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The Use Of Fungal Enzymes viz Protease, Cellulase, And Xylanase For Polishing Rice.

Loveleen Kaur Sarao1*, Maninder Arora2, V.K Sehgal3, Surekha Bhatia4

[1,2] *Department of Microbiology, Punjab Agricultural University, Ludhiana, 141004, Punjab, India.*[3,4]*Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana, 141004, Punjab, India.*

Abstract

This study involves the use of three fungal enzymes viz; cellulase, xylanase and protease to treat brown rice variety Pusa-44 prior to mechanical polishing. The effect of enzymatic pretreatment on the cooking and milling quality of rice was investigated. Three enzyme solutions were used viz; 100% (undiluted), 85% (85ml undiluted enzyme + 15 ml buffer) and 70% concentration (70ml undiluted enzyme + 30 ml buffer). The brown rice was treated with 3 ml of the enzyme solution for 1-3 min at 27℃, 37℃ and 47℃. According to response surface methodology the optimized conditions were 85% concentration of the enzyme, 2 min of the treatment time at a temperature of 42 ℃. As a result of the enzymatic pretreatment under the optimized conditions the head rice yield increased from 64.6% to 69%, percentage brokens decreased from 5% to 3.10%, polishing time decreased from 95 seconds to 54 seconds and the optimal cooking time reduced from 22.5 min to 16.54 min. hence, the enzymatic pretreatment prior to polishing increased the milling performance and cooking quality of the rice.

Keywords: Enzymatic pretreatment, Basmati rice, response surface methodology, fungal enzymes.

Introduction

Enzymes have been used for the processing of rapeseed to increase the dehulling efficiency (Sarkar et al, 1995), enzymatic hydrolysis of soyflakes to increase oil recovery (Aggarwal et al, 1997) and for treating Basmati-386 to improve its milling and cooking characteristic (Arora et al, 2007). Cereals are the most important food stuff and dominate the food sector as these are a versatile and reliable source of food. They are easy to store and can be used to produce a myriad of food products. Cereal processing thus forms a large and important part of food production chain (Owens, 2001) and rice is the most important crop in Asia (Smith, 2000). India produces 93 million tonnes of rice (Anonymous, 2007).

* Corrresponding author. Mailing Address :- 36-C Bhai Randhir Singh Nagar, Ludhiana-141002. Tel : +91-9915592736. E-mailaddress:loveleen_sarao84@yahoo.com

The commercial value of rice is due to its fine and slender grains; this characteristic also favours high percentage breakage during mechanical milling which is not desirable in the international market. Polishing rice up to an appropriate level is important to maintain quality of rice. The removal of pericarp or the bran layer is the primary step in the preparation of rice grains for human consumption (Hoseney, 1986). Loss of nutrients is observed if polishing is done more than required. This disadvantage of mechanical polishing can be overcome by enzymatic pretreatment prior to polishing to improve the milling characteristics. With the application of specific fungal enzymes, the hydrolysis of bran layer is possible in a selective manner, with minimum loss of grain mass. This technique can give higher head yield of rice from paddy and helps in retaining important components of the bran layer, and also reduces time of polishing (Morris and Bryce, 2004). The major constituents of the bran layer are Protein - 12-17%,Fat- 13-23%, Crude fiber- 6-14%,Ash-7-

15%,Carbohydrates-26-27%,Cellulose-11.20%,Arabinoxylans -8.30%,Mannans- 0.40%,Galactans-1.00%, Pentosans -5-8%,Uronic acid -0.40% (Choct, 1997 and Houston, 1972) . So the brown rice is treated with protease, cellulase and xylanase enzymes that would degrade some portion of the bran layer.

Material and Methods

The paddy variety Pusa-44 was procured from the local market to carry out the enzymatic pretreatment. Satake type rotary screen grade cleaner was used to grade the cleaned paddy. The moisture content of the paddy was adjusted to 14 $\pm$ 0.05% on wet basis (AOAC, 1984). This is considered to be the optimum level for milling the paddy samples. Response surface methodology (RSM) was used to design the experiments. To obtain the range of variables for milling the paddy, a second order Box and Behnken design was used.

