Enzymatic clarification of carrot juice by using response surface methodology

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Abstract: Carrot juice was treated with pectinase at various enzyme concentrations (0.01% to 0.1%), process temperatures (35^0C to 55^0C) and incubation time (40 to 120 min). The effect of these enzyme treatments on filterability, clarity, turbidity and viscosity of the juice were studied by employing a second order central composite design. The coefficient of determination (R^2) values for filterability, clarity, turbidity and viscosity were greater than 0.85. Statistical analysis showed that filterability, clarity, viscosity and turbidity were significantly (p<0.05) correlated to enzyme concentration, incubation temperature and incubation time. Enzyme concentration was the most important factor affecting the characteristics of the carrot juice as it exerted a highly significant influence (p<0.05) on all the dependent variables. An increase in process time and/or concentration of enzyme treatment was associated with an increase in filterability and clarity, and decrease in turbidity and viscosity. Based on response surface and contour plots, the optimum conditions for clarifying carrot juice were 0.092% enzyme concentration, incubation temperature of 54.2^0C and incubation time of 119 min.

Keywords: carrot juice, enzyme clarification, response surface methodology

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1 Introduction

Carrot is an important root vegetable and is often used for juice production (Yoon et al., 2005). Carrot juice has a high nutritional value as it is an important source of carotene, and carrot juice is preferably used as a natural source of pro-vitamin A in the carotenoids drinks (Demir et al., 2004; Yoon et al., 2005). In many countries, a steady increase of carrot juice consumption has been reported (Schieber et al., 2001). Juice clarification is an essential step before other specific treatments such as removing polyphenolic compounds, bitterness, tartness and acids with adsorbent resins (Carabasa et al., 1998; Johnson and Chandler, 1982; Lue, 1989), de-acidification by electrodialysis (Vera et al., 2003a; Vera et al., 2003b), recovery of natural color substances and concentration by membrane technologies (Alvarez et al., 2000; Bailey et al., 2000; Cassano et al., 2004). By complete removal of suspended solids, the efficiency of these post clarification treatments increases considerably.

Pretreatment of juice with enzymes causes hydrolysis and subsequent degradation of pectin. Separation of degraded pectin reduces the viscosity of the solution and removal of pectinious material, which tend to form a deposited foulant layer over the membrane surface. Consequently, depectinization directly leads to an improvement of the permeate flux (Alvarez et al., 1998; Chamchong and Noohorm, 1991; Sahin and Bayindirli, 1993). Pectinase hydrolyzes pectins resulting in pectin-protein complexes to flocculate. The enzymatic clarification is influenced by a number of variables including concentration of the enzyme, temperature and incubation time of the treatment (Neubeck, 1975; Baumann, 1981; Lanzarini and Pifferi, 1989). The present work involves optimization of

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different parameters affecting the depectinization rate. The general practice of optimization is by varying one and keeping the other parameters at an unspecified constant level. The major disadvantage of this single variable optimization is that it does not include interactive effects and does not depict the net effects of various parameters on the reaction rate. Thus to overcome this problem, the optimization studies have been conducted using response surface methodology (RSM).

RSM is an affective statistical technique for optimizing complex processes. RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions. It is less laborious and time-consuming than other approaches. It has constantly and successfully been demonstrated that it can be used in optimizing process variables (Montgomery, 2001; Dhingra and Paul, 2005; Yagci and Gogus, 2008; Alam et al., 2010; Alam et al., 2011).

Pectinase had been used for tangerine juice clarification (Chamchong and Noomhorm, 1991), pineapple juice clarification (Carneiro et al., 2002) prior to ultrafiltration and microfiltration. No attempt has been made to optimize the enzymatic clarification process for carrot juice. In this study, concentration of the enzyme, temperature and incubation time of the treatment were selected as the independent variables for optimization of the carrot juice using pectinase enzyme. These are the key factors that influence the mechanism of enzyme activity in the juice (Baumann, 1981). The purpose of the present work is to study the effect of enzyme concentration, temperature and incubation time on the filterability, clarity, turbidity and viscosity and to optimize the enzymatic clarification process of carrot juice using RSM.

