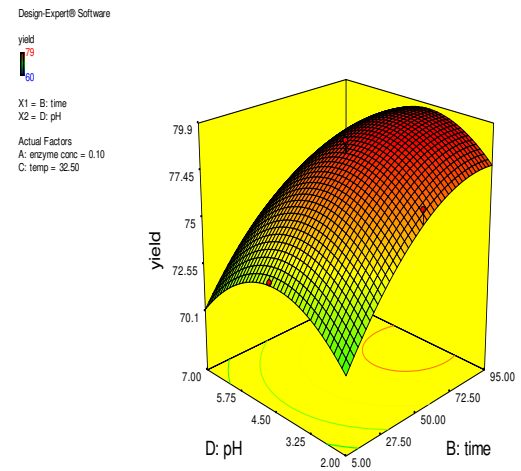

Fig: 1.5

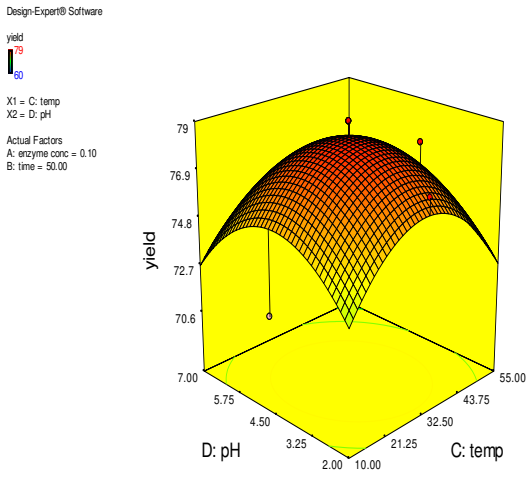

Fig: 1.6

The combined effect of Enzyme concentration, time, temperature, and pH on Yield was shown in Figs 1.1 to 1.6. The highest yield was obtained at enzyme concentration ranging from 0.1-0.14% %w/v, time ranging from 50-95 min, temperature ranging from 30-40 ^{0}C, and pH ranging from 2.5-4.5.

Combined Effect of Variables on Clarity using Soluble Enzyme:

Pectolytic enzymes break the pectin molecules when treated with pectinase enzymes and form pectin-protein flocs leaving behind the clear supernatant. From Table 3, it may be observed that clarity depended only on time as its linear effect was negative at P<0.25 but showed no quadratic effect. Concerning enzyme concentration (P<0.05) and temperature (P<0.25), it showed a positive effect on quadratic terms. It showed a significant interaction between enzyme concentration and time, enzyme concentration and temperature, time and temperature at P<0.25 with a negative effect and the interaction between time and pH was positive at P<0.05. It was interesting to note that there was no interaction between enzyme concentration and pH and temperature and pH.

The Analysis of Variance (ANOVA) for the percentage yield was shown in Table 5. It was evident from the data that the first and second-order terms were found to be significant and lack of fit was not significant.

Table 5: Analysis of Variance for Clarity using soluble enzyme

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	0.00288	0.00288	0.00021	3.83	0.007
Linear	4	0.00029	0.00029	0.000072	1.35	0.299
Square	4	0.002071	0.002071	0.000008	9.62	0.000
Interaction	6	0.000522	0.000522	0.000087	1.62	0.210
Residual Error	15	0.000807	0.000807	0.000054	--	--
Lack-of-Fit	10	0.00068	0.000680	0.000068	2.67	--
Pure Error	5	0.000128	0.000128	0.000026	--	--
Total	29	0.003691			--	--

(a) Fig 2.1, 2.2, and 2.3 show the combined effects of enzyme concentration with time, temperature, and pH on clarity. It was evident from the figure that the absorbance value decreased with enzyme concentration, time, and temperature up to a certain extent where lower absorbance indicates that the juice produced was clear. The absorbance value increased with pH. The maximum clarity (minimum absorbance) was obtained when enzyme concentration was at 0.1%v/v, while time was 50 min, the temperature was 30 ^{0}C, and the pH was set at 4.5.

(b) Fig 2.4 and 2.5 show the combined effect between time and temperature and time and pH respectively indicating that maximum clarity was obtained when time, temperature, and pH were set at 75-90 min, 30 ^{0}C, and 2.5 respectively.

(c) Fig 2.6 shows the combined effects of temperature and pH on clarity which indicate that when the temperature was set at 35 ^{0}C and pH 2.5, maximum clarity was obtained.