Experimental design. Response surface methodology (RSM) has been employed in the present study to obtain the optimum values of the enzymatic pretreatment variables. It is a collection of statistical techniques for designing an experiment, building models, evaluating the effects of factors, and searching for optimum conditions of factors for desirable factors (Li *et al*, 2006). These statistical methods have proved to be powerful and useful tools as they allow optimization of the response which is influenced by several variables. It gives information about the interaction between variables, process optimization and gives multiple responses at the same time. It is a powerful and efficient mathematical tool applied for optimization of any process, e.g. media components on fermentation (Adinarayana and Elliaiah, 2002; Park *et al*, 2002), for optimization of rosemary essential oil extraction by controlled pressure drop process (Rezzoug, Boutekedjiret and Allaf, 2005) and for the optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases (Nilsang, Lertsiri, Suphantharika and Assavanig, 2005). A Box and Behnken design (Box and Behnken, 1960) of three variables and three levels each having three centre point combinations was used. Most of the requirements of the enzymatic pre-treatment optimization process are fulfilled by this method, so this design was choosen. In this design, X_1, X_2, and X_3 are the coded variables, which are related to uncoded variables, i.e. variables in actual units linearly by the relation:

$$X_i = 2(\xi_i - \bar{\xi}_i)/d_i$$

Where,

ξ_i = Variable value in actual units of the i^{th} observation

ξ_1= Mean of highest and lowest variable value of ξ_i.

d_i = Difference between the highest and lowest variable value of ξ_i.

Based on this relationship, the independent pretreatment variables and their levels in the form of coded variables are given below in Table 1.

Table 1: Process variables and their levels.

Independent Variables	Symbol		Levels	
	Coded	Uncoded	Coded	Uncoded
Temperature (°C)	X_1	T	1	47
			0	37
			-1	27
Concentration (%)	X_2	C	1	100
			0	85
			-1	70
Time (mins.)	X_3	t	1	3
			0	2
			-1	1

Enzymes. Fungal enzymes protease produced from *Rhizopus microsporus var oligosporus* and cellulase and xylanase produced from *Scopulariopsis acremonium* via submerged fermentation were used for the enzymatic pre-treatment. Enzyme activities of the enzymes were expressed in International Units (IU). One IU was defined as one μmol of xylose (for xylanase activity), glucose (for carboxy methyl cellulase activity) or tyrosine (for protease activity) equivalents released per minute per ml under the following assay conditions by using xylose, glucose or tyrosine standard curve (Silva *et al*, 2005). Three different concentrations of the enzymes were prepared 100% concentration (undiluted enzyme), 85% concentration (85ml enzyme + 15 ml citrate phosphate buffer pH 5.2) and 70% concentration (70ml enzyme +30ml citrate phosphate buffer pH 5.2).

Enzymatic pre-treatment of paddy. A sample of 250 g paddy was cleaned and then shelled in Satake rubber roll

sheller to get brown rice. This brown rice obtained was graded to get head brown rice. Three ml of the fungal enzymes was sprayed on the head brown rice spread as thin film on a tray. The head brown rice was sprayed using different concentrations of the enzymes at different temperatures and was kept for 1-3 min. For polishing the pretreated samples, an abrasive type Satake laboratory polisher was used. The bran adhering to the polished white rice was removed by sieving. After weighing the white rice and the bran their percentages were calculated. The whole kernels were separated from the brokens by sizing of length.

Broken percentage. One of the prominent factors determining the quality of rice is the head rice. Head rice also increases the consumer acceptability. Higher value of percentage brokens lowers the quality. For the selected degree of polish i.e. 5%, percentage broken was obtained by taking the ratio of white rice obtained (broken) to weight of polished rice and it was expressed as percentage. The head rice yield was obtained by taking the ratio of the weight of the head rice to the weight of the paddy sample. This was also expressed as percentage.

$$\text{Percentage broken} = \frac{\text{weight of white rice (broken)}}{\text{weight of polished rice}} \times 100$$

Polishing time. Polishing time is defined as the time required to remove the bran layer to obtain the required degree of polish by the polisher. In this study the pretreated samples were polished to 5% degree of polish.