2 Material and methods

2.1 Juice extraction process

Carrot (Variety: PCB 5), commonly grown in Punjab was selected for the study and was procured from the vegetable farm of Punjab Agricultural University, Ludhiana, India. The carrots were washed, peeled and cut into small pieces. The juice was extracted using Sujata mixer. The TSS of the fresh juice was 6.5.

2.2 Enzymatic treatment

Commercial pectinase enzyme (source: *Aspergillus niger*) was obtained from Sisco Research Laboratory Pvt. Ltd. (SRL), Mumbai, India. The carrot juice with natural pH of 6.5 was treated with pectinase enzyme. The pH of the juice was kept at its natural pH. The pH range was optimal for the exogenous pectinases (Grassin and Fauquembergue, 1995). For each experiment, 200ml of juice was subjected to different enzyme treatment conditions. The independent process variables for the enzymatic treatment process were the pectinase enzyme concentration (C: 0.01%to 0.1%), juice temperature (T: 35°C to 55°C) and holding time (t: 40 to 120 min). The temperature of enzyme treatment was adjusted to the desired level using a water bath. At the end of the enzymatic treatment, the enzyme in the sample was inactivated by heating the juice at 90°C for 5 min in a water bath. The treated juices were centrifuged at 2,000 rpm for 10 min using a centrifuge and the supernatant was collected. The supernatant obtained were evaluated for their filterability, clarity, turbidity and viscosity.

2.2.1 Filterability

The centrifuged juice was filtered through a filter paper (Whatman No. 1). Filterability (s^{-1}) was determined from the reverse of the time taken for filtering 200 mL enzymatic treated carrot juice using gravity filtration through filter paper.

2.2.2 Clarity

The clarity of the juice obtained was determined by measuring the absorbance at a wavelength of 453 nm using RAYLEIGH UV2601 Spectrophotometer. Distilled water was used as the reference.

2.2.3 Turbidity

The turbidity of the juice was determined using a portable Turbidimeter and was expressed in nephelometric turbidity units (NTU).

2.2.4 Viscosity

The viscosity of clarified carrot juice was determined using Bohlin CVO 100 Rheometer at 100 rpm, temperature (25°C) and varying shear rates. The viscosity was expressed as an average of viscosity at various shear rates.

2.3 Experimental design

Response surface methodology was used to design the experiment. Design expert Software Version 8.0.2 was used to generate the experimental designs, statistical analysis and regression model. The Box-Behnken design with a quadratic model (Box and Draper, 1987) was employed. Three independent variables namely enzyme concentration (C), temperature (T) and time (t) were chosen. Each independent variable had 3 levels which were −1, 0 and +1. A total of 17 different combinations (including five replicates of the centre point each signed the coded value 0) were chosen in random order according to a Box-Behnken design configuration for three factors (Cochran and Cox, 1957). The experimental design in the coded and actual levels of variables is shown in Table 1. The responses function (y) measured were filterability, clarity, turbidity and viscosity of the carrot juice. These values were related to the coded variables by a second degree polynomial using the equation below.

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

Table 1 Experimental design for enzymatic clarification of carrot juice

Enzyme concentration, %		Temperature, ^{0}C		Time, min	
Actual (C)	Coded (X_1)	Actual (T)	Coded (X_2)	Actual (t)	Coded (X_3)
0.055	0	45	0	80	0
0.1	+1	35	-1	80	0
0.01	-1	45	0	40	-1
0.055	0	45	0	80	0
0.01	-1	55	+1	80	0
0.1	+1	45	0	40	-1
0.01	-1	35	-1	80	0
0.1	+1	55	+1	80	0
0.055	0	55	+1	40	-1
0.055	0	45	0	80	0
0.055	0	45	0	80	0
0.055	0	35	-1	40	-1
0.01	-1	45	0	120	+1
0.055	0	55	+1	120	+1
0.055	0	35	-1	120	+1
0.055	0	45	0	80	0
0.1	+1	45	0	120	+1

