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Extraction and Optimization of Guava Juice by Using Response Surface Methodology

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ABSTRACT

Raw guava juice is turbid, viscous and gray in color. This study was initiated to optimize the enzymatic clarification process of guava juice using response surface methodology. Guava juice was treated with pectinase at various enzyme concentrations (0.01-0.2%), temperatures (30-50°C) and time (30-150 min) of treatment. The effect of enzyme treatments on percentage yield, clarity, turbidity, titrable acidity and viscosity of the juice were studied by employing a second order central composite design. Based on response surface and contour plots, the optimum conditions for clarifying guava juice obtained were: 0.11% enzyme concentration, incubation temperature of 41.30°C and incubation time of 115.98 min with percent yield (80.03%), clarity (1.34 Abs), turbidity (1.07 Abs), titrable acidity (0.525%), viscosity (2.4 cps) and desirability of 0.942. Statistical analysis showed that percentage yield, clarity, viscosity and turbidity were significantly ($p<0.05$) correlated to enzyme concentration, incubation temperature and incubation time.

Key words: Guava juice, enzymatic clarification, response surface methodology, pectinase, guava pulp

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the most cultivated fruit crop in many tropical and subtropical countries. It is rich in ascorbic acid (vitamin C), vitamin A, dietary fiber, pectin, sugars, folic acid, potassium, manganese, copper and certain dietary minerals. Guava is also consumed for medicine purposes in pharmacological activities. These are also processed and preserved in the form of jam, pulp, puree, squash, nectar and juice. The ripened guava contains 0.58-0.66% pectin, 1.8-7.2% reducing sugar, 9.0-16.2% total sugar, 9.0-16.8° Brix total solids, 0.07-0.58% acidity, 88.0-260.6 mg/100 g ascorbic acid.

Natural Fruit juice consumption pattern has significantly changed in recent years and has became a potent alternative to traditional caffeine-containing beverages (Jagtiani *et al.*, 1999; Joseph and Priya, 2011; Sanda *et al.*, 2011; Sevda *et al.*, 2011a; Sevda and Rodrigues, 2011a). Guava juice contains as much as four times higher Vitamin C than orange juice and with its unique flavour and aroma, is able to compete in the market, in any form (guava juice or guava wine) (Anderson and Badrie, 2005; Reddy *et al.*, 2006). Raw guava juice is grey in color, very turbid and viscous (Viquez *et al.*, 1981). Pectin substances (polysaccharide content) are responsible for the turbidity and viscosity of guava juice and these are composed of partially methyl-esterified

galacturonic acid residues linked by a-1,4-glycosidic bonds. Pectinases hydrolyze pectin and therefore, are used in fruit juice clarification (Ceci and Lozano, 1998; Alam *et al.*, 2010; Abudabos, 2012; Singh *et al.*, 2012; Kilara, 1982; Kuddus *et al.*, 2011). Reports are there in which litchi juice (Vijayanand *et al.*, 2009), pineapple juice (Pal and Farhath, 2010), pomegranate juice (Shalini and Gupta, 2010; Sevda and Rodrigues, 2011b; Sevda *et al.*, 2011b), apple juice (Kashyap *et al.*, 2001) have been clarified by using pectinase. Effect of pectinase treatment and pectin hydrolysis depends on several physicochemical factors like incubation time, enzyme concentration, temperature of incubation and initial pH of the pulp (Grassin and Fauquembergue, 1996). One factor method used for the general process parameter optimization by varying one variable and keeping other constant, but it does not include interactive effects among the variable and so it does not optimize the net effects of various parameters on the reaction rate. So to overcome this problem and to see the interaction with all parameter in lesser experiment optimization studies have been carried out using response surface methodology (Montgomery, 2001; Myers, 1976).

Response Surface Methodology (RSM) is one of the most efficient tools to optimize parameters in biosystems (Frank, 2001; Seth and Mishra, 2011; Jaiswal *et al.*, 2011). It is a statistical approach which uses quantitative data from appropriate experiments to simultaneously determine and solve multivarient equations thus providing an optimum solution (Kalil *et al.*, 2000; Daramola *et al.*, 2007). RSM significantly reduces the number of experiments needed to evaluate multiple parameters and their interactions (Montgomery, 2001), thus making it convenient and time-efficient (Myers, 1976). Therefore, the present work is aimed to study the effect of pectinase treatment on Guava pulp and to optimize the process conditions by response surface methodology.

MATERIALS AND METHODS

Experimental materials: Ripped Guava fruits (Psidium guajava Linn) were purchased from local market (Nashik variety) of Matunga, Mumbai. Pectinase used was kindly supplied as a gift sample by M/S Lumis Biotech, Mumbai, India.

Method of pulp preparation: The guava fruits were washed, peeled and cut into small pieces. By using mixer blinder pulp was prepared. A ratio of 2:1 (Pulp: Distilled water; w/v) was utilized in the extraction process. The pH and total soluble solids content of the pulp obtained were 4.0 and 10.8, respectively.

Enzymatic treatment: The pulp was treated with pectinase enzyme at its natural pH 4.0. For each experiment, 150 g pulp was subjected to different enzyme treatment conditions as shown in Table 1. Based on preliminary experiments, the range of the variables for enzymatic treatment was selected.

The independent variables for the enzymatic treatment were the concentration of pectinase used, X1 (0.01-0.2%), Incubation temperature, X2 (30-50°C) and Time of incubation, X3 (30-150 min). At the end of the enzymatic treatment, the enzyme in the sample was inactivated by heating the suspension at 90°C for 5 min in a water bath. The treated juices were centrifuged at 3000 g for 10 min and the supernatant was collected.

Percentage yield determination: Percent yield was measured by measuring the volume obtained per 100 g of pulp.