Optimal cooking time. The process of preparation of food stuff by the action of heat is known as cooking. To calculate the optimum cooking time the following procedure was adopted. Two gram of whole kernel (white rice) obtained after grading were taken in glass tubes containing 20 ml distilled water. The glass tubes were immersed in boiling water bath. Each rice sample was cooked for five different periods of 10, 15, 20, 25 and 30 minutes. Thereafter, the tubes were taken out and the contents of each tube were poured on the filter paper to soak extra water. Manual rolling of the kernels was done on the filter paper to remove surface moisture, till they lost the glistening appearance associated with the surface film of water. The rice kernels were weighed and the graph of moisture gained (%) vs. cooking time was plotted and from graph when the rice weight showed 250 percent increase that limit was considered as the optimum cooking time (Thapar *et al*, 1998).

Contour plotting. A contour plot has lines or curves of constant response values drawn on a graph or plane whose coordinate axis represents the levels of the independent variables and the response is visualized perpendicular to the plane of the paper. Series of the contour plots of equal responses were generated which provided useful information in interpreting contribution of the response (Cox and Cochran, 1964; Montgomery, 2004). As three variables are involved in the pre-treatment process, the value of one variable was fixed to obtain the contours. The non variant parameters were set at optimum point and a new relationship was developed and plotted between the dependent and independent variables.

Results and Discussions

To develop a response surface model the data was analysed employing multiple regression technique. A linear model and a second order model with and without interaction terms were tested for their adequacies to describe the response surface and R^2 values were calculated. A second order polynomial of the following form was fitted to the data of all the responses and the results are given in Table 2

$$y' = \beta_0 + \sum_{i=1}^{n}\beta_i x_i + \sum_{i=1}^{n}\beta_i x_i^{\,2} + \sum_{i=1}^{n-1}\sum_{j=1}^{n-1}\beta_{ij} x_i x_j + \varepsilon$$

where β_0, β_i, β_{ij} are the constant coefficients usually determined by least squares method, $x_i x_j$ are coded independent variables and ε is the error involved in estimating the coefficients β from the experimental data.

For optimization, the Analysis of Variance (ANOVA) for the overall effect of three factor variables on the response variable according to fitted model was done and the least significant factor affecting the response variable was selected Table 3.

Table 2 Treatment factors and the response of the treatment on Pusa-44

S.NO	Treatment Factors			Responses		
	Temperature (x_1)	Concentration (x_2)	Time (x_3)	Broken percentage (%)	Polishing time(s)	Optimal Cooking Time (min)
1	-1	-1	0	4.33	63	18.12
2	1	-1	0	3.45	54	18.50
3	-1	1	0	4.62	60	19.70
4	1	1	0	3.52	55	16.54
5	-1	0	1	3.40	71	19.30
6	1	0	-1	3.33	63	17.40

7	-1	0	-1	4.34	67	19.50
8	1	0	1	3.46	63	16.55
9	0	1	-1	4.21	68	18.45
10	0	-1	1	4.25	61	18.50
11	0	-1	-1	4.26	66	18.50
12	0	1	1	4.10	63	18.10
13	0	0	0	3.13	63	17.42
14	0	0	0	3.10	61	17.66
15	0	0	0	3.25	67	17.66
Control				4.50	95	22.50

Table 3: Analysis of Variance (ANOVA) for Model Fitting – Pusa-44

Source	Degree of freedom	Sum of squares		
		Broken percentage	Polishing time	Optimal cooking time
Model	9	3.50661*	262.830*	12.0993*
Linear	3	1.19793*	104.500	7.4687
Quadratic	3	2.12256*	146.330*	1.1714
Cross product	3	0.18613	12.000	3.4592*
Residual	5	0.34513	29.170	0.5928
Total	14	3.77290	292.000	10.7870
Correlation coefficient (R^2)		0.910	0.900	0.953

