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EFFECT OF CRUDE PECTINASE ON APPLE JUICE QUALITY CHARACTERISTICS BY DESIRABILITY APPROACH

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ABSTRACT
The present study analyzed the efficiency of crude pectinase produced from *Bacillus subtilis* using hazelnut shell hydrolysate during clarification of apple juice. Response surface methodology (RSM) based desirability analysis was carried out to optimize the enzyme load, temperature, and time with respect to lightness, turbidity and clarity. The apple juice clarification was performed at different enzyme loads (0.3-0.7%), pH (4-7), temperature (30-50 °C) and time (2-6 h). The RSM results revealed optimum clarification conditions of 0.3% (w/v) enzyme load, 45.8 °C temperature, and 2 h of time resulting in 70.40 lightness, 49.44 turbidity and 88.33% clarity. To confirm the validity of RSM models, RMSE values of L value, turbidity and clarity were calculated as 4.65, 5.28 and 9.15, respectively. As a conclusion, maximum clarity at a low enzyme load and time makes the enzyme useful for juice industry.
Keywords: Clarification, crude bacterial pectinase, desirability optimization methodology, response surface methodology, depectinization

İSTENEBİLİRLİK YAKLAŞIMI İLE ELMA SUYU KALİTE ÖZELLİKLERİ ÜZERİNE HAM PEKTİNAZ ENZİMİNİN ETKİSİ

ÖZ
Bu çalışmada elma suyunun berraklaştırılması sırasında fındık kabuğu hidrolizatı kullanılarak *Bacillus subtilis*'ten üretilen ham pektinaz enziminin etkinliği analiz edilmiştir. Açıklık-koyuluk, bulanıklık ve berraklık açısından enzim yükü, sıcaklık ve süreyi optimize etmek için istenebilirlik analizine dayalı cevap yüzey metodu kullanılmıştır. Elma suyu berraklaştırması, farklı enzim miktarları (% 0.3-0.7), pH (4-7), sıcaklık (30-50 °C) ve süre (2-6 saat) kullanarak gerçekleştirilmiştir. RSM sonuçları, %0.3 (w/v) enzim miktarı, 45.8 °C ve 2 saat optimum berraklaştırma koşullarında 70.40 açıklık-koyuluk, 49.44 bulanıklık ve %88.33 berraklık ile sonuçlanmıştır. RSM modellerinin geçerliliğini doğrulamak için, L değeri, bulanıklık ve berraklığın RMSE değerleri sırasıyla 4.65, 5.28 ve 9.15 olarak hesaplanmıştır. Sonuç olarak, düşük enzim miktarı ve sürede elde edilen maksimum berraklık enzimi meyve suyu endüstrisi için kullanılabilir yapmaktadır.
Anahtar kelimeler: Berraklaştırma, ham bakteriyal pektinaz, istenebilirlik optimizasyon metodu, cevap yüzey metodu, depektinizasyon

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INTRODUCTION

Haze and turbidity in freshly pressed juice are caused mainly by pectin and other polysaccharides. Based on the National Nutrient Database for Standard Reference (US), carbohydrate content is very high in raw apples (13.8%), pineapples (13.1%) and tomato (4.0%). A considerable challenge for fruit processing contributes a stable cloud in fruit juice which includes high amount of pectin. Transparency and homogeneity are the two main characteristics for fruit juices (Sakhale et al., 2016). Clarification of fruit juices is desirable to comply with international standards. Effective enzymatic clarification subsequently leads to higher juice yield and short clarification process (Whitaker, 1984; Sandri et al., 2011; Sandri et al., 2013).