Table 1: Experimental design indicating coded and actual values of independent variables

Experiment no	Enzyme conc. (X1) (%)	Temperature (X2) (°C)	Time (X3) (min)
1	0.20(+1)	50 (+1)	150 (+1)
2	0.01 (-1)	40 (0)	90 (0)
3	0.20 (+1)	40 (0)	90 (0)
4	0.10 (0)	30 (-1)	90 (0)
5	0.10 (0)	50 (+1)	90 (0)
6	0.10 (0)	40 (0)	30 (-1)
7	0.10 (0)	40 (0)	150 (+1)
8	0.20 (+1)	50 (+1)	150 (+1)
9	0.10 (0)	50 (+1)	150 (+1)
10	0.20 (+1)	30 (-1)	150 (+1)
11	0.10 (0)	30 (-1)	150 (+1)
12	0.20 (+1)	50 (+1)	30 (-1)
13	0.10 (0)	50 (+1)	30 (-1)
14	0.20 (+1)	30 (-1)	30 (-1)
15	0.01 (-1)	30 (-1)	30 (-1)
16	0.10 (0)	40 (0)	90 (0)
17	0.10 (0)	40 (0)	90 (0)
18	0.10 (0)	40 (0)	90 (0)
19	0.10 (0)	40 (0)	90 (0)
20	0.10 (0)	40 (0)	90 (0)

Clarity and turbidity determination: Clarity and turbidity of the juice obtained was determined by measuring the absorbance at 525 nm and at 660 nm, respectively, using a UV-VIS spectrophotometer.

Titrable acidity determination: Titrable acidity was determined by the method of Ranganna (1977).

Viscosity determination: Viscosity was measured using a Brookfield viscometer.

Experimental and statistical design: Response Surface Methodology (RSM) is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equations simultaneously (Montgomery, 2001; Myers, 1976). RSM is used to determine the optimum enzyme concentrations, for the production of guava juice. A central composite rotatable experimental design (CCRD) for three independent variables was used. The medium components (independent variables) selected for the optimization were enzyme concentration, incubation temperature and time. Regression analysis was performed on the data obtained from the design experiments.

Coding of the variables was done according to the Eq. 1:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i} \quad i = 1, 2, 3, ..., k \tag{1}$$

where, x_i, dimensionless value of an independent variable; X_i, real value of an independent variable; X_{cp}, real value of an independent variable at the center point and ΔX_i, step change of real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable I.

The experiments were carried out in duplicate, which was necessary to estimate the variability of measurements, i.e., the repeatability of the phenomenon. Replicates at the center of the domain in three blocks permit the checking of the absence of bias between several sets of experiments. The relationship of the independent variables and the response was calculated by the second order polynomial (Eq. 2):

$$Y = \beta_0 + \sum_{i=1}^{} \beta_i X_i + \sum_{i=1}^{} \beta_{ii} X_i X_j + \sum_{i<j} \sum_{j} \beta_{ij} X_i X_j \quad (2)$$

Y is the predicted response, β_0 a constant, β_i the linear coefficient, β_{ii} the squared coefficient and β_{ij} the cross-product coefficient, k is number of factors.

The second order polynomial coefficients were calculated using the software package Design Expert Version 6.0.10 to estimate the responses of the dependent variable. Response surface plots were also obtained using Design Expert Version 6.0.10.

Central Composite Design (CCD) was employed to study the combined effect of three independent variables as enzyme concentration, temperature and incubation time which were coded as A, B and C, respectively.

The minimum and maximum values for enzyme concentration were in range of 0.01 and 0.20, temperature 30 and 50°C and incubation time varies between 30 to 150 min. As shown in Table 1 that complete design consists of 20 experiments (including six centre points) which were carried out randomly.

RESULTS AND DISCUSSION

RSM was employed for optimizing the parameter of guava juice extraction. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms are presented in Table 2. The Statistical analysis indicates that the proposed model was adequate, possessing significant fit and with very satisfactory values of R^2. for all the responses. The R^2. value for precentage yield, clarity, turbidity, titrable acidity and viscosity were 0.9883, 0.9718, 0.9705, 0.8637 and 0.9803, respectively, closer the value of R^2 to the unity the better the emperical model fits the actual data. The smaller the values of R^2 the less relevant the dependent variables in the model have to explain of the behaviors variations (Little and Hills, 1978;

Table 2: Regression coefficients and R^2 values for four dependent variables for enzymatic clarified guava juice

Regression coefficient	Yield (%)	Clarity (Abs)	Turbidity (Abs)	Titrable acidity (%)	Viscosity (cps)
b_0	78.279400	1.358525	1.080706	0.525092	0.943582
A	10.162300	-0.276500	-0.278860	0.043480	-8.368510
B	1.615379	-0.025570	-0.033770	0.005633	-0.559650
C	4.337049	-0.069050	-0.045450	-0.005860	-5.150640
A^2	-8.917980	0.161945	0.200445	-0.026620	8.306654
B^2	-2.249450	0.150545	0.205870	0.005754	-0.826480
C^2	-2.749450	0.075045	0.079870	0.001754	1.886884
AB	-2.228710	0.029909	0.042028	-0.016510	0.649091
AC	-1.199410	0.047797	0.038559	-0.001880	5.822861
BC	3.327874	-0.011870	-0.035820	-0.005650	-1.267340
R^2	0.988300	0.971800	0.970500	0.863700	0.980300

A: Enzyme concentration, B: Temperature, C: Incubation time

Mendenhall, 1975). The probability (p) values of all regression models as shown in Table 2 were less than 0.00001, with significant fit for model.

Effect of enzyme concentration, temperature and time: The application of RSM yielded following regression equation, which is empirical relation between (Y) and the test variable in coded units for final Equation in Terms of Coded Factors:

Percent yield (%) = $+78.28+10.16*A+1.62*B+4.34*C-8.92*A^2-2.25*B^2-2.75*C^2-2.23*A*B-1.20*A*C+3.33*B*C$

Clarity = $+1.36-0.28*A-0.026*B-0.069*C+0.16*A^2+0.15*B^2+0.075*C^2+0.030*A*B+0.048*A*C-0.012*B*C$

Turbidity = $+1.08-0.28*A-0.034*B-0.045*C+0.20*A^2+0.21*B^2+0.080*C^2+0.042*A*B+0.039*A*C-0.036*B*C$

Titrable acidity = $+0.53+0.043*A+5.633E-003*B-5.859E-003*C-0.027*A^2+5.754E-003*B^2+1.754E-003*C^2-0.017*A*B-1.878E-003*A*C-5.650E-003*B*C$

Sqrt(viscosity) = $+0.94-8.37*A-0.56*B-5.1*C+8.31*A^2-0.83*B^2+1.89*C^2+0.65*A*B+5.82*A*C-1.27*B*C$

Further statistical analysis (Table 3) was then performed. This analysis is a joint test on all the variables involving one particular factor (Floros and Chinnan, 1987).