* Significant ($P<0.05$)

Effect of variables on percentage brokens. The enzymatic pretreatment reduced the number of percentage brokens. This decrease is due to the effect of cellulase, protease and xylanase enzymes which breaks down the bran layer and facilitates its easy removal. The percentage brokens were found to decrease with increase in concentration of the enzyme from 70% concentration to 85% concentration. When the concentration of the enzyme was increased to 100%, the percentage brokens were found to increase again (Table 2, Fig 1 .1-1.3) which may be due to the over softening of the bran layer. The increase in treatment temperature from 27℃ to 37℃ caused a decrease in percentage brokens. The broken percentage increased again when the temperature was increased to 47℃. The increase in treatment time from 1 to 2 min decreased the percentage brokens. A further increase in treatment time upto 3 min led to increase in percentage brokens. This may be due to increase in brittleness of the grain as a result of the prolonged treatment time. Least percentage of brokens i.e. 3.10% was obtained with a pretreatment time of 2 min, 85% concentration of the enzyme and at 37℃ temperature. Similarly Arora *et al* (2007) also reported a reduction in the percentage brokens by commercial cellulase pretreatment brokens of Basmati-386. The least percentage brokens i.e 3.23% were obtained at 37℃ temperature, with 0.35g $100ml^{-1}$ concentration of the enzyme at a treatment time of 2 min. The head rice yield was found to increase as a result of the enzymatic pretreatment. The head rice yield increased to 69% in case of Pusa-44.

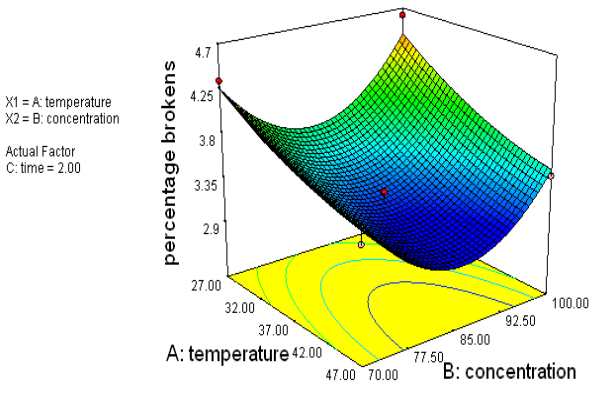


Figure 1.1 Surface plot for percentage brokens (Concentration vs. Temperture)–Pusa-44