The enzymatic hydrolysis of pectin is influenced by several variables including incubation period, temperature, pH and enzyme concentration (Lee et al., 2006; Rai et al., 2004; Sin et al., 2006). Also during clarification, the cost of the enzyme is important. In order to develop economically and environmentally feasible clarification process, a rational design and modeling approach is inevitable for better-describing the effect of process parameters on the clarification. The optimization of operating parameters for pectinase treatments with respect to sweet orange juice (Rai et al., 2004), banana juice (Lee et al., 2006), sapodilla juice (Sin et al., 2006) and star fruit juice (Abdullah et al., 2007) have already been reported. Despite several studies on the optimization of depectinization during enzymatic treatment such as commercial pectinases of fungal origin (Chen et al., 2012; Pinelo et al., 2010; Alam et al., 2014), clarification of fruit juices using bacterial and fungal crude pectinase enzymes are among reported ones. Besides, current literature supports the use of crude enzyme as crude exo-polygalacturonase from *B. subtilis* CM5 was more efficient than commercial pectinase (Pectinex) (13.3% more yield) in carrot juice (Swain and Ray, 2010). However, there is no information about influence of parameters on clarification in preliminary study of Swain and Ray (2010). According to Joshi et al. (2011), use of pectin methyl esterase enzyme from *Aspergillus niger* by apple pomace increased the yield of apple and pear juices. Sandri et al. (2011) tested the efficiency of two crude pectinolytic extracts (*A. niger* T0005007-2 and *A. oryzae* IPT 301) during clarification of apple, butia palm fruit, blueberry, and grape juices. Kumar and Sharma (2015) investigated effects of concentration of crude (*A.niger* NCIM 548) and commercial enzymes (such as cellulase, pectinase, hemicellulase), time and temperature on the enzymatic degumming of pineapple (*Ananas comosus*) mill juice to improve filtration rate, clarity, relative viscosity and percentage overrun. From these studies, it can be seen that pectinases originated from fungi are widely used in beverage industry to clarify fruit juices. Potential use of pectinase produced from *Bacillus* sp. MBRL576 by submerged fermentation to clarify banana, apple and carrot juices has been reported (Bhardwaj and Garg, 2014). Uzuner and Cekmecelioglu (2015) reported potential use of bacterial pectinase originated from *B. subtilis* grown on hazelnut shell hydrolysate in clarification of carrot juice. Optimization of crude pectinase clarification conditions with respect to several responses has not been reported yet. In the present study, a novel approach was applied that dealt with the use of crude bacterial pectinase to obtain low cost clarification process of apple juice for industrial applications such as juice industry.

As a result, the aim of this study was to investigate the enzymatic clarification of apple juice as a model fruit using crude bacterial pectinase produced by *Bacillus subtilis* and also evaluate the performance of clarification process. Optimal enzyme load, temperature and time to provide the highest lightness (L value) and clarity percentage and also lowest turbidity were determined using the Box-Behnken response surface methodology (RSM).

MATERIALS AND METHODS

Materials

The 'Golden Delicious' apples were supplied from a local market in Ankara, Turkey and hazelnut shells were supplied from a local plant in Ordu, Turkey. Pectinex 3XL (from *Aspergillus*

niger) was purchased from Sigma-Aldrich (Darmstadt, Germany).

Preparation of apple juice

After washing with tap water and cutting into halves, apple halves were subsequently squeezed with a fruit and vegetable juicer with a yield of 51.65±2.90% and manually filtered through two layers of cheesecloth to obtain raw apple juice.

Crude enzyme preparation

The ground hazelnut shells were pretreated with dilute acid (H_2SO_4) and subsequently hydrolyzed with cocktail enzyme (Viscozyme L, Sigma-Aldrich, Denmark) as described in our previous study (Uzuner and Cekmecelioglu, 2014). Enzyme was produced in 500 mL erlenmeyer flask containing 100 mL total hydrolyzate was supplemented with 5% hazelnut shell instead of glucose and pre-specified amounts of nutrients (0.5% (w/v) yeast extract, 0.02% (w/v) K_2HPO_4, 0.2% (w/v) pectin and 0.02% (w/v) $MgSO_4.7H_20$) and incubated at 30 °C and pH 7.0 for 72 h using *Bacillus subtilis* according to our previous study (Uzuner and Cekmecelioglu, 2015).

Enzymatic clarification

The pH and brix of the apple juice obtained were 3.75±0.07 and 12.65±0.92 °Bx respectively. For each experiment, 100 mL of juice was subject to different enzyme treatment conditions as mentioned in experimental design and shown in Table 1. Based on preliminary experiments, the range of the variables for clarification process was selected. Samples of apple juice (100 mL) were mixed with laboratory produced crude pectinase enzyme (4.00 U/mL) (16) in a flask and placed in a shaking water bath at 120 rpm to control different incubation temperatures (30, 40 and 50 °C) and incubation time (2, 4 and 6 h). Aliquots were taken at the end of the enzymatic treatment and immediately heated the mixture at 90 °C for 5 min to inactivate the enzyme. After cooling, samples were centrifuged at 6272x *g* for 10 min to recover the samples.

Analysis of response variables

Clarity test (%)

The clarification of the juice was evaluated by measuring absorbance at 660 nm. The degree of clarification was expressed by percentage of clarity calculated using (equation 1),

$$Clarity = \frac{\left[Abs_{untreated\ sample} - \left(Abs_{control} - Abs_{sample}\right)\right]}{Abs_{untreated\ sample}} * 100 \quad (1)$$

where $Abs_{untreated}$ is the sample with no enzyme and heating, $Abs_{control}$ is the sample with no enzyme heated at 50 °C, Abs_{sample} is sample with enzyme heated at 50 °C

Turbidity

Turbidimeter (Model-FT. Myers, FL) was used to measure turbidity of the raw and clarified juice samples. The results were measured as a nephelometric turbidity units (NTU).