Response on percent yield: The values of various responses at different experimental combinations for coded variables are given in Table 3. Effect of temperature and time on percent

Table 3: Effect of enzyme concentration , temperature and incubation time on five dependent variable and their actual and predicated value

Coded variable			Yield (%)		Clarity (Abs)		Turbidity (Abs)		Titrable acidity (%)		Viscosity (cps)	
X1	X2	X3	Actual	Predicated	Actual	Predicated	Actual	Predicated	Actual	Predicated	Actual	Predicated
0.20	50	150.0	80	80.38	1.475	1.44	1.290	1.25	0.525	0.53	2.4	2.4
0.01	40	90.0	61	59.20	1.810	1.80	1.560	1.56	0.49	0.45	307	304
0.2	40	90.0	78	79.52	1.260	1.24	1.034	1.00	0.52	0.54	3.6	3.6
0.1	30	90.0	71	73.01	1.649	1.57	1.414	1.36	0.54	0.53	2.4	2.4
0.1	50	90.0	79	76.71	1.460	1.51	1.254	1.28	0.525	0.53	2.4	2.4
0.1	40	30.0	69	69.90	1.626	1.54	1.329	1.24	0.535	0.53	55.2	57
0.1	40	150.0	80	78.82	1.332	1.39	1.087	1.14	0.522	0.54	1.2	1.2
0.2	50	150.0	80	80.38	1.475	1.47	1.290	1.25	0.525	0.53	2.4	2.4
0.01	50	150.0	66	66.91	1.832	1.84	1.626	1.65	0.48	0.48	10	9.6
0.2	30	150.0	76	74.95	1.412	1.46	1.252	1.31	0.57	0.55	3.6	3.4
0.01	30	150.0	52	52.56	2.0132	1.97	1.937	1.87	0.42	0.44	118	114
0.2	50	30.0	68	67.45	1.460	1.51	1.269	1.25	0.57	0.56	2.6	2.4
0.01	50	30.0	48	49.18	2.132	2.18	1.937	1.89	0.48	0.49	991	980
0.2	30	30.0	76	77.11	1.481	1.44	1.273	1.34	0.555	0.56	2.5	2.4
0.01	30	30.0	49	48.15	2.100	2.10	1.883	1.97	0.435	0.44	859	867
0.1	40	90.0	77	77.11	1.378	1.39	1.111	1.11	0.515	0.52	2.4	2.4
0.1	40	90.0	78	77.11	1.387	1.39	1.131	1.11	0.52	0.52	2.4	2.4
0.1	40	90.0	77	77.11	1.378	1.39	1.111	1.11	0.515	0.52	2.4	2.4
0.1	40	90.0	76	75.33	1.368	1.39	1.034	1.11	0.51	0.52	2.4	2.4
0.1	40	90.0	77	77.11	1.378	1.39	1.111	1.11	0.515	0.52	2.4	2.4

yield at a constant enzyme concentration is shown in Fig. 1(a). It was observed that with increase in both temperature and time, the percentage yield also increased but after a certain level of temperature it became constant and started decreasing above 46°C. This may be due to the denaturing of enzyme at higher temperature.

Percent yield kept on increasing with increase in the incubation time. Effect of enzyme concentration and temperature on percent yield at a constant incubation time is shown in Fig. 1(b). As the enzyme concentration increases, the percent yield is also increases with increasing in temperature. In this, change in enzyme concentration affects the percent yield. At the lowest level of temperature, percent yield is less; with increase in temperature percent yield also increases. Effect of enzyme concentration and time on percent yield of guava juice at a constant temperature is shown in Fig. 1(c). As the time and enzyme concentration increases, the percent yield also increases. Actual and predicated values for percent yield is shown in Fig. 1(d) and percent yield