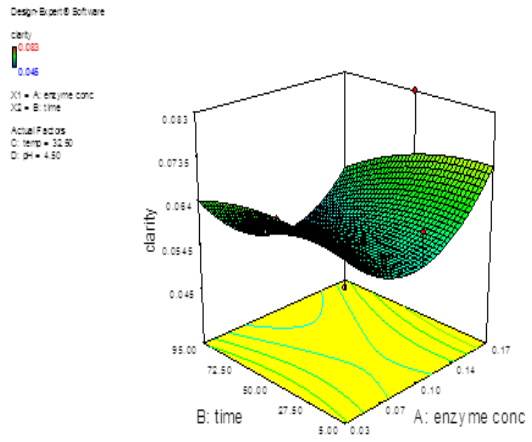

Fig: 2.1

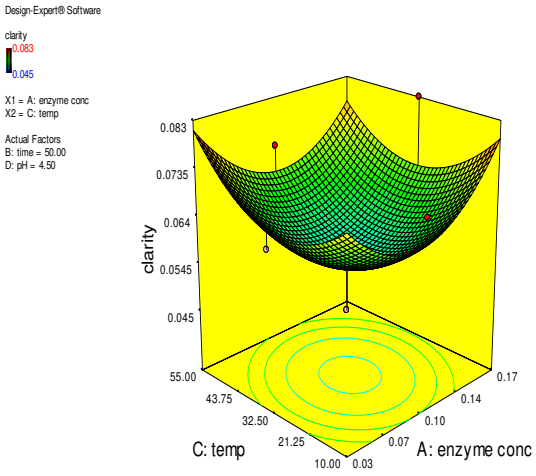

Fig: 2.2

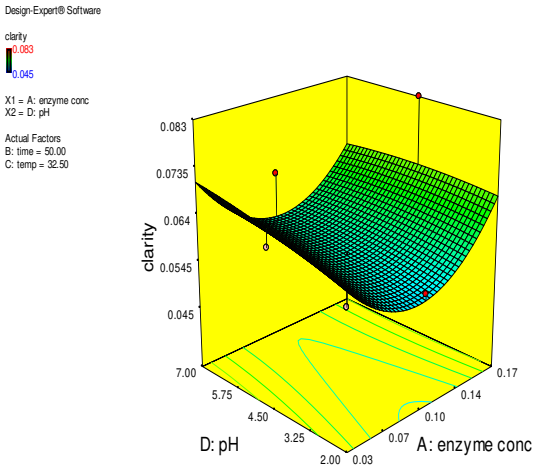

Fig: 2.3

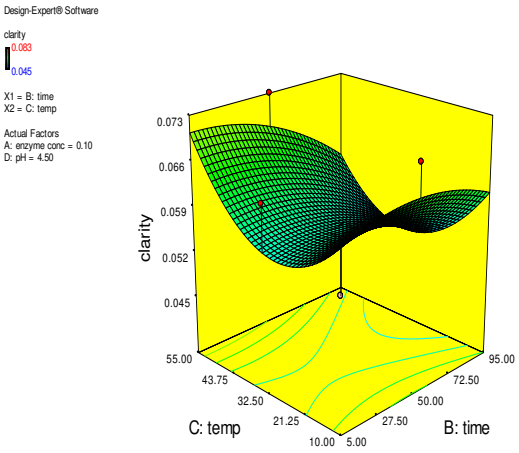

Fig: 2.4

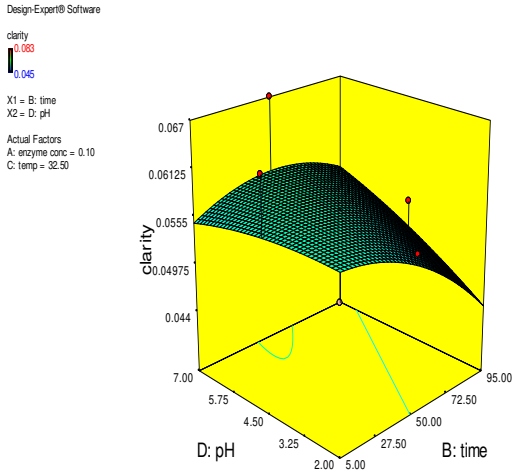

Fig: 2.5

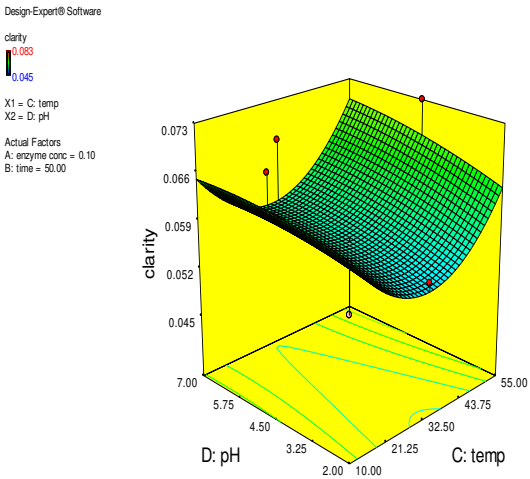

Fig: 2.6

The combined effect of Enzyme concentration, time, temperature, and pH on clarity was shown in Figs 2.1 to 2.6 The maximum clarity was obtained at enzyme concentration ranging from 0.1-0.14%, w/v, time ranging from 50-95 min, temperature ranging from 30-40 ^{0}C and pH ranging from 2.5-4.5.

Combined Effect of Variables on Viscosity using Soluble Enzyme:

From Table 3, it was evident that the enzyme concentration (P<0.25), time (P<0.01), temperature (0.01), and pH (P<0.05) affected negatively in the linear terms. It was observed that the enzyme concentration, temperature, and pH did not show any quadratic effect whereas, the quadratic effect was positive with time at p<0.01. The interaction effect was positive concerning enzyme concentration and temperature (P<0.25), time and temperature (P<0.01), and time and pH (P<0.01) whereas the effect was negative concerning enzyme concentration and time (P<0.05) and enzyme concentration and pH (P<0.25). There was no interaction effect between time and temperature. The fruit juices having high viscosity may lead to problems during the filtration process, so that clarified juice with low viscosity is preferred.