Color measurement

The color parameters (CIE L* a* b*) of the raw and clarified juice samples were measured using a Hunter Laboratory Color Flex Spectrophotometer (Hunter Associates Laboratory Inc., Reston VA, USA), where L value indicates lightness.

Total phenolic content analysis

The Folin-Ciocalteu assay was used for the determination of total phenolic content in apple juice samples colorimetrically at 760 nm (Spanos et al., 1990), as follows: 5 mL of 10 fold diluted Folin-Ciocalteu reagent and 4 mL Na_2CO_3 solution (75g/L) were mixed with 1 mL of 1:5 diluted sample and incubated at 50 °C in a water bath for 5 min. Phenolics were expressed as gallic acid equivalents in mg/L.

Experimental design and statistical analysis

The relationships between responses of L value, turbidity and clarity and independent variables of temperature, enzyme load, and time were studied using the Box-Behnken Design (BBD) with a quadratic model using MINITAB 16.0 (Minitab Inc. State College, PA, USA). The BBD matrix was constructed using three independent

variables each having three levels, which were enzyme load (X_1; 0.3, 0.5, and 0.7%), temperature (X_2; 30, 40, and 50 °C), and time (X_3; 2, 4, and 6h) (Table 1).

Table 1. Design summary: Coded and uncoded variables of the response surface design for clarification of apple juice.

Levels/ Run order	Enzyme load (%) X_1	Temp (°C) X_2	Time (h) X_3	L value	Turbidity (NTU)	Clarity (%)	TPC (mg/L)
Low (-1)	0.3	30	2				
Middle (0)	0.5	40	4				
High (+1)	0.7	50	6				
1	0.5	30	2	61.63± 2.26^{bc}	196.75± 2.90^{b}	75.24±1.35^{b}	96.68±0.31^{g}
2	0.7	40	6	63.26±1.87^{b}	89.65±1.65^{e}	75.0±1.14^{bc}	146.68±2.82^{de}
3	0.7	50	4	62.57±2.54^{b}	59.35±0.46^{g}	75.46±1.28^{b}	136.95±8.45^{def}
4	0.5	40	4	54.05±0.62^{def}	123.05±3.61^{d}	72.58±4.56^{bc}	265.49±1.88^{a}
5	0.5	30	6	50.56±0.66^{f}	277±4.81^{a}	66.24±70.69^{cd}	110.62±6.88^{fg}
6	0.5	50	2	58.42±1.22^{bcd}	75.29±1.90^{f}	72.73±0.00^{bc}	252.43±4.69^{ab}
7	0.5	50	6	60.34±1.03^{bc}	56.81±1.85^{gh}	70.0±1.29^{bcd}	221.24±10.64^{c}
8	0.3	50	4	63.11±0.62^{b}	79.86±1.68^{f}	73.64±3.86^{bc}	233.63±6.88^{bc}
9	0.7	30	4	57.56±0.28^{cde}	49.40±1.17^{h}	75.63±1.82^{b}	126.99±4.38^{efg}
10	0.3	30	4	63.23±0.21^{b}	143.9±0.28^{c}	73.81±0.67^{bc}	130.75±2.19^{def}
11	0.3	40	2	71.88±0.05^{a}	62.51±0.12^{g}	92.45±0.71^{a}	159.73±0.00^{d}
12	0.7	40	2	62.13±1.14^{bc}	59.42±0.099^{g}	74.19±2.28^{bc}	158.19±1.56^{d}
13	0.3	40	6	53.26±0.23^{ef}	137.85±1.20^{c}	63.71±3.42^{d}	251.77±20.65^{abc}

*Within the same column, values not preceded by the same letter are significantly different ($p<0.05$).

The levels of these variables were determined by preliminary studies. The uncoded and coded independent variables and also the overall BB experimental design are given in Table 2, in which L value, turbidity and clarity are reported as averages of three replicates of each run (total 15 runs).

A quadratic mathematical equation was used to fit the collected data.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (2)$$

where Y_1, Y_2 and Y_3 are L value, turbidity and clarity, respectively. b's are regression coefficients, and X_1, X_2, X_3 are enzyme load, temperature and time, respectively. From regression analysis of the Box-Behnken design, optimal conditions for clarity, turbidity and L value were defined. To validate the model, additional five check points at the defined clarification conditions were carried out in duplicate. Analysis of variance (ANOVA) and regression were performed at 95% confidence interval to define the coefficients of the predictive model and significant terms. Analysis of variance (ANOVA) was performed to determine whether statistically significant effect of enzyme load, temperature and time ($p<0.05$). The pairwise comparisons were made by Tukey's test. Performance of the predictive model was compared by root mean square error (RMSE) value, which reflect the goodness of the predictions, as follows:

$$RMSE = \left(\frac{1}{N} \sum_{i=1}^{N} \left(X_{pred,i} - X_{\exp,i} \right)^2 \right)^{0.5} \quad (3)$$

where X_{exp} is the experimental value and X_{pred} is the predicted value of % clarity, N is the number of data.