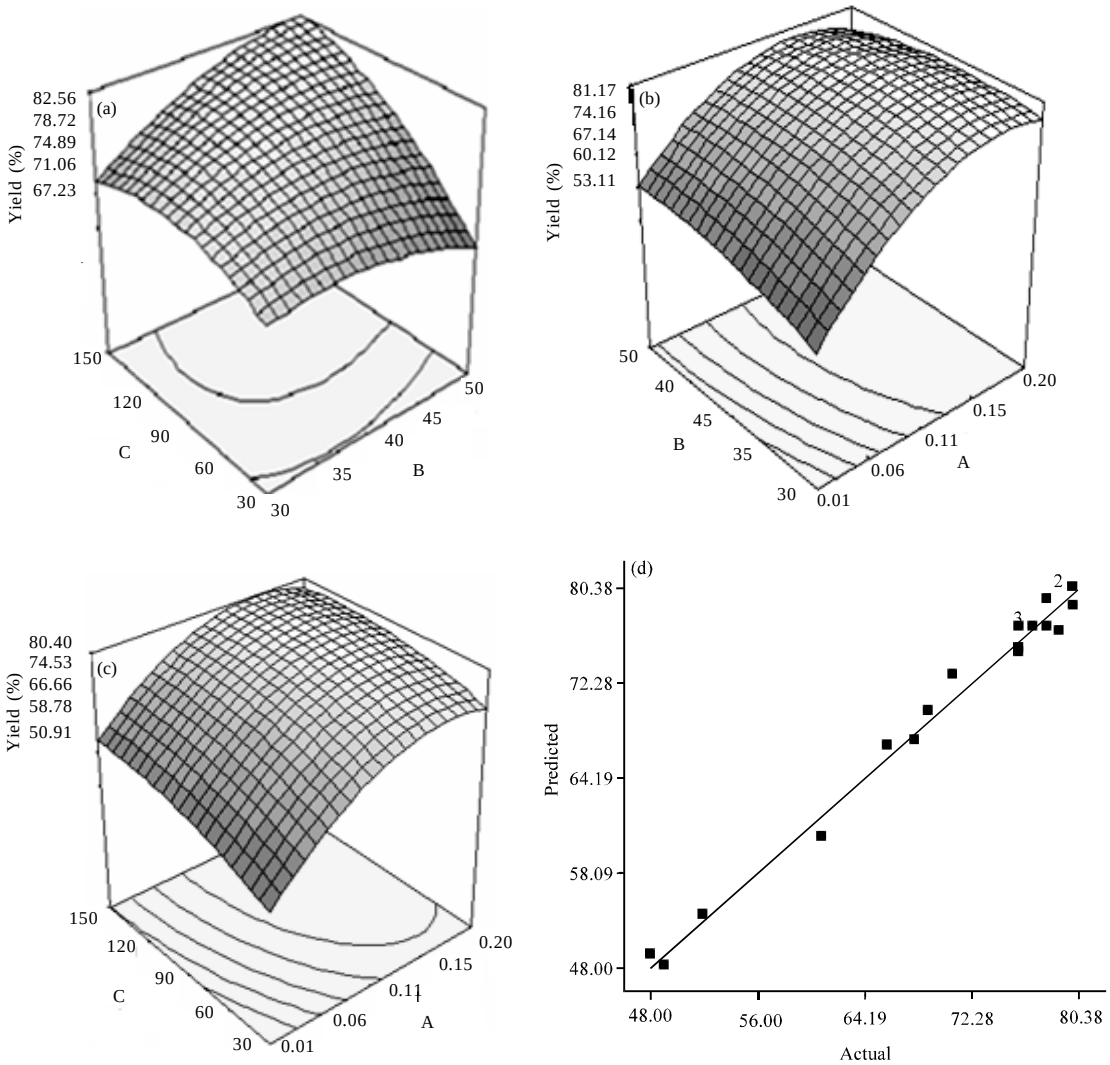

Fig. 1(a-d): Response surface for percent yield of guava juice as a function of (a) Temperature (B) and time (C), (b) Enzyme concentration (A) and temperature (B), (c) Enzyme concentration (A) and time (C) and (d) Actual and predicated values

value vary from 48 to 81%. These results shown higher values or near similar of juice yield in compare to literature (Sharma *et al.*, 1999). These above all figures indicate the 3D surface for the effects of the independent variables on percent yield. The total amount of extracted juice was increased because of degradation of pectin lads to reduction of water holding capacity and consequently, free water is related to the system and reduces the viscosity thus facilitating percent yield.

Response on clarity: Figure 2(a) shows the response surface for the clarity as a function of enzyme concentration (A) and incubation temperature (B). Lower the values of Optical Density (OD) relates to high clarity juice. The figure shows as the enzyme concentration increases, the OD value decreases so clarity of juice increases.

By giving the enzyme treatment, pectinase enzyme breakdown the pectin molecule, which facilitates the formation of pectin-protein flocs, leaving a clear supernatant and significantly removing the colloidal aspect of the juices. Clarity is an important index of clarified juice. It can be concluded from this Fig. 2(a) that, the clarity of juice increased with increase in the enzyme concentration and temperature. Response surface for the clarity as a function of incubation temperature (B) and incubation time (C) is shown in Fig. 2(b). The figure shows that high clarity

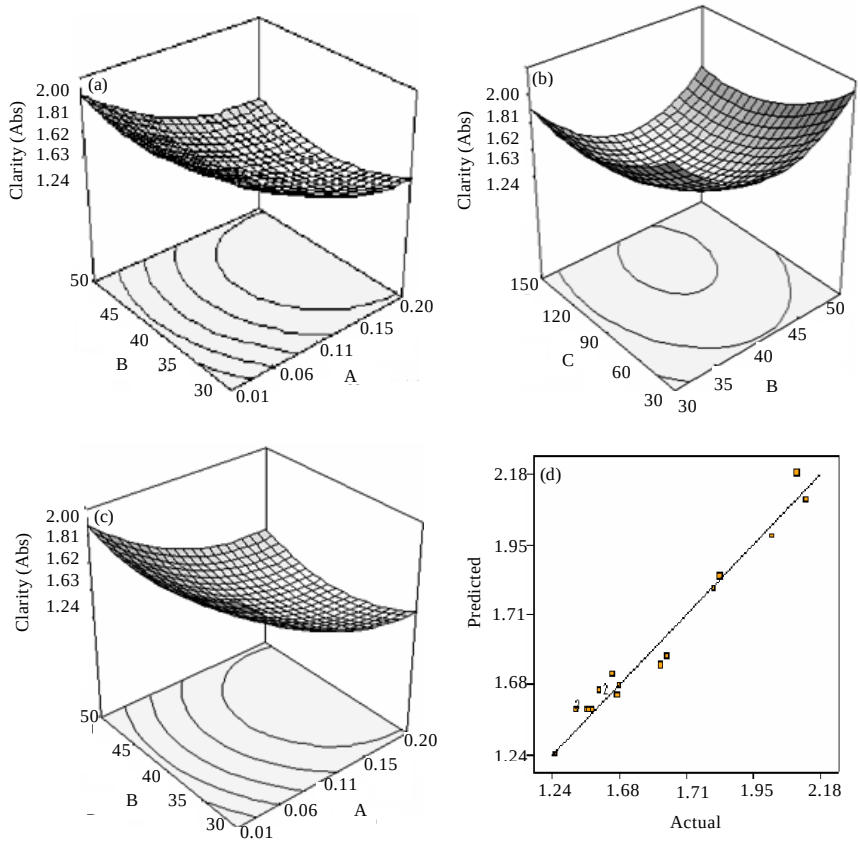

Fig. 2(a-d): Response surface for clarity of guava juice as a function of (a) Enzyme concentration (A) and temperature (B), (b) Temperature (B) and time (C), (c) Enzyme concentration (A) and temperature (B) and (d) Actual and predicated values

values were obtained in the temperature range of 38 to 45°C for corresponding time (higher than 82 min). In general, the time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara, 1982; Lee *et al*., 2006). Higher the temperature above 45°C , reduces the enzyme activity and hence the clarity is also decreased. High the incubation time gives high clarity values because enzymes breakdown more pectin and juice gets higher clarity. Figure 2(c) shows the response surface for the clarity as a function of enzyme concentration (A) and incubation time (C). At lower enzyme concentration juices shows less clarity. It can be concluding from the Fig. 2(c) that, the clarity of juice was increased with increasing the enzyme concentration and incubation time. Actual and predicated values for clarity are shown in Fig. 2(d), clarity value varies from 1.24 to 2.18 (optical density @ 525 nm).