The Analysis of Variance (ANOVA) for the viscosity was shown in Table 6. It was evident from the data that the first and second-order terms were found to be significant and lack of fit was not significant.

Table 6: Analysis of Variance for viscosity using soluble enzyme

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	23876359	23876359	1705454	20.25	0.000
Linear	4	14454244	14454244	3613561	42.91	0.000
Square	4	5511464	5511464	1377866	7.01	0.018
Interaction	6	3910651	3910651	651775	7.74	0.001
Residual Error	15	1263054	1263054	84204	--	--
Lack-of-Fit	10	1263051	1263051	126305	189457.61	0.000
Pure Error	5	3	3	1	--	--
Total	29	25139413	--	--	--	--

(a) Fig 3.1, 3.2, and 3.3 show the combined effects of enzyme concentration with time, temperature, and pH on viscosity. The minimum viscosity was obtained when enzyme concentration is at 0.1%v/v, while time was 60 min, the temperature was 40 ^{0}C, and ph was set at 4.5.

(b) Figv3.4 and 3.5 show the combined effect between time and temperature and time and pH respectively indicating that minimum viscosity was obtained when time, temperature, and pH were set at 50-75 min, 30 ^{0}C, and 4.5 respectively.

(c) Fig 3.6 shows the combined effects of temperature and pH on clarity which indicate that when the temperature was set at 45 ^{0}C and pH 4.5, minimum viscosity was obtained.