2.4 Optimization

Response surface methodology was applied to the experimental data using a commercial statistical package, design expert Software Version 8.0.2 (Stat ease Inc., Minneapolis, USA, Trial Version). The same software was used for the generation of response surface plots, superimposition of contour plots and optimization of process variables. The optimization of enzymatic process aimed at finding the levels of independent variables viz. enzyme concentration, temperature and process time, which could give maximum possible value of filterability, clarity and minimum value of turbidity and viscosity of carrot juice.

3 Results and discussion

The experimental values for filterability, clarity, turbidity and viscosity under different treatment combinations are presented in Table 2. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms are presented in Table 3. The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R^2 for all the responses. The R^2 values for filterability, clarity, turbidity and viscosity were 0.975, 0.877, 0.947 and 0.888 respectively. The closer the value of R^2 to the unity, the better the empirical model fits the actual data (Little and Hills, 1978; Mendenhall, 1975). The probability (p) values of all regression models were less than 0.01, with no lack-of-fit.

3.1 Effects of enzyme concentration, temperature and time on selected responses

The effect of different enzyme treatment conditions on the selected responses i.e. filterability, clarity, turbidity and viscosity are reported (Table 3) by the coefficient of the second order polynomials. To aid visualization, the response surfaces for filterability, clarity, turbidity and viscosity are shown in Figure 1.

Figure 1a shows the contour map for the effect of the independent variables on the filterability. The filterability of carrot juice varied from 0.00142-0.00729 s^{-1}, irrespective of the enzyme concentration, temperature and incubation time (Table 2). As shown in Table 3, filterability was positively related to the linear effect of enzyme concentration ($p < 0.05$), temperature ($p < 0.05$) and incubation time ($p < 0.10$) and the quadratic terms of these variables were not found to be significant except the temperature resulting in an increase in filterability with

enzyme concentration at all temperatures. It can be seen from Table 3 that the interaction term of enzyme concentration and temperature affected the filterability of juice. At higher level of temperature, the filterability of the juice was found to increase rapidly with an increase in incubation time (Figure 1a). At the highest level of incubation time, the filterability of the juice increase to a certain level and then increase at a slower rate. During the enzymatic treatment, pectinase breaks down the pectin molecules. Degradation of pectin leads to a reduction of water holding capacity, and consequently, free water is released to the system and reduces the viscosity and thus facilitating filtration (Lee et al., 2006).

The clarity of carrot juice varied from 0.68 to 2.51Abs, irrespective of the enzyme concentration, temperature and incubation time (Table 2). The clarity was significantly affected by the linear ($p < 0.05$) and quadratic ($p < 0.1$) term of enzyme concentration. The clarity of the juice increased with the increase in the concentration of pectinase enzyme however, for temperature the affect was vice versa (Table 3). Figure 1b clearly shows that at higher temperatures the clarity of the juice followed parabolic pattern, owing to a significant quadratic term which is corroborated by Table 3. The temperature increased the rate of enzymatic reactions, hence the rate of clarification, as long as the temperature was below denaturation temperature for the enzyme. In general, the time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara 1982).

Table 2 Effect of enzyme concentration, temperature and time on four dependent variables