Optimization and verification

Optimization including the numerical and graphical optimization and point prediction was carried out to establish the optimum level of three independent variables, enzyme load (X_1), temperature (X_2) and time (X_3) to achieve desirable responses such as maximum clarity, maximum L value and minimum turbidity. The optimum value of multiple responses was determined by using MINITAB optimizer tool.

RESULTS AND DISCUSSION

Composition of untreated apple juice

The chemical composition of apples (Golden Delicious) varies with geographic location, fruit season, maturity, light exposure and harvesting time (Kahle et al., 2005). Lightness (L value) and turbidity (NTU) values of apple juice were '41.16±2.84', '458.1±11.03', respectively. pH and Brix of apple juice were also measured as 3.75±0.07 and 12.65±0.92 °Bx, respectively. These results agree well with those of Usaga et al. (2015) who studied the effects of pressing and insoluble solids on the UV treatment of cloudy apple juice. Total phenolic content of apple juice was also found as 358.85±65.70 mg/L.

Depectinization of apple juice and response surface optimization

The efficacy of crude enzyme during clarification of apple juice was evaluated by RSM optimization response surface. The values of L, turbidity, clarity and total phenolic content of apple juice under different conditions are presented in Table 1.

Effects of clarification parameters on L value, turbidity and clarity

L value

L values of apple juice ranged between 53.14-71.88% with crude pectinase under various enzyme load, temperature and time (Table 1). Second order polynomial regression models with interaction terms are presented in Table 2.

Table 2. Revised ANOVA results and estimated regression coefficients for the coded clarification model*

Term	L value		Turbidity (NTU)		Clarity (%)	
	Coeff	*P* value	Coeff	*P* value	Coeff	*P* value
Regression	-	0.000	-	0.000	-	0.000
Linear	-	0.000	-	0.000	-	0.000
Enzyme load	-0.75	0.083*	-0.013	0.880*	0.038	0.783*
Temp.	1.43	0.002	-0.87	0.000	-	-
Time	-3.33	0.000	0.28	0.001	-0.75	0.000
Square	-	0.000	-	0.000	-	0.001
Temp*Temp	1.63	0.014	0.183	0.115*	-	-
Time*Time	2.64	0.000	-0.322	0.009	-0.21	0.292*
Enz.load*Enz.load	6.52	0.000	-0.495	0.000	0.80	0.000
Interaction	-	0.000	-	0.000	-	0.000
Temp*Time	3.25	0.000	-0.42	0.000	-	-
Temp*Enz.load	1.28	0.039	-0.39	0.010	-	-
Time*Enz.load	4.94	0.000	-	-	1.03	0.000
Lack-of-fit	-	0.121	-	0.415	-	0.676
Constant	53.47	0.000	0.59	0.000	-0.21	0.242*
R^2	0.95		0.93		0.79	
R^2 (adj)	0.92		0.88		0.74	
R^2-pred	0.85		0.79		0.65	
PRESS	139.2		3.5		9.9	

*Result is insignificant when $P>0.05$

Among the variables studied, temperature and time showed significant effects (p<0.05) on L value. Linear (p>0.05) effect of enzyme load was not significant; while the quadratic (p<0.05) effect significantly affected L value during clarification process. Interactions between temperature and time, temperature and enzyme load, and time and enzyme load (p<0.05) showed positive significant effects (Table 2).

Turbidity

Turbidity of apple juice ranged between 49.40-277.00% with crude pectinase under various enzyme load, temperature and time. Temperature and time were found to be the significant main factors (p<0.05) affecting turbidity. Linear (p>0.05) effect of enzyme load was not significant; while the quadratic (p<0.05) effect significantly affected turbidity during clarification process. Interactions between temperature and time and temperature and enzyme load (p<0.05) showed negative significant effects (Table 2).

At constant temperature (40 °C), apple juice turns to turbid, when increasing with time. At constant temperature, the turbidity was found to decrease with enzyme load and temperature up to 0.7% and 40 °C, respectively (Table 1). Pectin caused the turbidity of fruit juices. Turbidity was mostly associated with enzyme load. During the clarification process, the amount of pectin in the juices decreased, therefore turbidity of the juices also decreased (Alverez et al., 1998).