Response on turbidity: Response surface for the turbidity as a function of enzyme concentration (A) and incubation temperature (B) is shown in Fig. 3(a). As the enzyme concentration increases, the turbidity decreases. Temperature range 38 to 44°C and enzyme concentration 0.11 and above showed good results. Figure 3(b) shows the response surface for the turbidity as a function of temperature (B) and incubation time (C). Figure 3 shows that temperature near 40°C and incubation temperature above 85 minute gives the lowest turbidity, temperature near 30°C is not optimum for enzyme and thus near this temperature range enzyme not showing the maximum activity and thus juice shows high value of turbidity. Response surface for the turbidity as a function of enzyme concentration (A) and incubation time (C) is shown in Fig. 3(c). Time and enzyme concentration significantly ($p<0.001$) affected the turbidity in both linear and quadratic manner. Both independent variables showed positive effect on quadratic terms. As the clarification process took place, the amount of pectin in the juices decreased, therefore reducing the turbidity of the juices (Alvarez *et al*., 1998). Increase in enzyme concentration and incubation time might decrease turbidity. Pectin was the main cause of turbidity (Girard and Fukumoto, 1999). The interaction between incubation time and enzyme concentration was also significant ($p<0.001$) and its effect was positive on turbidity meaning that the action of enzyme was dependent on the incubation time during enzyme treatment. Actual and predicated values for turbidity are shown in Fig. 3(d), clarity value varies from 1.02 to 1.96 (optical density @ 660 nm). Enzyme treatment to juice reduces the turbidity by degrading the pectin content in the juice.

Response on titrable acidity: Effect of temperature and time on titrable acidity at a constant enzyme concentration is shown in Fig. 4(a). In this when both temperature and time is increasing than titrable acidity is also increasing but after a certain level of temperature it remains constant and after 46°C it starts decreasing because at higher temperature enzyme activity is decreases. Titrable acidity is constantly increased with increasing the incubation time. Effect of enzyme concentration and temperature on titrable acidity of guava juice on a constant incubation time is shown in Fig. 4(b). As the enzyme concentration increases the percent yield is also increase with increasing in temperature. In this change in enzyme concentration affect the titrable acidity. At the lowest level of temperature, titrable acidity is less, with increasing in temperature percent yield is also increased.

Effect of enzyme concentration and time on titrable acidity of guava juice at a constant temperature is shown in Fig. 4(c). As the time and enzyme concentration is increases the titrable acidity is also increases. Actual and predicated values for titrable acidity is shown in Fig. 4(d),

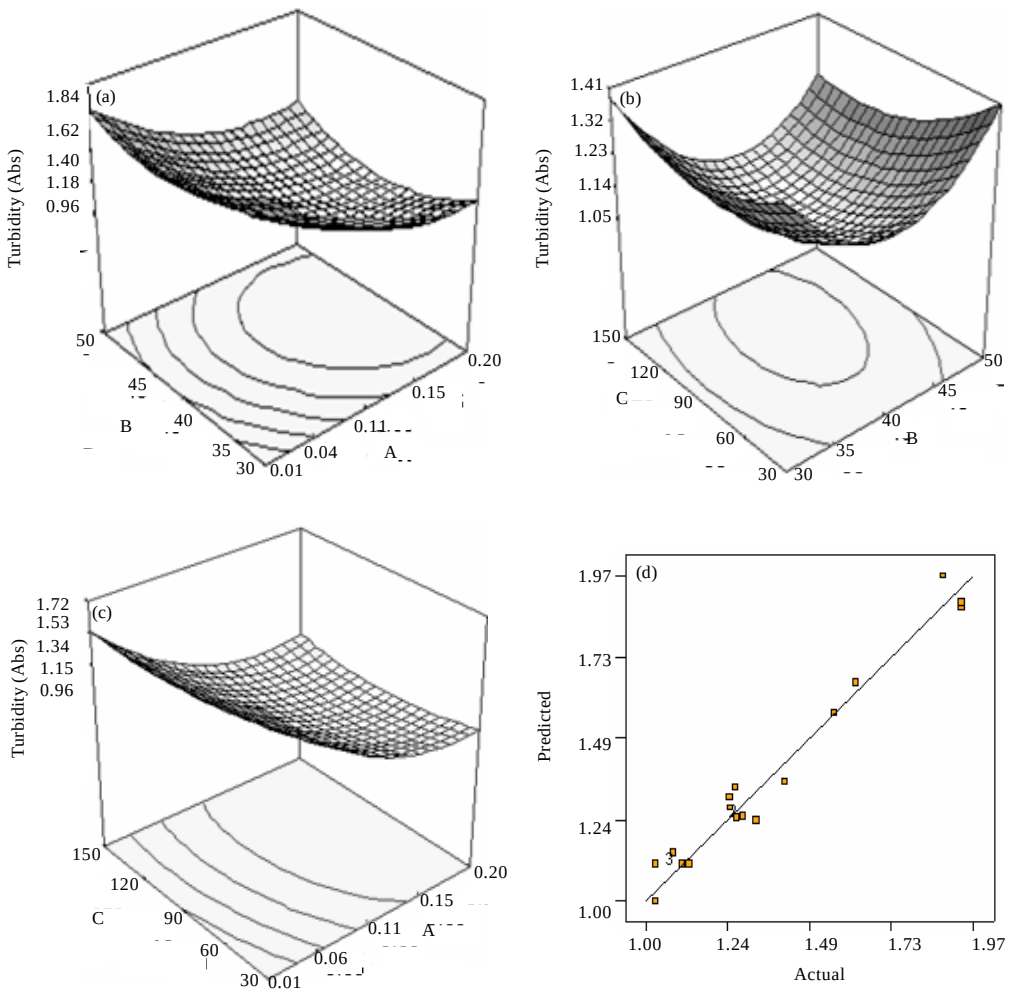

Fig. 3(a-d): Response surface for turbidity as a function of (a) Enzyme concentration (A) and temperature (B), (b) Temperature (B) and time (C), (c) Enzyme concentration (A) and time (C) and (d) Actual and predicated values

titrable acidity value varies from 0.42-0.57%. These above all figures indicate the 3D surface for the effects of the independent variables on titrable acidity.