Independent variables			Dependent variables			
Enzyme concentration, %	Temperature, ^{0}C	Incubation time, min	Filterability, s^{-1}	Clarity, Abs	Turbidity, NTU	Viscosity, cps
0.055	45	80	0.002273	1.52	753.00	0.02413
0.1	35	80	0.002632	2.36	268.00	0.02500
0.01	45	40	0.002208	1.42	1057.00	0.02522
0.055	45	80	0.002315	1.58	647.00	0.01866
0.01	55	80	0.004202	0.68	783.00	0.01844
0.1	45	40	0.002597	2.03	657.00	0.02630
0.01	35	80	0.00142	0.88	700.00	0.02841
0.1	55	80	0.007299	1.85	490.00	0.01400
0.055	55	40	0.005	1.13	705.00	0.01330
0.055	45	80	0.00237	1.79	698.00	0.01795
0.055	45	80	0.002283	1.97	725.00	0.01995
0.055	35	40	0.001669	1.31	414.00	0.02790
0.01	45	120	0.002247	1.69	802.00	0.02428
0.055	55	120	0.005556	1.05	681.00	0.01040
0.055	35	120	0.002353	1.71	371.00	0.02630
0.055	45	80	0.002222	1.29	710.00	0.02380
0.1	45	120	0.003546	2.51	545.00	0.02275

Table 3 Regression coefficients (uncoded variables) from polynomial model and their significance

Regression coefficients	Filterability		Clarity		Turbidity		Viscosity	
	Coefficient value	*F* value	Coefficient value	*F* value	Coefficient value	*F* value	Coefficient value	*F* value
Intercept	0.00229*	30.38	1.63*	5.56	706.60*	14.05	0.0216*	6.17
Enzyme (A)	0.00075*	32.03	0.51*	30.10	-172.75*	57.81	-0.00104	1.17
Temperature (B)	0.00175*	174.10	-0.19**	4.32	113.25*	24.84	-0.00643*	44.87
Incubation Time (C)	0.00028**	4.42	0.13	2.02	-54.25*	5.70	-0.00124	1.37
A*B	0.00047*	6.34	-0.078	0.34	34.75	1.17	-0.00025	0.036
A*C	0.00023	1.47	0.054	0.17	35.75	1.24	-0.00065	0.23
B*C	-0.00003	0.03	-0.122	0.87	4.75	0.02	-0.00032	0.057
A^2	0.00030	2.71	0.212	2.75	38.08	1.48	0.00286	4.68
B^2	0.00130*	50.33	-0.399*	9.77	-184.43*	34.68	-0.00229	3.02
C^2	0.00006	0.10	0.071	0.30	20.58	0.43	0.00087	0.44
C.V. %	12.21		16.67		9.93		12.59	
R^2	0.975		0.878		0.947		0.888	

Note: * significant at 5%, ** significant at 10% level of significance.