Clarity

Clarification percentages of apple juice ranged between 63.71-92.45% with crude pectinase under various enzyme load, temperature and time. Time was found to be the only significant main factor (p<0.05) affecting clarity. Linear (p>0.05) effect of enzyme load was not significant; while the quadratic (p<0.05) effect significantly affected clarity during clarification process. Interactions between time and enzyme load (p<0.05) showed a positive significant effect (Table 2).

According to Kilara (1982), the rate of enzymatic clarification process increases with increasing temperature to denaturation temperature (40–60 °C). Therefore, moderate temperature should be used during clarification of apple juice. At constant temperature, apple juice turns to clear, when increasing with enzyme load. Increase in enzyme load may cause an increase in rate of clarification by exposing the positively charged protein beneath. Thus electrostatic charged between cloud particles is reduced, which cause these particles to aggregate to larger particles and eventually settle down (Sin et al., 2006). However, clarity decreased with increasing time (Table 2).

However, Alam et al. (2014) showed that an increase in time and/or enzyme load was increased the clarification of carrot juice subjected to commercial pectinase at various enzyme loads (0.01% to 0.1%), temperature (35 °C to 55 °C) and time (40 to 120 min).

Total phenolic content (TPC)

Total phenolic content was 358.85±65.70 mg/L in the control samples. After depectinization, total phenolic content of apple juice ranged between 96.68-265.49 mg/L with crude pectinase under various enzyme load, temperature and time. Temperature and enzyme load were found to be the only significant main factors (p<0.05) affecting TPC. Linear (p>0.05) effect of time was not significant; while the quadratic (p<0.05) effect significantly affected TPC during clarification process (data not shown). The TPC was found to increase with temperature and increase with enzyme load up to 0.50 (%) and decreases slowly thereafter (Table 2).

Fitting the model

RSM is a frequently used technique for modeling and determining optimal process conditions. The BBD response surface analysis of experimental results for L value, turbidity and clarity under various enzyme load, temperature and time (Table 1), was used to identify the best pretreatment conditions in the ranges tested. A second-order polynomial equation was developed to identify the relationship between predicted values of L value (Y_1), turbidity (Y_2) and clarity (Y_3) as a function of enzyme load (X_1), temperature (X_2) and time (X_3). Since the original turbidity and clarity values were non-normal distributed

(p>0.05), Johnson transformation was applied to the original data of turbidity and clarity.
According to ANOVA results, the insignificant terms were excluded and the model was re-written in terms of coded factors (Table 2). The model for the L value, turbidity and clarity can be derived by using the coefficients (Table 3).

Table 3. Regression equations for apple juice quality characteristics

Responses	Predicted models	R^2
L value (Y_1)	$Y_1=53.47+1.43*X_2-3.33*X_3+1.63*X_2^2+2.64*X_3^2+6.52*X_1^2+3.25*X_2*X_3+1.28*X_1*X_2+4.94*X_1*X_3$	0.95
Turbidity (Y_2)	$Y_2=0.59-0.87*X_2+0.28*X_3-0.322*X_3^2-0.495*X_1^2-0.42^*X_{3^*}X_2-0.39*X_{1^*}X_2$	0.93
Clarity (Y_3)	$Y_3=-0.75*X_3-080X_1^2+1.03*X_{1^*}X_3$	0.79

The degree of efficacy of varying treatment conditions on the clarity can be deduced by comparing the magnitude of the coefficients of the second order model (Table 3). The most important factor was time with the highest coefficient (3.33, 0.75) for L value and clarity, respectively whereas the most important factor was temperature with the highest coefficient (0.87) for turbidity (Table 3).

L value, turbidity and clarity were found fairly adequate to represent the data with R^2 of 0.95, 0.93 and 0.79, respectively. Predicted R^2 values for L value, turbidity and clarity found to be 0.85, 0.79 and 0.65, respectively. The insignificant lack of fit values for L value, turbidity and clarity ((p = 0.121 >0.05), (p = 0.415 >0.05) and (p = 0.676 >0.05) respectively), also proved that the model fit the experimental data well (Table 2). The coefficient of variation (CV) describes the dispersion of the data and smaller values of CV give better reproducibility. CV values were 9.5% and 8.5% for L value and clarity, respectively.

Verification of optimal clarification conditions

The process parameters need to be optimized for maximum L value and clarity and minimum turbidity of apple juice. The multiple response optimization of clarification parameters was carried out using response surface methodology-based on the desirability approach. Approaches based on desirability analysis are an objective function that ranges from zero to one (least to most desirable, respectively) at the goal.