Response on viscosity: From Table 2, it may be observed that the apparent viscosity depends on the duration of enzymatic treatment as its linear effect is negative and quadratic effect is positive. The effect of enzyme concentration on viscosity is also significant and it has linear negative effect, positive quadratic effect and positive interaction effect. From Fig. 5(a) it is evident that at a fixed time, the apparent viscosity of juice decreases with increase in enzyme concentration. It is clear from the figure that the decrease is not linear in nature. This fact is corroborated by the observations of the values of coefficients presented in Table 2. It may be observed from Table 2 that coefficients corresponding to quadratic and interactions terms are significant. Therefore, the variation of apparent viscosity with enzyme concentration is non-linear in nature.

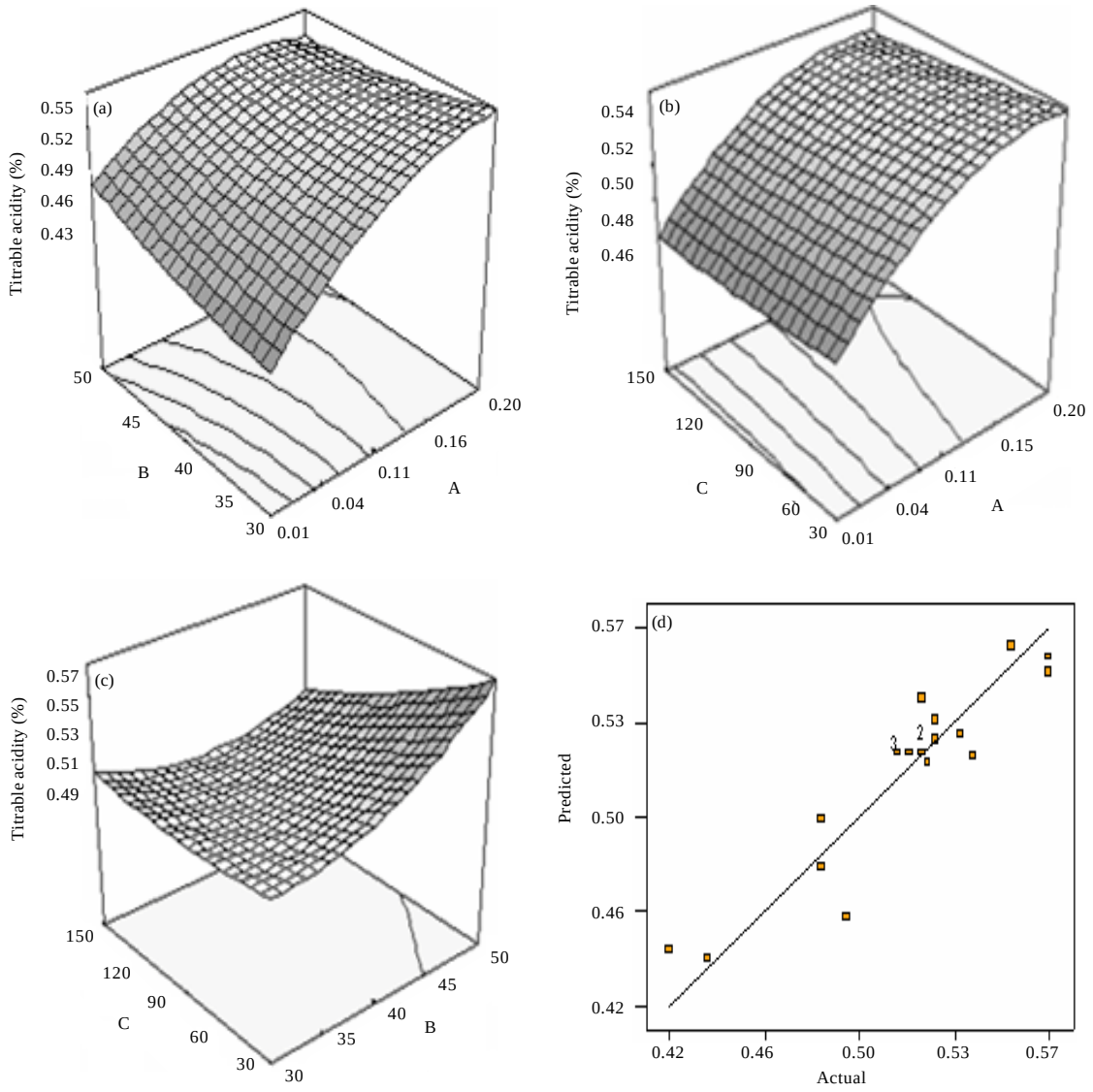

Fig. 4(a-d): Response surface for titrable acidity as a function of (a) Enzyme concentration (A) and temperature (B), (b) Enzyme concentration (A) and time (C), (c) Temperature (B) and time (C) and (d) Actual and predicated values

Figure 5(b) at fixed enzyme concentration viscosity decrease incubation time and temperature increases. The variation of apparent viscosity with enzyme concentration and duration of the enzymatic treatment at constant temperature is presented in Fig. 5(c). It may be observed from the figure that the viscosity of juice decreases with time of treatment almost linearly at constant temperature and enzyme concentration. This signifies that the linear effect of duration of treatment is dominant over the quadratic effect. It may also be observed from the figure that the viscosity decreases with enzyme concentration at fixed temperature and duration of the treatment. The pectinaceous substances posses a higher water holding capacity and developed a cohesive network structure (Koffi *et al*., 1991; Girard and Fukumoto, 1999). Actual and predicated values for viscosity are shown in Fig. 5(d). Degradation of pectin by enzyme led to reduction of water holding