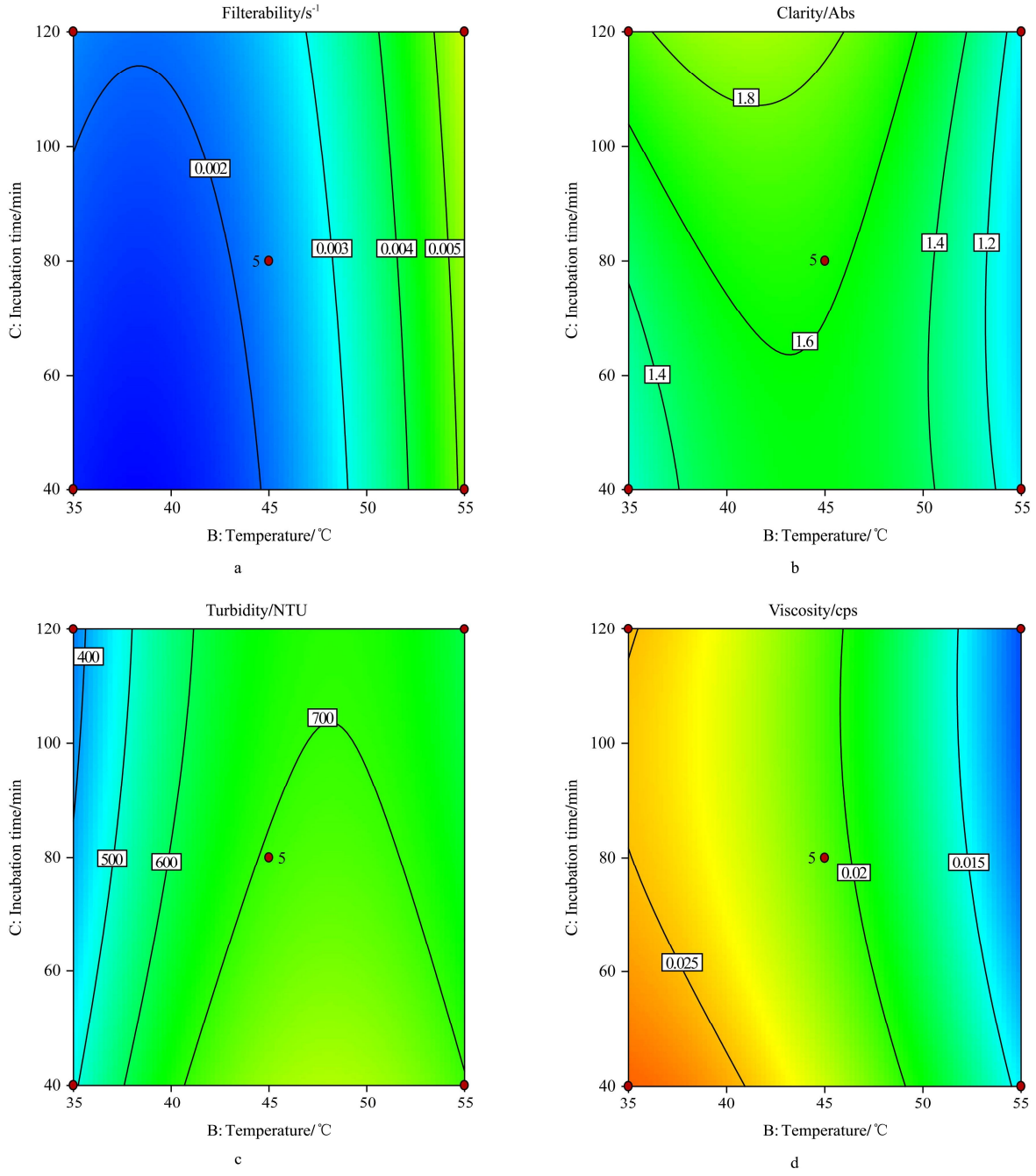


Figure 1 Contour plots of selected responses of carrot juice as a function of incubation time and process temperature at constant enzyme concentration of 0.055%

The turbidity of the juice varied from 268 to 1,057 NTU, irrespective of the enzyme concentration, temperature and incubation time (Table 2). Turbidity was significantly affected by the linear term of enzyme concentration ($p < 0.05$), temperature ($p < 0.05$) and incubation time ($p < 0.05$) and the quadratic term of process temperature ($p < 0.05$). An increase in enzyme concentration drastically decreased turbidity (Table 3). Figure 1c clearly shows the positive effect of temperature on turbidity. The turbidity decreased markedly with the increase in enzyme concentration, irrespective of incubation time and process temperature. The increase in enzyme concentration and incubation time decreased the turbidity of the juice (Table 3). 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).

