J Food Sci Technol (September–October 2011) 48(5):560–568
DOI 10.1007/s13197-010-0175-1

ORIGINAL ARTICLE

Efficacy of xylanase purified from *Aspergillus niger* DFR-5 alone and in combination with pectinase and cellulase to improve yield and clarity of pineapple juice

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Revised: 13 September 2010 / Accepted: 8 November 2010 / Published online: 22 December 2010

Abstract Pineapple is one of the fruits having xylan rich hemicellulose content more than pectin. Therefore, the efficacy of absolutely purified xylanase from *A. niger* DFR-5 alone and in combination with pectinase and cellulase on juice yield and clarity was studied. Xylanase provided maximum yield (71.3%) and clarity (64.7%) of juice in comparison to control responses (61.8% yield and 57.8% clarity). When used together, a synergistic effect of xylanase, pectinase and cellulase on process responses was observed indicating the necessity of a cock-tail of hydrolytic enzymes for complete cell wall degradation. Overall, an increase in juice yield by 52.9% was observed. The process was numerically optimized with the constraint of 'minimum' pectinase and cellulase and 'maximum' xylanase and incubation time for 'maximum' juice yield and clarity. The closeness of observed response (90.2% yield and 80.9% clarity) to the predicted one (89.6% yield and 80.3% clarity) indicated the validity of developed model.

Keywords *Aspergillus niger* · Xylanase · Juice yield · Pineapple · Statistical design

Introduction

Hemicellulose is one of the main components of plant cell wall polysaccharides and xylan is the major constituent of it. Xylan stands next to cellulose in abundance and is composed of a linear backbone of β-1,4-linked D-xylopyranose residues which is further substituted, depending on plant sources to a varying degree with glucuronopyranosyl, 4-0-methyl-D-glucopyranosyl, α-L-arabinofuranosyl, acetyl, as well as linked to feruloyl and coumaryl components of lignin (Shallom and Shoham 2003). Xylanase (EC 3.2.1.8) catalyze the hydrolysis of xylan into shorter sugar residues which have wide applications in industry (Goulart et al. 2005). Nature is abound with bacteria and fungi capable of producing xylanase to solubilize this complex component to simple molecules for completing the carbon cycle (Badhan et al. 2007). Filamentous fungi are useful producers of xylanase because they are capable of producing high levels of extracellular enzymes and can be cultivated very easily (Pal and Khanum 2010b).

Recent years have seen a surge of interest in xylanases because of their applications in the food and beverage industries (bakery goods, coffee, starch, plant oil and juice manufacture), feedstock improvement (increasing animal feed digestibility) and the quality improvement of lignocellulosic residues (Bakri et al. 2008).

Pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavor and refreshing sugar-acid balance (Bartolome et al. 1995). Pineapple juice is largely consumed world-wide mostly as a canning industry by-product, in the form of single strength, reconstituted or concentrated and in the blend composition to obtain new flavors in beverages and other products (Arthey 1995; deCarvalho et al. 2008). More recently, the consumer market has been equally receptive to limpid fruit juice either obtained by the traditional clarification processes using gelatine, bentonite or by processes using ultrafiltration and microfiltration membranes (deCarvalho et al. 2008). Enzymes are used to ensure optimal juice yield and a quality product that ensure consumer appeal.

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Mostly juices are pectin rich but Grassin and Fauquembergue (1996) noted that pineapple juice contains a small amount of pectin but high hemicellulose content. Our laboratory is actively engaged in the studies on microbial metabolites including xylanase (Pal and Ramana 2009, 2010; Pal et al. 2009; Pal and Khanum 2010a, b). Since the major fraction of hemicellulose is composed of xylan, the application of xylanase in pineapple juice clarification is worth studying. Keeping these points in view, the study was conducted to evaluate the efficacy of absolutely purified xylanase from *Aspergillus niger* DFR-5 alone and in conjunction with other cell wall degrading enzymes namely pectinase and cellulase to improve the yield and clarity of pineapple juice.

Materials and methods

Materials and processing

The pineapple fruits *(Ananas comosus* L.) were obtained from the local market of Mysore and used immediately or stored at 5 °C for not more that 5 days before being used. The enzyme xylanase was produced and purified up to absolute homogeneity from a local isolate *A. niger* DFR-5 in our own laboratory (Pal and Khanum 2010a) while pectinase and cellulase were procured from Sigma-Aldrich Co. All others reagents were of analytical grade.