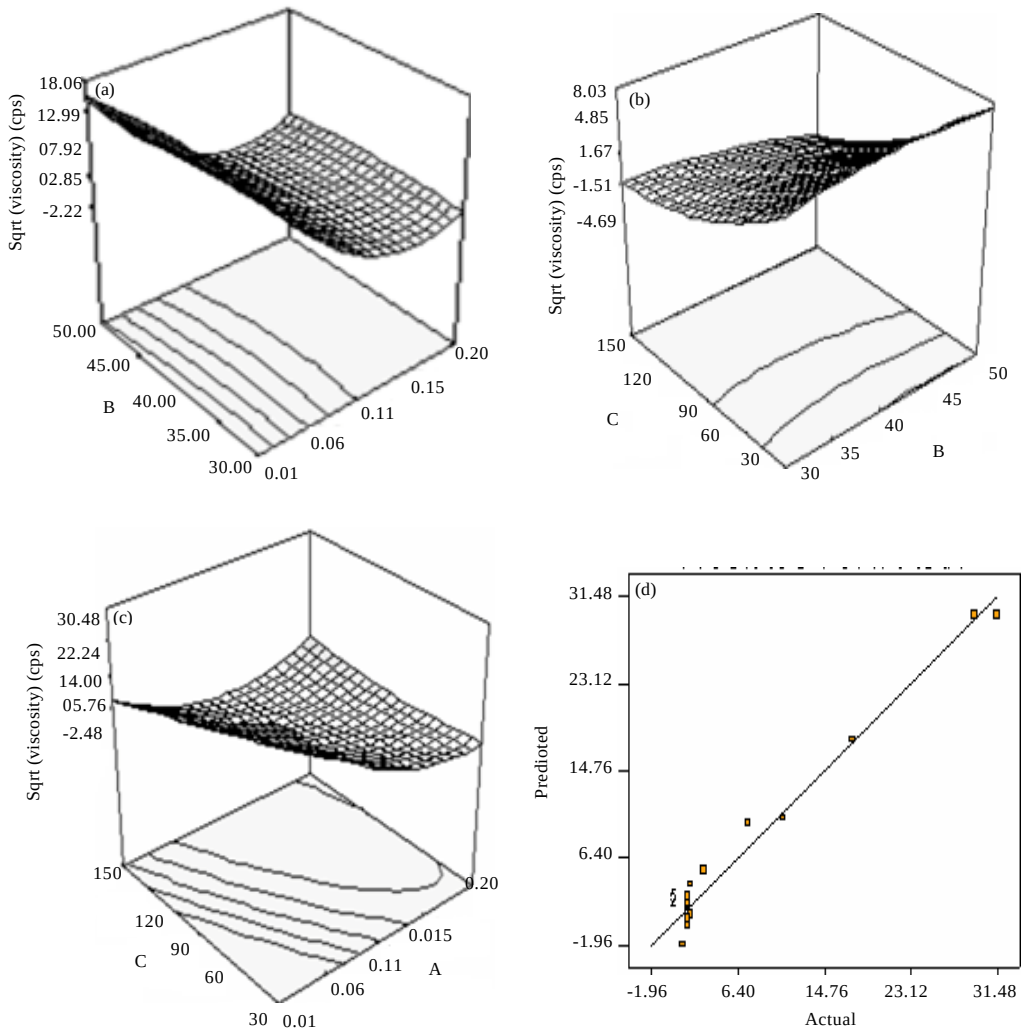

Fig. 5(a-d): Response surface for viscosity as a function of (a) Enzyme concentration (A) and temperature (B), (b) Temperature (B) and time (C), (c) Enzyme concentration (A) and time (C) and (d) Actual and predicated values

capacity and therefore, free water was released to the system to further reduce the viscosity also temperature increases the rate of enzymatic reaction.

Optimization: The different conditions (enzyme concentration, temperature and incubation time) for enzyme treatment revealed that all these variables markedly affect the percent yield, clarity, turbidity, titrable acidity and viscosity of the guava juice. These can be related to the enzyme treatment conditions by second order polynomials. Figure 1, 2, 3, 4 and 5 show the optimum conditions of the clarification process to yield maximum percent yield, clarity, titrable acidity and minimum turbidity and viscosity. It was noted that the optimum conditions for extraction of guava juice were slightly different. There are a number of combinations of variables that could give maximum levels of percent yield, clarity, titrable acidity and minimum turbidity and viscosity. Since

the optimum response for each dependent variable did not fall exactly in the same region, the superimposition of all the contour plots obtained was done.

Considering the cost of enzyme, the best combinations of process variables for response functions are found. The process variables for best combination of response function are enzyme concentration 0.11%, temperature 41.30°C and incubation time 116 min. The response functions were calculated from the final polynomial and the response were percent yield (80.03%), clarity (1.34 Abs), turbidity (1.07 Abs), titrable acidity (0.525%) and viscosity (2.4 cps) and desirability of 0.942.

From the systematic study of the variation of the operating variables and measurement of the dependent properties, response functions are established employing an appropriate statistical analysis. Using the contour plots or the response surface methodology, the optimum set of the operating variables are obtained graphically in order to obtain the desired levels of five properties of the guava juice.

CONCLUSION

From the systematic study of the variation of the operating variables and measurement of the dependent properties, response functions are established employing an appropriate statistical analysis. Using the contour plots or the response surface methodology, the optimum set of the operating variables are obtained graphically in order to obtain the desired levels of properties of the guava juice. Based on response surface and contour plots, the optimum conditions for clarifying the Guava juice were: 0.11% enzyme concentration, incubation temperature of 41.30°C and incubation time of 115.98 min.

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REFERENCES

Abudabos, A.M., 2012. Effect of enzyme supplementation to normal and low density broiler diets based on corn-soybean meal. Asian J. Anim. Vet. Adv., 7: 139-148.

Alam, M.S., A. Singh and B.K. Sawhney, 2010. Response surface optimization of osmotic dehydration process for aonla slices. J. Food Sci. Technol., 47: 47-54.