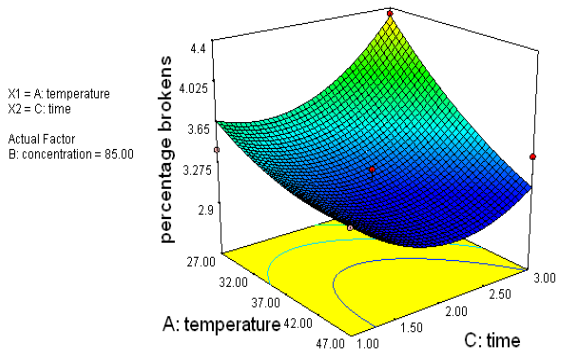


Figure 1.2 Surface plot for percentage brokens (Time vs. Temperature) – Pusa-44

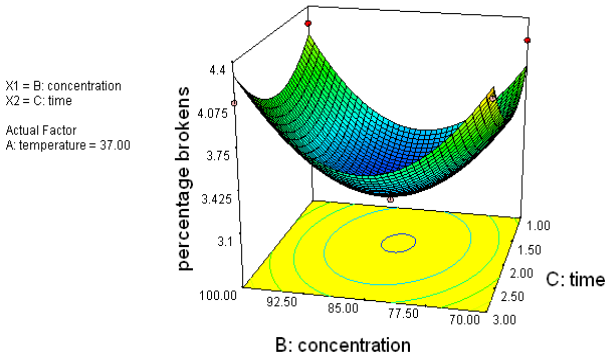


Figure 1.3 Surface plot for percentage brokens (Time vs. Concentration) –Pusa-44

Effect of variables on polishing time. In this study the polishing time decreased as a result of enzymatic pretreatment. The enzymatic pretreatment leads to the degradation of the bran layer, thereby its easy removal. The polishing time increased with increase in concentration of the enzymes from 70% concentration to 85% concentration because the increase in concentration saturated the active sites of the substrate leading to its low activity (Sarkar *et al*, 1998). It decreased again with further increase in enzyme concentration upto 100%. The polishing time also decreased with increase in temperature from 27℃ to 47℃. With the increase in treatment time from 1min to 2 min the polishing time showed a reduction. The polishing time increased again when the pretreatment time was increased to 3 min (Table 2, Fig 3.1-3.3). The lowest value for polishing time for Pusa-44 the treatment time of 2 min at 47℃ with an enzyme concentration of 70% had the lowest polishing time (54 seconds). Similarly, Arora *et al* (2007) reported a decrease in polishing time by treating Basmati-386 with commercial cellulase. They reported that a treatment time of 2 min at 47℃ temperature with an enzyme concentration of 0.15 g $100ml^{-1}$ gave the lowest polishing time which was 51 seconds.

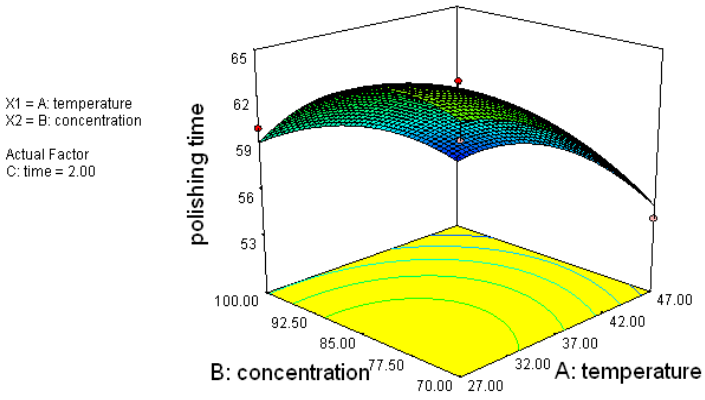


Figure 3.1 Surface plot for polishing time (Temperature vs. Concentration) - Pusa-44