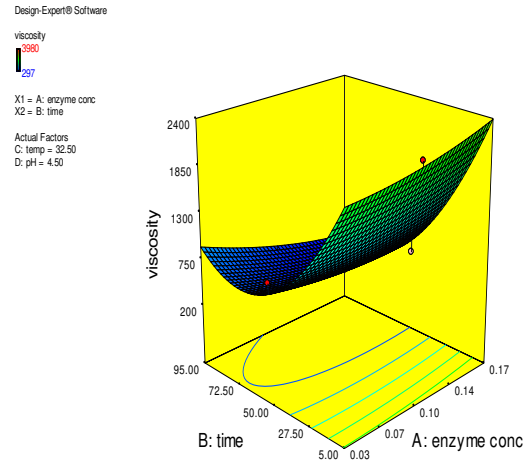

Fig: 3.1

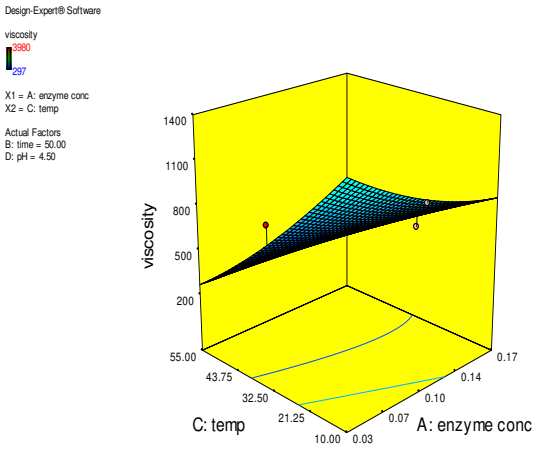

Fig: 3.2

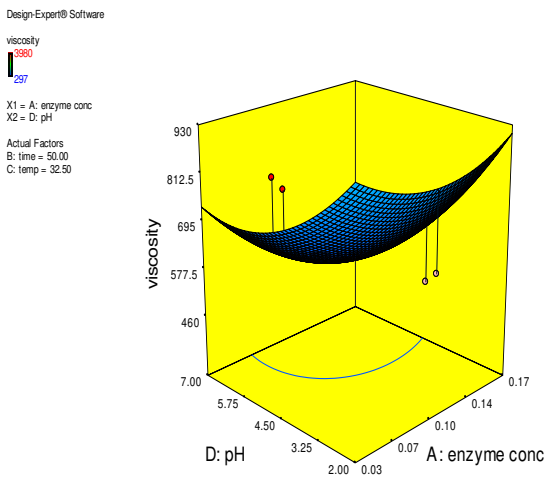

Fig: 3.3

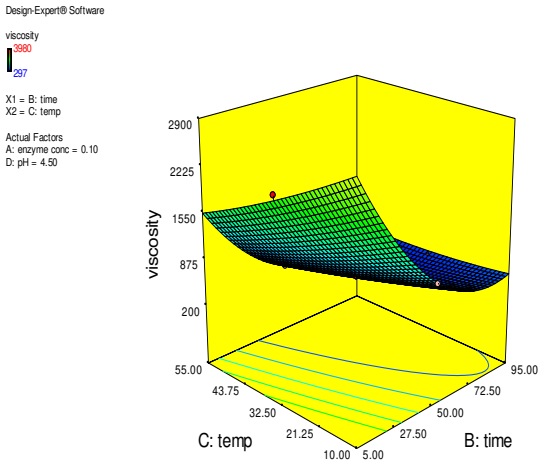

Fig: 3.4

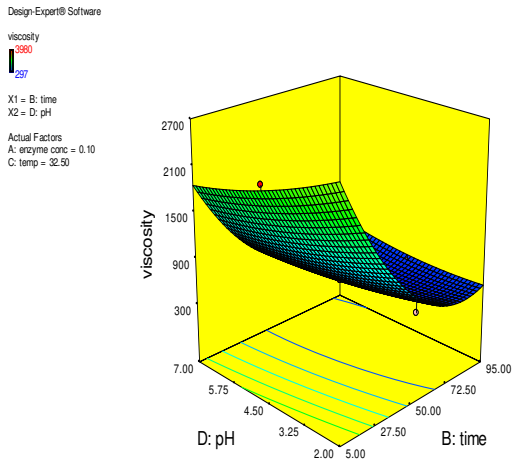

Fig: 3.5

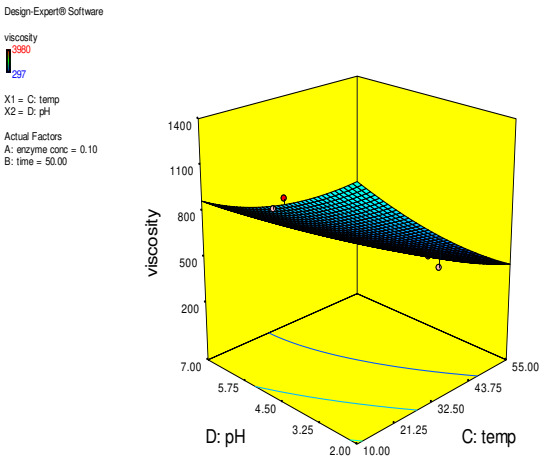

Fig: 3.6

The combined effect of Enzyme concentration, time, temperature, and pH on viscosity was shown in Figs 3.1 to 3.6. Minimum viscosity was obtained at enzyme concentration ranging from 0.1-0.17% w/v, time ranging from 50-95 min, temperature ranging from 30-40 ^{0}C, and pH ranging from 2.5-4.5.

Conclusions

RSM was an efficient tool that helped in optimizing the parameters at a faster rate and hence less costly since the number of experiments was reduced.

From the Central composite design, for the liquefaction of guava fruit pulp, the maximum percentage yield was 79.7%, clarity was 0.0475 abs and minimum viscosity was 486.66 cP at the following optimum process variables, enzyme concentration 0.12%v/v, time 95 min, temperature 32.7 ^{0}C and pH 2.75 for the soluble enzyme.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

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