Xylanase activity was assayed by the method of Khanna and Gauri (1993). The solution of xylan and the enzyme extract at appropriate dilution was incubated at 37 °C for 30 min and the reducing sugars were determined by the dinitrosalicylic acid method described by Miller (1959), with xylose as the standard. The released xylose was measured at 540 nm using a spectrophotometer (Shimadzu Co., Kyoto Japan). One unit of xylanase is defined as the amount of enzyme required to release 1 μmol of reducing sugar as xylose equivalent/min under the assay conditions.

The laboratory purified xylanase (purified preparation contains ~3943 IU/ml) was added in terms of IU while commercial enzyme preparations viz. pectinase (~1 IU/mg) and cellulase (~4 IU/mg) were added in per cent basis (*w*/*w*) to pineapple pulp.

To make the pulp, pineapple fruit was washed under running tap water before removing the crown and peel. The flesh intact with the core was sliced to approximately 2×2×1 cm before being mashed in a warring blender for 1–2 min. The pulp was adjusted to pH 5.0 and used in further experiments.

Individual effect of hydrolytic enzymes on process response

The enzymes xylanase, pectinase and cellulase were mixed with pineapple pulp at a concentration of 1000 IU/100 g, 0.3 and 0.3%, respectively. The content was allowed to incubate at 37 °C for 270 min followed by pasteurization to inactivate the enzymes. The content was immediately cooled and centrifuged at 10, 000 rpm for 15 min. The supernatant juice was taken out and the process responses namely yield and clarity were recorded.

Interactive effect of hydrolytic enzymes on process response

Experimental design

A central composite rotatable deign (CCRD) was used to determine the effects of four independent variables viz. xylanase, pectinase, cellulase and incubation time on juice yield and clarity. The experiments were conducted at 37 °C. The range and levels of the variables are given in Table 1. The levels of variables were chosen based on the preliminary experiments. To design the experiments, the test factors were coded according to the following equation

$$x_i = X_i - X_0/\delta X_i$$

Where, x_i is the dimensionless coded value of the *i*th independent variable; X_i the natural value of the *i*th

Table 1 The experimental domain

Independent variables	Coded level				
	$-\alpha$	−1	0	+1	$+\alpha$
X_1, Xylanase (IU/100 g pulp)	500	750	1000	1250	1500
X_2, Pectinase (%, *w*/*w*)	0.10	0.20	0.30	0.40	0.50
X_3, Cellulase (%, *w*/*w*)	0.10	0.20	0.30	0.40	0.50
X_4, Incubation time (min)	90	180	270	360	450

independent variable; X_0 the natural value of the ith independent variable at the center point and δX_i the step change value. Once the experiments were performed, the experimental results were fitted with a 2nd order polynomial equation

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$$

Where, Y is the predicted response; b_0 the intercept; b_1, b_2, b_3, b_4 the linear co-efficient; b_{11}, b_{22}, b_{33}, b_{44} the squared co-efficient and b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34} the interaction co-efficients.

Methodology

The pineapple pulp was brought at 37 °C and requisite amount of enzymes were added, mixed well and incubated for required time (Table 2). At the end of incubation period, the pulp was pasteurized at 97 °C for 5 min in a water bath to denature the enzymes, cooled and centrifuged at 10, 000 rpm for 15 min. The supernatant was collected and analyzed for juice yield and clarity.

Response analysis

Volume of the filtrate was measured and yield was expressed as % yield (v/w). Juice clarity was measured by measuring the transmittance (%T) of juice at 650 nm since at this wavelength other browning components do not interfere with the measurements (Sreenath and Santhanam 1992). The results are expressed as Mean±SD of three experiments.

Data analysis

All statistical experimental designs and results analysis were carried out using Design-Expert and Minitab software. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2, and its statistical significance was checked by Fisher's F-test. The significance level of each regression co-efficient was determined by Student's t-test. The level of significance was given as p-value.

Optimization strategy

The process was numerically optimized with respect to 'minimum' pectinase and cellulase and 'maximum' xylanase and incubation time.

Results and discussion

Individual effect of hydrolytic enzymes on process response

The activities of different cell wall hydrolytic enzymes on pineapple pulps were measured in terms of juice yield and clarity. The yield and clarity of juice without any treatment was 61.8 and 57.8%, respectively and addition of all the hydrolytic enzymes increased both the responses. The maximum juice yield of 71.3% was recorded with xylanase treatment followed by pectinase (68.2%) while the cellulase treatment gave the lowest yield of 66.5% (Fig. 1). The increase in juice yield in all the cases was accompanied with an increase in clarity.