The optimum clarifying conditions under these constraints were found to be 0.3% of enzyme load, 45.8 °C of temperature and 2h of time. Sin et al. (2006) reported that sapodilla juice was clarified at different incubation times (30–120 min), temperature (30–50 °C) and enzyme load (0.03–0.10%) with optimal conditions of enzyme load (0.1%), temperature (40 °C) and time (120 min). A similar behavior of clarity was observed for the changes in incubation time in the study of Sin et al. (2006) and Alam et al. (2014). As time increased, fine particles in juice slowly settle down (Sin et al., 2006).

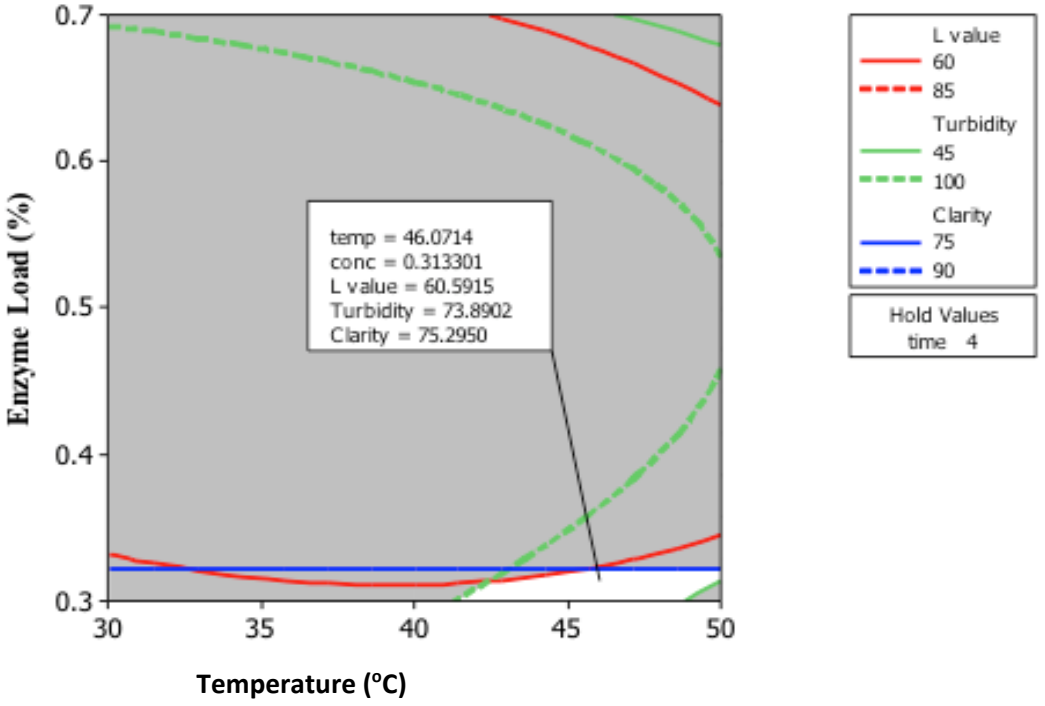
The overlaid contour plots for multi-response of clarification process are shown in Figure 1a-c.

Contour plots are used to illustrate the relationship between experimental factors and one response. The advantage of overlaying the contour plots is to visualize how experimental factors affect many responses simultaneously for any given set of conditions. The white area of the plots shows the range of enzyme load, temperature and time where the criteria for three response variables are satisfied (Figure 1a-c). At optimum time parameter, both temperature and enzyme load parameters are to be maintained within a very narrow range as shown in Figure 1a. Figure 1b shows that holding enzyme load at optimum conditions, a feasible range was

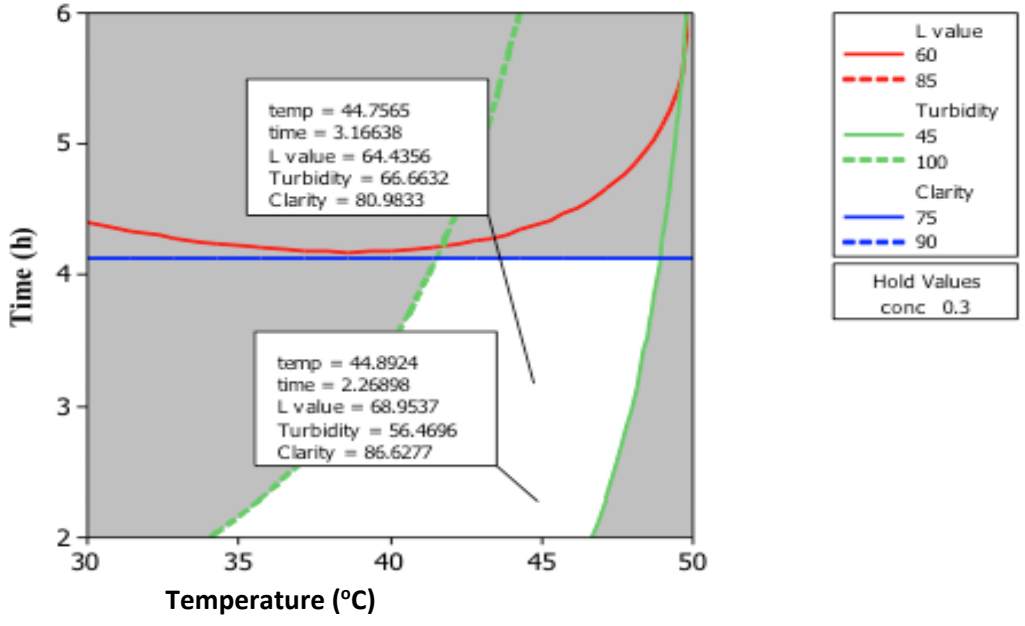
obtained within temperature at (34-47 °C) and time at (2-4 h). Two feasible zones (Figure 1c) were obtained when both the clarification process parameters are optimum (see lower left corner).