Alvarez, S., R. Alvarez, F.A. Riera and J. Coca, 1998. Influence of depectinization on apple juice ultrafiltration. Colloid Surface A, 138: 377-382.

Anderson, C. and N. Badrie, 2005. Physico-chemical quality and consumer acceptance of guava wines. J. Food Sci. Technol., 42: 223-226.

Ceci, L. and J. Lozano, 1998. Determination of enzymatic activities of commercial pectinases for the clarification of apple juice. Food Chemi., 61: 237-241.

Daramola, M.O., K.J. Keesman and F. Spenkelink, 2007. Process modelling of ultrafiltration units: An RSM approach. J. Applied Sci., 7: 3687-3695.

Floros, J.D. and M.S. Chinnan, 1987. Optimization of pimiento pepper lye peeling process using response surface methodology. Trans. ASAE, 30: 560-565.

Frank, R., 2001. Blending response surface methodology and principle components analysis to match a target product. Food Quail. Prefer., 12: 457-465.

Girard, B. and L.R. Fukumoto, 1999. Apple juice clarification using microfiltration and ultrafiltration polymeric membranes Food Sci. Technol., 32: 290-298.

Grassin, C. and P. Fauquembergue, 1996. Application of pectinase in beverage. Prog. Biotechnol., 14: 453-462.

Jagtiani, J., H.T. Chang and W.S. Sakai, 1999. Guava: Tropical Fruit Processing. Academic Press, New York.

Jaiswal, N., O. Prakash, M. Talat, S.H. Hasan and R.K. Pandey, 2011. Application of response surface methodology for the determination of optimum reaction conditions (Temperature and pH) for starch hydrolysis by a-Amylase. Asian J. Biochemi., 6: 357-365.

Joseph, B. and R.M. Priya, 2011. Phytochemical and biopharmaceutical aspects of *Psidium guajava* (L.) essential oil: A review. Res. J. Med. Plant, 5: 432-442.

Kalil, S.J., F. Maugeri and M.I. Rodrigues, 2000. Response surface analysis and simulation as a tool for bioprocess design and optimization. Proc. Biochem., 35: 539-550.

Kashyap, R.D., P.K. Vohra, S. Chopra and R. Tewari, 2001. Application of pectinase in the commercial sector: A review. Bioresour. Technol., 77: 215-227.

Kilara, A., 1982. Enzyme and their uses in the processed apple industry: A review. Prog. Biochem., 23: 35-41.

Koffi, E.K., C.A. Sims and R.P. Bates, 1991. Viscosity reduction and prevention of browning in the preparation of clarified banana juice. J. Food Qual., 14: 209-218.

Kuddus, M., Roohi, J.M. Arif and P.W. Ramteke, 2011. An overview of cold-active microbial a-amylase: Adaptation strategies and biotechnological potentials. Biotechnology, 10: 246-258.

Lee, W.C., S. Yusof, N.S.A. Hamid and B.S. Baharin, 2006. Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). J. Food Eng., 73: 55-63.

Little, T.M. and F.J. Hills, 1978. Agricultural Experimentation Design and Analysis. John Wiley and Sons, New York, USA, pp: 170.

Mendenhall, W., 1975. Introduction to Probability and Statistics. 4th Edn., Duxbury Press, North Settuate, MA., pp: 273.

Montgomery, D.C., 2001. Response Surface Methodology. Jhon Wiley, New York, pp: 416-419.

Myers, R., 1976. Response Surface Methodology. John Wiley, New York, pp: 139-153.

Pal, A., Farhath, 2010. Efficacy of xylanase purified from *Aspergillus niger* DFR-5 alone and n combination with pectinase and cellulose to improve yield and clarity of pineapple juice. J. food Sci. technol., 48: 560-568.

Ranganna, S., 1977. Manual of Analysis of Fruits and Vegetable Products. Tata McGraw-Hill, New Delhi, India, Pages: 634.

Reddy, L.V.A., Y.H.K. Reddy and O.V.S. Reddy, 2006. Wine production by guava piece immobilized yeast from Indian cultivar grapes and its volatile composition. Biotechnology, 5: 449-454.

Sanda, K.A., H.A. Grema, Y.A. Geidam and Y.M. Bukar-Kolo, 2011. Pharmacological aspects of *Psidium guajava:* An update. Int. J. Pharmacol., 7: 316-324.

Seth, D. and H.N. Mishra, 2011. Optimization of honey candy recipe using response surface methodology. Am. J. Food Technol., 6: 985-993.

Sevda, S. B. and L. Rodrigues, 2011a. Fermentative behavior of saccharomyces strains during guava (Psidium Guajava L.) must fermentation and optimization of guava wine production. J. Food Proc. Technol., 2: 118-119.

Sevda, S.B. and L. Rodrigues, 2011b. The making of pomegranate wine using yeast immobilized on sodium alginate. Afr. J. Food Sci., 5: 299-304.

Sevda, S.B., L. Rodrigues and C. Joshi, 2011a. Effect of heat shock on yeast cell and its effect on glycerol production in pomegranate wine. J. Food Bev. Wor., 38: 48-50.

Sevda, S.B., L. Rodrigues and C. Joshi, 2011b. Influence of heat shock on yeast cell and its effect on glycerol production in guava wine. J. Biochem. Technol., 3: 230-232.

Shalini, R. and D.K. Gupta, 2010. Utilization of pomace from apple processing industries: A review. J. Food Sci. Technol., 47: 365-371.

Sharma, S., K. Rajat, R. Prasad and P. Vasuderian, 1999. Biology and potential of *Psidium guajava*. J. Sci. Ind. Res., 58: 414-421.

Singh, A., S. Kumar and H.K. Sharma, 2012. Effect of enzymatic hydrolysis on the juice yield from bael fruit (*Aegle marmelos* Correa) pulp. Am. J. Food Technol., 7: 62-72.

Vijayanand, P., S.G. Kulkarni and G.V. Prathibha, 2009. Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice. J. Food Sci. Technol., 47: 235-239.

Viquez, F., C. Laetreto and R.D. Cooke, 1981. A study of the production of clarified banana juice using pectinolytic enzymes. Internati. J. Food Sci. Technol., 16: 115-125.