The middle lamella comprised of pectin and primary wall comprised of cellulose and hemicellulose makes the cell wall of pineapple fruit. The middle lamella acts as an intercellular substance to bind/connect the cells together. The pectinase enzyme results in cell separation by pectin hydrolysis (Qin et al. 2005). The primary wall is a strong network composed of cellulose, hemicellulose and embedded lignin. The progressive degradation of cellulose fibrils weakens the wall making the juice recovery easier. The maximum effect of xylanase on juice yield and corresponding clarity can be explained with the fact that hemicellulose is the most abundant constituent of pineapple fruit cell wall and xylan is major fraction of it (Grassin and Fauquembergue 1996). The degradation of xylan by the xylanase resulted in release of bound water and a corresponding increase in juice yield was observed. All the enzymes were used together to deduce their interactive effects on yield and clarity in the next experiment.

Interactive effect of hydrolytic enzymes on process response

The experimental and predicted values for juice yield and clarity under different treatment conditions are presented in Table 2. It is clear that the minimum yield of 68.2% was observed in run number 23 (X_1, X_2, X_3 at centre level and X_4 at -α level) while the maximum of 94.5% was recorded in run number 16 (all the variables at +1 levels). The clarity of juice varied in the range from 68.2 to 84.1% in different experiments. It is clear from the comparison of run number 17 and 18, 19 and 20, 21 and 22 and 23 and 24 that increase in concentrations of all the enzymes and incubation period from their negative α to positive α level increased the response variables substantially. An increase in juice yield by 52.9% in comparison to control (61.8%) was observed using the cock-tail of cell wall hydrolytic enzymes. It is worth mentioning that, when the xylanolytic, cellulolytic and the pectinolytic enzyme preparations were

Table 2 Experimental design and results

Run No.	Coded level				Observed response[a]		Predicted response	
	X_1	X_2	X_3	X_4	Yield (%)	Clarity (%T)	Yield (%)	Clarity (%T)
1	−1.00	−1.00	−1.00	−1.00	77.5±3.4	69.1±2.5	78.3	70.1
2	1.00	−1.00	−1.00	−1.00	82.5±4.2	74.8±3.2	81.2	73.4
3	−1.00	1.00	−1.00	−1.00	81.6±3.8	72.9±3.1	80.0	71.6
4	1.00	1.00	−1.00	−1.00	86.5±4.4	77.2±3.4	85.7	76.8
5	−1.00	−1.00	1.00	−1.00	78.6±3.6	70.3±2.8	78.5	70.2
6	1.00	−1.00	1.00	−1.00	82.7±3.8	73.8±2.9	82.5	74.0
7	−1.00	1.00	1.00	−1.00	83.8±3.6	75.2±3.2	83.1	74.6
8	1.00	1.00	1.00	−1.00	89.2±4.3	79.6±3.8	89.9	80.3
9	−1.00	−1.00	−1.00	1.00	89.8±4.2	80.1±3.9	87.5	76.6
10	1.00	−1.00	−1.00	1.00	89.9±4.0	80.2±3.8	89.6	80.4
11	−1.00	1.00	−1.00	1.00	86.6±3.8	77.2±3.4	85.8	76.7
12	1.00	1.00	−1.00	1.00	92.2±3.9	82.3±3.7	90.7	81.0
13	−1.00	−1.00	1.00	1.00	85.9±3.5	76.4±3.3	85.7	76.5
14	1.00	−1.00	1.00	1.00	88.9±3.6	79.4±3.6	88.9	80.7
15	−1.00	1.00	1.00	1.00	87.2±3.7	78.1±3.4	86.9	78.1
16	1.00	1.00	1.00	1.00	94.5±4.2	84.1±3.9	92.8	82.8
17	−2.00	0.00	0.00	0.00	81.4±3.7	72.7±2.8	82.6	73.8
18	2.00	0.00	0.00	0.00	90.2±3.9	80.6±3.3	91.5	81.8
19	0.00	−2.00	0.00	0.00	82.8±3.2	74.0±3.1	83.2	74.4
20	0.00	2.00	0.00	0.00	86.8±3.5	77.6±3.2	88.9	79.4
21	0.00	0.00	−2.00	0.00	82.4±3.1	73.7±2.8	84.9	76.0
22	0.00	0.00	2.00	0.00	87.2±3.7	77.9±2.9	87.2	77.9
23	0.00	0.00	0.00	−2.00	76.4±3.1	68.2±1.8	76.6	68.5
24	0.00	0.00	0.00	2.00	86.4±3.8	77.1±3.4	88.7	79.0
25	0.00	0.00	0.00	0.00	87.3±3.7	78.1±3.5	87.6	78.0
26	0.00	0.00	0.00	0.00	88.1±3.8	77.9±3.4	87.6	78.0
27	0.00	0.00	0.00	0.00	87.6±3.6	78.0±3.7	87.6	78.0
28	0.00	0.00	0.00	0.00	87.8±3.7	78.1±3.5	87.6	78.0
29	0.00	0.00	0.00	0.00	87.2±3.5	78.2±3.2	87.6	78.0
30	0.00	0.00	0.00	0.00	87.4±3.6	78.0±3.4	87.6	78.0

[a] Values are Mean±SD of three experiments

used individually, their efficacy was less but when used together, a synergistic effect on juice yield was observed. This is because; the action of individual enzyme exposes the remaining polysaccharides for their complete and rapid degradation. As a result, the cells get collapsed at a faster rate resulting in a higher yield (Qin et al. 2005). The findings showed that a consortium of enzymatic preparations is essential for optimal hydrolysis of pineapple pulp to get better results.