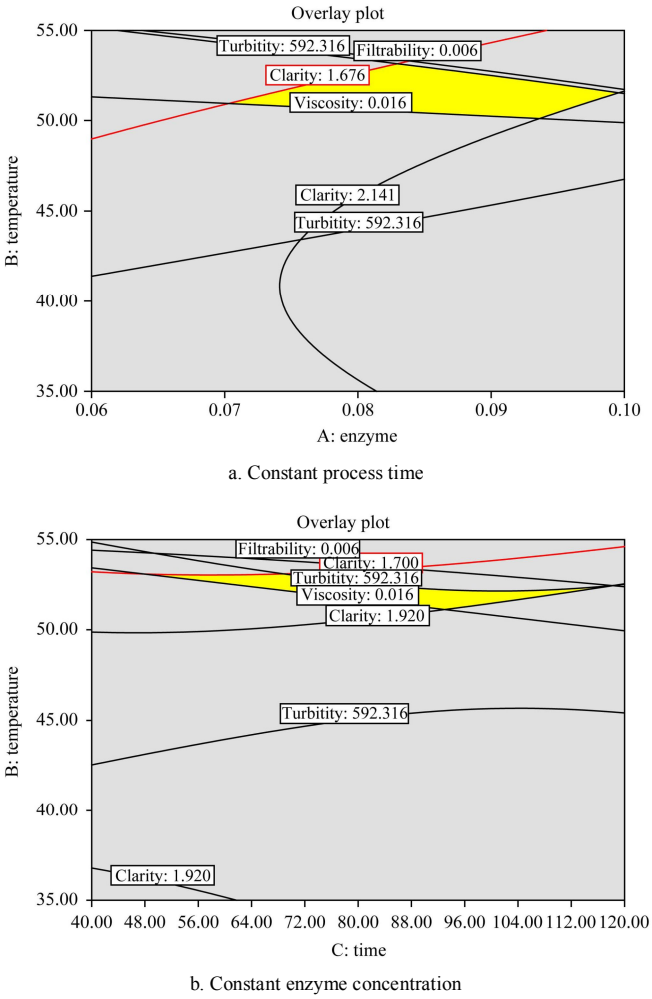
The viscosity of the juice varied from 0.0104 to 0.0284 cps, irrespective of the enzyme concentration, temperature and incubation time (Table 2). It is pertinent from table 3 that the linear term of enzyme concentration, temperature and incubation has negative

effect on viscosity. Among the selected variables, only process temperature showed significantly higher affect on viscosity ($p < 0.05$). Moreover, at the lowest level of temperature, the viscosity of the juice initially decreased and then increased at a slower rate. At the highest level of temperature, viscosity decreased marginally (Figure 1d). The temperature increases the rate of enzymatic reactions. Upon enzyme treatment, degradation of pectin leads to a reduction of water holding capacity, free water was released to the system thus reducing the viscosity of the juice (Lee et al., 2006).

3.2 Optimization

The optimum conditions of the clarification process to yield maximum filterability and clarity and minimum turbidity and viscosity, respectively. There are a number of combinations of variables that could give maximum level of filterability and clarity and minimum level of 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. Figure 1 shows the superimposed contour plot for optimization of filterability, clarity, turbidity and clarity keeping the incubation time constant at the central point. The zone of optimization, as shown in the superimposed contour plot, depicts enzyme concentration to be in the range of 0.07% and 0.1% and temperature between 49^{0}C and 54.5^{0}C. Figure 2 (a and b) shows the superimposed contour plot of filterability, clarity, turbidity and viscosity keeping the enzyme concentration constant at the central point. The zone of optimization, as shown in the superimposed contour plot, depicts process temperature to be in the range of 51^{0}C and 54.5^{0}C and incubation time between 49 min and 119.5 min. During juice clarification by ultrafiltration or microfiltration, the cost of enzyme treatment is important. Therefore, considering the cost of enzyme, the best combinations of process variables for response functions are found.

The optimum operating condition for enzymatic clarification by numerical optimization was 0.092% of enzyme concentration, 54.2^{0}C of process temperature and 119 min of incubation time. The response functions corresponding to this operating condition were 0.0066 s^{-1} filterability, 1.75 Abs clarity, 565.475 NTU turbidity and 0.014 cps viscosity.



a. Constant process time

b. Constant enzyme concentration

Figure 2 Superimposed contour plots for optimization of filterability, clarity, turbidity and viscosity

4 Conclusions

Response surface methodology can be used successfully for optimizing the enzymatic clarification of carrot juice. The operating variables (pectinase enzyme concentration, temperature and incubation time) for enzyme treatment markedly affected the filterability, clarity, turbidity and viscosity of the carrot juice. The regression coefficients of second order polynomials obtained can be used for optimum enzyme treatment conditions for desired responses within the range of conditions applied in this study. The optimum set of the operating variables are obtained graphically in order to obtain the desired levels of these properties of the carrot juice which is suitable for the subsequent membrane based clarification.

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