To confirm the validity of RSM models, five optimum check points were selected to perform over the entire experimental domain (Table 4).

a)



b)



c)

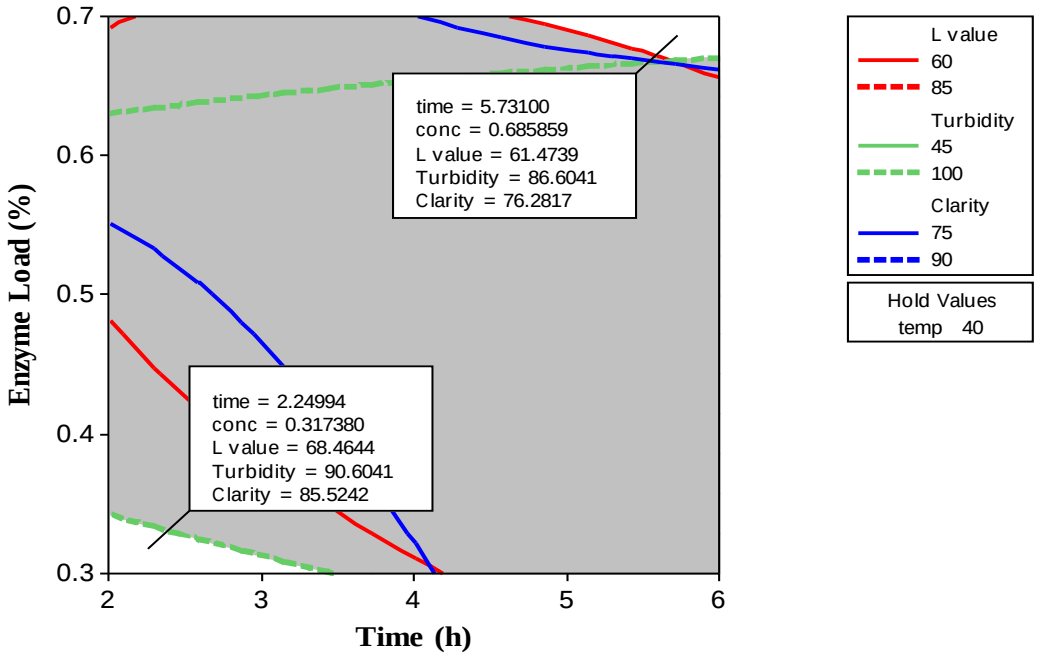


Fig. 1. Over laid surface plots showing the effect of a) pH and time, b) Enzyme load and time, c) Enzyme load and pH on clarification of apple juice.

Table 4. Verification of clarification model

Temperature (°C)		35	35	45	45	45	RMSE
Enzyme Load (%)		3	5	4	3	4	
Time (h)		0.3	0.3	0.4	0.3	0.5	
L value	Experimental	61.52	53.61	61.35	61.14	60.00	4.65
	Predicted	66.67	56.78	56.28	65.2	54.59	
Turbidity (NTU)	Experimental	108.00	114.40	111.40	74.94	108.30	5.28
	Predicted	98.43	110.25	108.77	79.13	110.72	
Clarity (%)	Experimental	77.70	75.40	81.25	64.58	72.92	9.15
	Predicted	81.57	69.22	72.56	81.57	71.62	

The resultant experimental data were compared with that of the predicted values in Table 4. The constructed model was also assessed using error analysis. The RMSE values of L value, turbidity and clarity were calculated as 4.65, 5.28 and 9.15, respectively which both indicate low error or high accuracy of prediction.