Increase in plum juice yield from 48 to 77.5% using pectinolytic enzyme has been reported from our laboratory, earlier (Singh and Das Gupta 2005). A clarification rate of 25–27% in the orange juice treated with xylanase alone has been reported by Olfa et al. (2007) while the juice yield of 85.1% from guava fruit using a mixture of pectinase, cellulase and hemicellulase preparation has been achieved

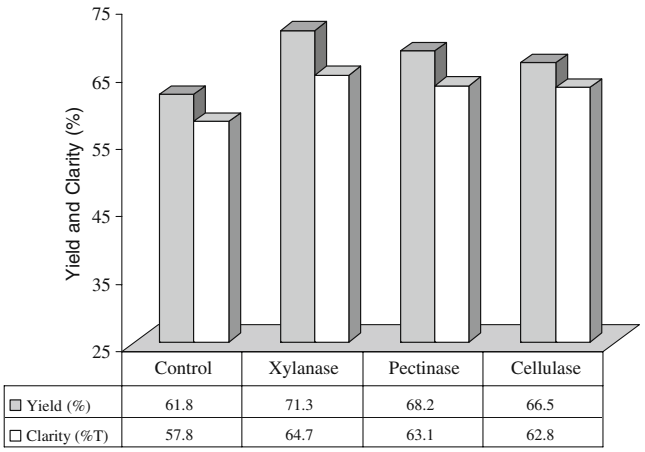

Fig. 1 Individual effect of cell wall hydrolytic enzymes on yield and clarity of pineapple juice (n=3)

by Ahmad et al. (2009). More recently, the effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice has been studied by Vijayanand et al. (2010).

The effects of change in variables on process response can better be explained in terms of their statistical coefficients which are presented in Table 3. The variables, analyzed for their linear, quadratic and interactive effects gave the following equations (in terms of coded unit) to predict the juice yield and clarity within the experimental domain-

Juice yield (%)=87.57+2.21 (xylanase)+1.41 (pectinase)+0.58 (cellulase)+3.03 (incubation time)–0.13 (xylanase^2)–0.38 (pectinase^2)–0.38 (cellulase^2)–1.23 (incubation time^2)+0.69 (xylanase X pectinase) + 0.26 (xylanase X cellulase)–0.21 (xylanase X incubation time) + 0.71 (pectinase X cellulase)–0.86 (pectinase X incubation time)–0.51 (cellulase X incubation time)

Juice clarity (%T)=78.05+2.00 (xylanase)+1.24 (pectinase)+0.48 (cellulase)+2.61 (incubation time)–0.068 (xylanase^2)–0.28 (pectinase^2)–0.28 (cellulase^2)–1.07 (incubation time^2)+0.47 (xylanase X pectinase) + 0.11 (xylanase X cellulase)–0.23 (xylanase X incubation time) + 0.73 (pectinase X cellulase)–0.71 (pectinase X incubation time)–0.42 (cellulase X incubation time)

The significance of each coefficient was determined by Student's *t*-test. The sign and magnitude of the co-efficients indicate the effect of the variable on the response. The smaller the *p*- and larger the *t*-value, the more significant is the corresponding co-efficient (Myers and Montogomery 2002). Analysis of result shows that the juice yield was significantly (1% level) affected by xylanase, pectinase and incubation time at linear level. The effect of cellulase was also significant but at 10% level of significance. Positive values of the linear coefficients of the model indicate that the yield/recovery increased with increase in the level of independent variables. The effect of all the variables was negative at quadratic level and only the incubation time significantly (at 1% level) affected the process yield. The interaction between pectinase and cellulase was found significant on process yield at 10% level while interaction between pectinase and incubation time was significant at 5% level of significance. The remaining interactions were statistically non-significant (Table 3).

The juice clarity, measured in terms of % transmittance (%T) at 650 nm, was significantly affected by all the independent variables except cellulase at their linear level. The statistically significant effect of incubation time at quadratic level was also observed. It is worth mentioning that the effect of all the variables was positive at linear level while negative at quadratic level. The interactive effect of pectinase with cellulase and incubation time on juice clarity was found significant at 10% level (Table 3).

The juices obtained after enzymatic treatment had more yield and corresponding clarity compared to untreated one because of the reduction in xylan, pectin and cellulose content. Practically most of the major polysaccharides were liquefied when the consortium of enzymes was used. Degradation of the polysaccharides like pectin leads to a reduction in water holding capacity and consequently, free water is released to the system which increases the yield

Table 3 Co-efficients of the regression equation

Term	Yield (%)			Clarity (%T)		
	Co-efficient	*t*-value	*p*-value	Co-efficient	*t*-value	*p*-value
Constant	87.57	136.82	< 0.0001	78.05	134.56	< 0.0001
A	2.21	6.86	< 0.0001	2.00	6.83	< 0.0001
B	1.41	4.38	0.0005	1.24	4.23	0.0007
C	0.58	1.79	0.0942	0.48	1.64	0.1219
D	3.03	9.40	< 0.0001	2.61	8.94	< 0.0001
A^2	−0.13	−0.42	0.6789	−0.068	−0.25	0.8077
B^2	−0.38	−1.25	0.2295	−0.28	−1.02	0.3216
C^2	−0.38	−1.25	0.2295	−0.28	−1.02	0.3216
D^2	−1.23	−4.08	0.0010	−1.07	−3.91	0.0014
A×B	0.69	1.74	0.1016	0.47	1.31	0.2100
A×C	0.26	0.67	0.5155	0.11	0.30	0.7707
A×D	−0.21	−0.54	0.5977	−0.23	−0.65	0.5280
B×C	0.71	1.81	0.0907	0.73	2.04	0.0590
B×D	−0.86	−2.19	0.0449	−0.71	−1.97	0.0672
C×D	−0.51	−1.30	0.2131	−0.42	−1.17	0.2603

A=Xylanase; B=Pectinase; C=Cellulase; D=Incubation time

Table 4 Regression analysis (ANOVA) for process response

Response	Source	SS	DF	MS	F-value	Prob>F
Yield	Model	469.38	14	33.53	13.49	<0.0001
	Residual	37.28	15	2.49		
	Total	506.66	29			
	CV=1.84%; $R_{=}^2$ 0.926; Table $F_{14,\ 15\ (1\%)}$=3.56					
Clarity	Model	358.01	14	25.57	12.48	<0.0001
	Residual	30.75	15	2.05		
	Total	388.76	29			
	CV=1.87%; $R_{=}^2$ 0.921; Table $F_{14,\ 15\ (1\%)}$=3.56					

SS Sum of Square, *DF* Degree of Freedom, *MS* Mean Square

and clarity of juice (Demir et al. 2000). Several researches have shown that enzymatic treatment results in higher yields of fruit and vegetable products (Sreenath et al. 1994; Demir et al. 2000; Will et al. 2000). The increase in yield has been attributed to the partial or complete degradation of the cell wall and middle-lamina pectins, other polysaccharides and cell substances (Dorreich 1996) thus increasing press capacity which results in increased juice yield and other health beneficial compounds (Demir et al. 2000).

In general, the juice yield and clarity goes hand-in-hand and the time required to obtain a clear juice is inversely proportional to the concentrations of enzymes used (Kilara 1982). The interaction effect between pectinase and incubation time was positively significant on juice yield and clarity meaning that action of enzyme was dependent on the incubation period.

The adequacy of the developed model was tested employing the Fisher's *F*-test. If the model is a good predictor of the experimental results, the calculated *F*-values should be several times greater than tabulated *F*-value. The F-tests [($F_{\text{yield (14, 15)}}$=13.49>$Ft_{\text{(14, 15)}}$=3.56) and ($F_{\text{clarity (14, 15)}}$=12.48>$Ft_{\text{(14, 15)}}$=3.56)] with a very low probability value (P model<0.0001) indicated the model was highly significant for juice yield and clarity and there is a quadratic relationship between the independent variables and response variables (Table 4). The goodness of fit of the model was examined by determination coefficients (R_{yield}^2=0.926, R_{clarity}^2=0.921), which implied that the sample variations of more than 92% was attributed to the variables and only less than 8% of the total variation could not be explained by the model. The closer the value of R^2 to the unity, the better the empirical model fits the actual data. A lower value of coefficients of variation (CV_{yield}=1.84%, CV_{clarity}=1.87%) showed the experiments conducted were precise and reliable (Myers and Montogomery 2002).