CONCLUSIONS

This study assesses how process variables (enzyme load, temperature and time) affect clarification of apple juice. Polynomial regression models successfully defined the clarification as well. Maximal lightness and clarification yield (70.40 and 88.33%, respectively) and minimum turbidity (49.44) were observed with 0.3%

enzyme load at 45 °C for 2 h. Different fruit juices can be treated with the produced bacterial pectinase as part of future work.

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REFERENCES

Abdullah, A.G.L., Sulaiman, N.M., Aroua, M.K., Megat Mohd Noor, M.J. (2007). Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. *J Food Eng Essex*, 81(1): 65-71.

Alam, MdS., Ahuja, G., Gupta, K. (2014). Enzymatic clarification of carrot juice by using response surface methodology. *Agric Eng Int: CIGR J*, 16(3): 173-179.

Alvarez, S., Alvarez, R., Riera, F.A., Coca, J. (1998). Influence of depectinization on apple juice ultrafiltration. *Colloids Surf A: Physicochem Eng Asp*, 138: 377-382.

Bhardwaj, V., Garg, N. (2014). Production, purification of pectinase from *Bacillus sp.* MBRL576 isolate and its application in extraction of juice. *Int J Sci Res*, 3(6): 648-652.

Chen, X., Xu, F., Qin, W., Ma, L., Zheng, Y. (2012). Optimization of enzymatic clarification of green asparagus juice using response surface methodology. *J Food Sci*, 77(6): 665-670.

Joshi, V.K., Parmar, M., Rana, N. (2011). Purification and characterization of pectinase produced from apple pomace and evaluation of its efficacy in fruit juice extraction and clarification. *Indian J Nat Prod Resour*, 2(2): 189-197.

Kahle, K., Kraus, M., Richling, E. (2005). Polyphenol profiles of apple juices. *Mol Nutr Food Res,* 49: 797-806.

Kilara, A. (1982). Enzymes and their uses in the processed apple industry: A review. *Process Biochem,* 23: 35–41.

Kumar, S., Sharma, H.K. (2015). Enzymatic degumming of pineapple (*Ananas comosus*) mill juice using crude and commercial enzymes. *J Food Measur Char,* 9(3): 414-425.

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

Pinelo, M., Zeuner, B., Meyer, A.S. (2010). Juice clarification by protease and pectinase treatments indicates new roles of pectin and protein in cherry juice turbidity. *Food Bioprod Process,* 88: 259-265.

Rai, P., Majumdar, G.C., Dasgupta, S., De, S. (2004). Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. *J Food Eng*, 64: 397-403.

Sakhale, B.K., Pawar, V.N., Gaikwad, S.S. (2016). Studies on effect of enzymatic liquefaction on quality characteristics of Kesar mango pulp. *Int Food Res J*, 23(2): 860-865.

Sandri, I.G., Fontana, R.C., Barfknecht, D.M., Silveira, M.M. (2011). Clarification of fruit juices by fungal pectinases. *LWT-Food Sci Technol*, 44 (10): 2217-2222.

Sandri, I.G., Lorenzoni, C.M.T., Fontana, R.C., Silveira, M.M. (2013). Use of pectinases produced by a new strain of *Aspergillus niger* for the enzymatic treatment of apple and blueberry juice. *LWT-Food Sci Technol*, 51(2): 469-475.

Sin, H.N., Yusof, S., Hamid, N.S.A., Rahman, R.A. (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *J Food Eng,* 73: 313-319.

Spanos, G.A., Wrolstad, R.E., Heatherbell, D.A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *J Agric Food Chem,* 38: 1572-1579.

Swain, M.R., Ray, R.C. (2010). Production, Characterization and application of a thermostable exo-polygalacturonase by *Bacillus subtilis* CM5. *Food Biotechnol,* 24: 37-50.

Usaga, J., Worobo, R.W., Moraru, C.I., Padilla-Zakour, O.I. (2015). Time after apple pressing and insoluble solids influence the efficiency of the UV treatment of cloudy apple juice. *LWT Food Sci Technol* 62(1): 218-224.

Uzuner, S., Cekmecelioglu, D. (2014). Hydrolysis of hazelnut shells as a carbon source for bioprocessing applications and fermentation. *Int J Food Eng*, 10(4): 799-808.

Uzuner, S., Cekmecelioglu, D. (2015). Enhanced pectinase production by optimizing fermentation conditions of *Bacillus subtilis* growing on hazelnut shell hydrolyzate. *J Mol Catal B: Enzym*, 113: 62-67.

Whitaker, J.R. (1984). Pectic substances, pectic enzymes and haze formation in fruit juices. *Enzyme Microb Technol*, 4: 341-349.