Figures 2, 3 and 4 represent some of the 3-D surface graphics showing the interactive effects of variables on process response (yield and clarity). Each figure presented

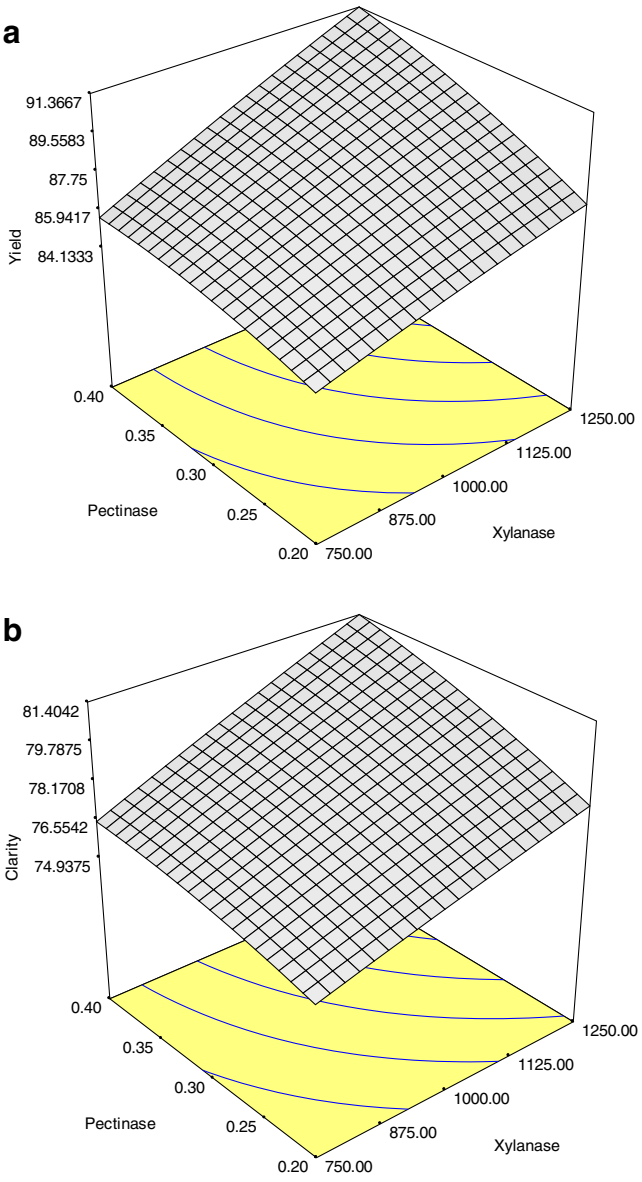

Fig. 2 Effect of xylanase and pectinase on **a** yield and **b** clarity of pineapple juice

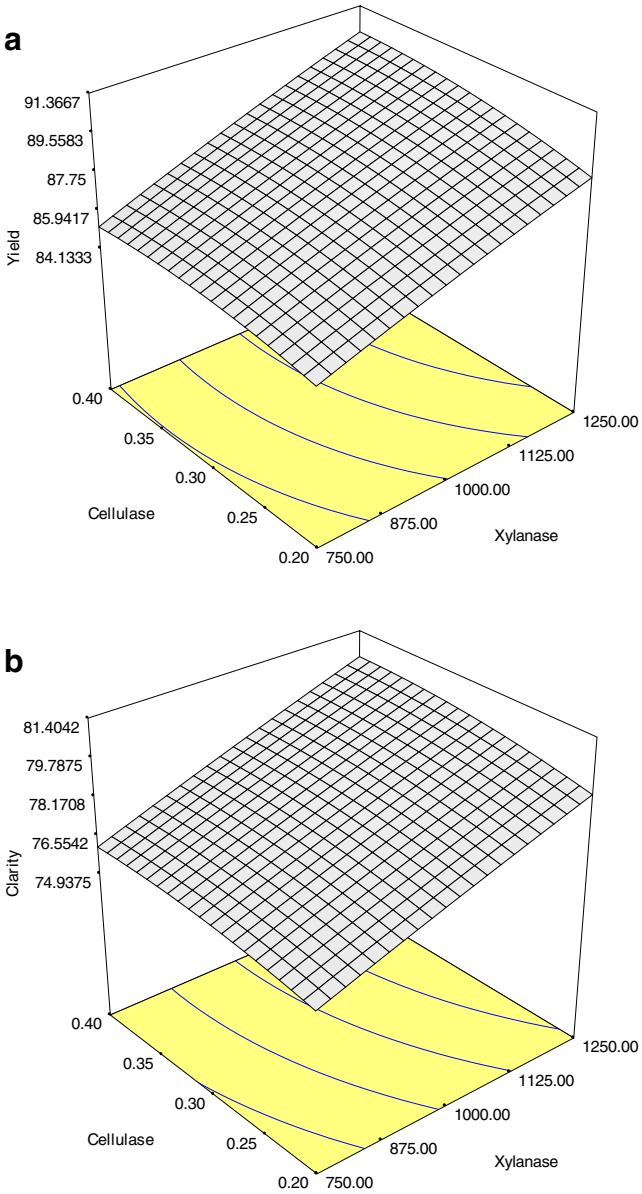

Fig. 3 Effect of xylanase and cellulase on **a** yield and **b** clarity of pineapple juice

the effect of two variables on response while keeping the other variables at zero level. From Figs. 2 and 3, it is clear that juice yield and clarity increased with increase in enzyme concentrations. However, the effects of xylanase on process responses were more prominent. Figure 4 shows the effect of xylanase and incubation time and it can be inferred that juice yield and clarity increased with increase in incubation time indicating the importance of reaction time on process response.

The simultaneous effect of variations in levels of all the independent factors on process response can be seen in the perturbation graph (Fig. 5). The plot reveals that the juice yield and clarity were most sensitive to xylanase concentration and incubation time while least sensitive to cellulase treatment. The results can again be explained with the facts that pineapple fruit cell wall is rich in xylan and needs time to be degraded by xylanase (Grassin and Fauquembergue 1996).

Process optimization

During the enzyme assisted juice clarification, the cost of enzyme treatment is important. The process was numeri-

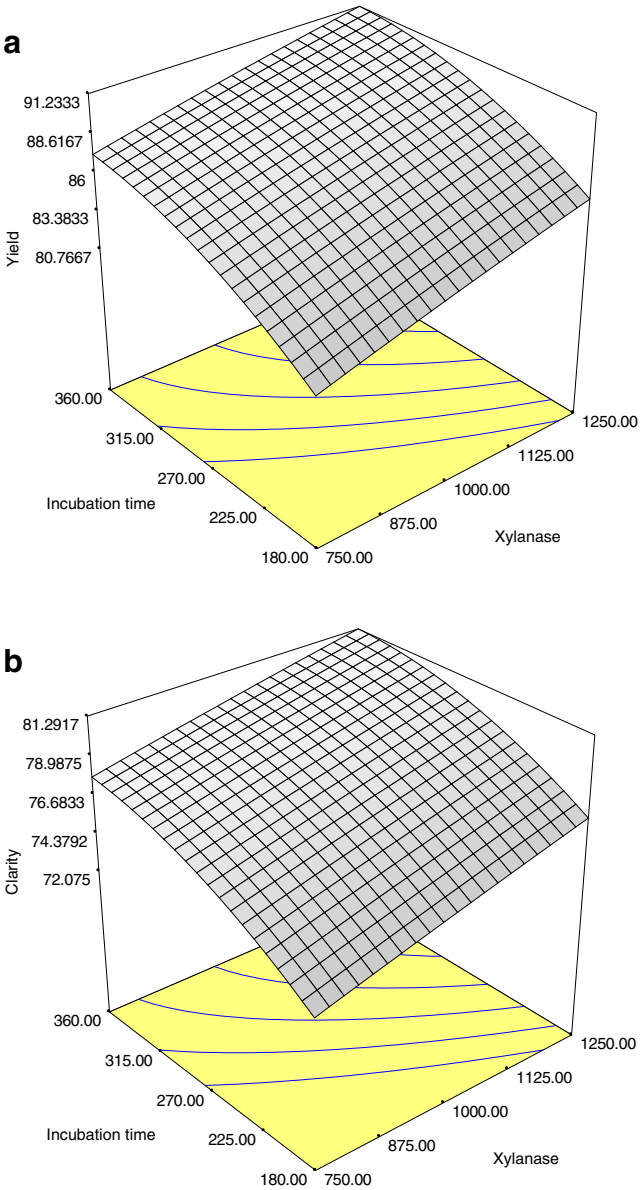

Fig. 4 Effect of xylanase and incubation time on **a** yield and **b** clarity of pineapple juice

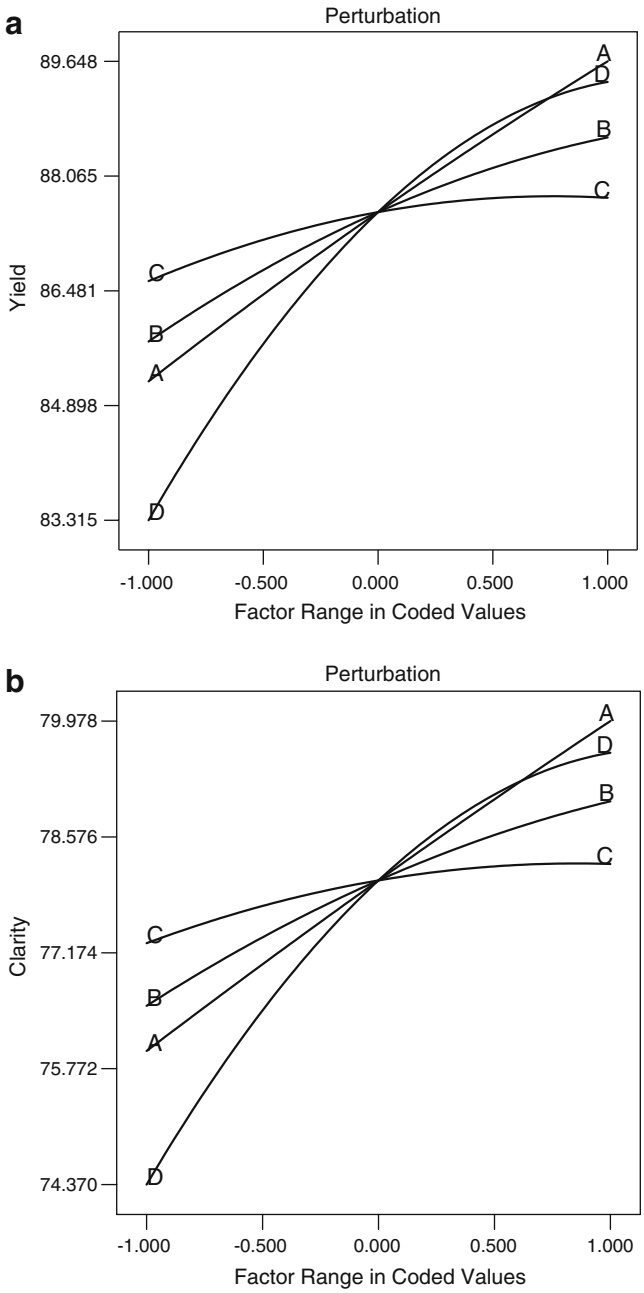

Fig. 5 Perturbation graph showing the effect of independent variables on **a** yield and **b** clarity of pineapple juice (A=xylanase, B=pectinase, C=cellulase and D=incubation time)

cally optimized using Design-Expert statistical software. Since the enzymes pectinase and cellulase were procured so they were kept at their minimum level to economize the process. The xylanase being our own product was kept at highest level along with incubation time. Both the responses (juice yield and clarity) were given a goal to maximize. All the responses and independent variables were given equal importance.

By using the given criteria, a solution having maximum desirability of 90.6% was selected and experiments were conducted. The optimized levels of the variables along with observed (90.2% yield and 80.9% clarity) and predicted (89.6% yield and 80.3% clarity) responses are presented in Table 5. The closeness of the observed and predicted responses indicates the validity of developed model.

Conclusion

Enzymatic clarification of fruit juices is a process that takes place now-a-days in the juice processing industry and the optimization of process conditions is necessary to facilitate the process. Among the various cell wall degrading enzymes, xylanase provided maximum effect on yield (71.3%) and clarity (64.7%) of pineapple juice as compared to control (61.8% yield and 57.8% clarity). A synergistic effect of xylanase purified from *A. niger* DFR-5, pectinase and cellulase on juice yield and clarity was observed. Overall, an increase in juice yield by 52.9% in comparison to control (61.8%) was observed using the cock-tail of cell wall hydrolytic enzymes. The process was numerically optimized with the constraint of 'minimum' pectinase and cellulase and 'maximum' xylanase and incubation time for 'maximum' juice yield and clarity. The closeness of observed response to the predicted one validated the developed model. In conclusion, the bio-technological potential of xylanase purified from *A. niger* DFR-5 could be of great importance to the juice clarification industry.

Table 5 Constraints, criteria for optimization, solution along with predicted and observed response value

Constraints	Goal	Importance	Solution	Observed response*
Xylanase (IU/100 g pulp)	Maximize	4	1250	–
Pectinase (%, *w*/*w*)	Minimize	4	0.20	–
Cellulase (%, *w*/*w*)	Minimize	4	0.20	–
Incubation time (min)	Maximize	4	359	
Yield (%, *v*/*w*)	Maximize	4	89.6	90.2±2.8
Clarity (%T)	Maximize	4	80.3	80.9±2.2

*(*n*=3); Desirability=90.6%

Acknowledgements The authors are grateful to Dr. A.S. Bawa, Director, Defence Food Research Laboratory, Mysore, for providing all the necessary facilities, constant guidance and encouragement during this investigation.

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