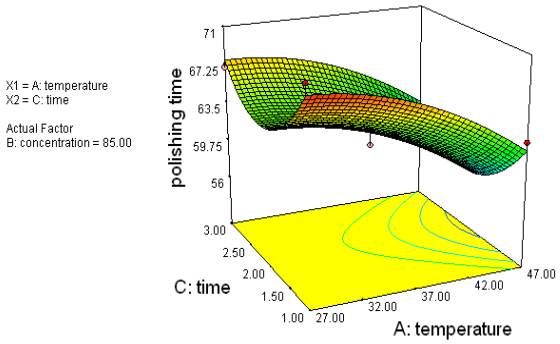


Figure 3.2 Surface plot for polishing time (Temperature vs. Time) - Pusa-44

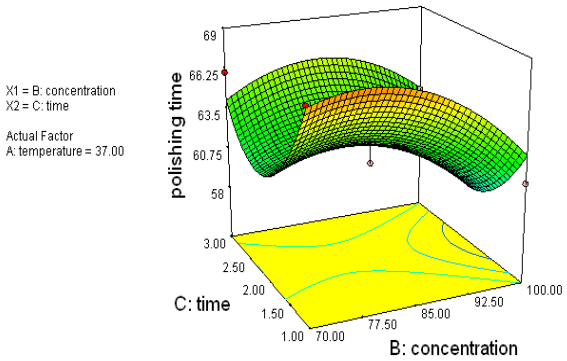


Figure 3.3 Surface plot for polishing time (Concentration vs. Time) - Pusa-44

Effect of variables on optimal cooking time. A reduction of the optimal cooking time of rice is a desirable characteristic. A reduction in optimal cooking time was observed as a result of enzymatic pretreatment. As the concentration of the enzyme increased from 70% concentration to 100% concentration the optimal cooking time was found to decrease. The increase in treatment temperature from 27℃ to 47℃ caused a lowering of the optimal cooking time. The treatment time had no specific effect on the cooking time. The minimum cooking time was found at the treatment time of 2 min at 47℃ with an enzyme concentration of 100% for Pusa-44 (16.54 min) as presented in Table 2, Fig 5.1-5.3 . The reason for this decrease may be attributed to the fact that the outer bran layer which has an oily layer over it, acts as a barrier for the diffusion of water into the kernel during cooking which was degraded by lipase enzyme This enzyme gets activated along with other enzymes at pretreatment conditions (Arora *et al*, 2007). This degradation of the oily layer increased the rate of water diffusion into the kernel hence decreasing the optimal cooking time. Similarly Arora *et al* (2007) used commercial cellulase for pretreatment of Basmati-386 and reported a reduction in cooking time. The treatment time of 3 min, temperature 47℃ and an enzyme concentration of 0.35g 100 ml^{-1} concentration gave an optimal cooking time of 16 min.

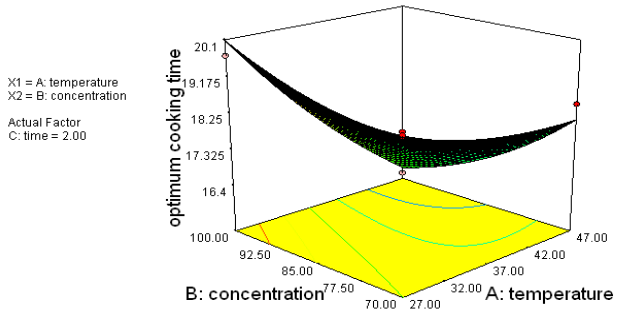


Figure 5.1 Surface plot for optimal cooking time (Temperature vs. Concentration) - Pusa-44

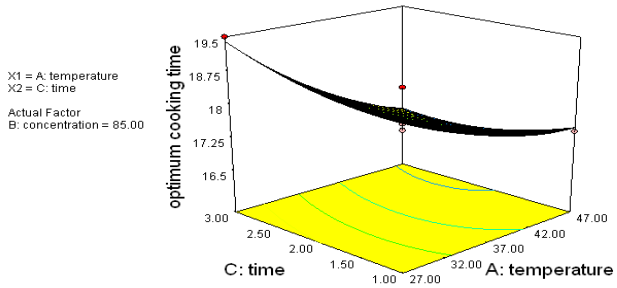


Figure 5.2 Surface plot for optimal cooking time (Temperature vs. Time) -Pusa-44

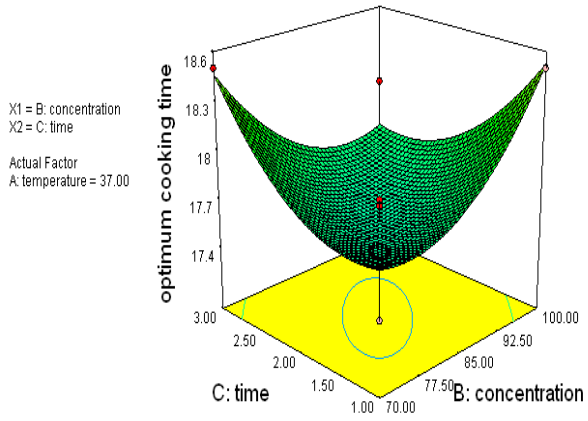


Figure 5.3 Surface plot for Optimal Cooking Time (Concentration vs. Time) - Pusa-44

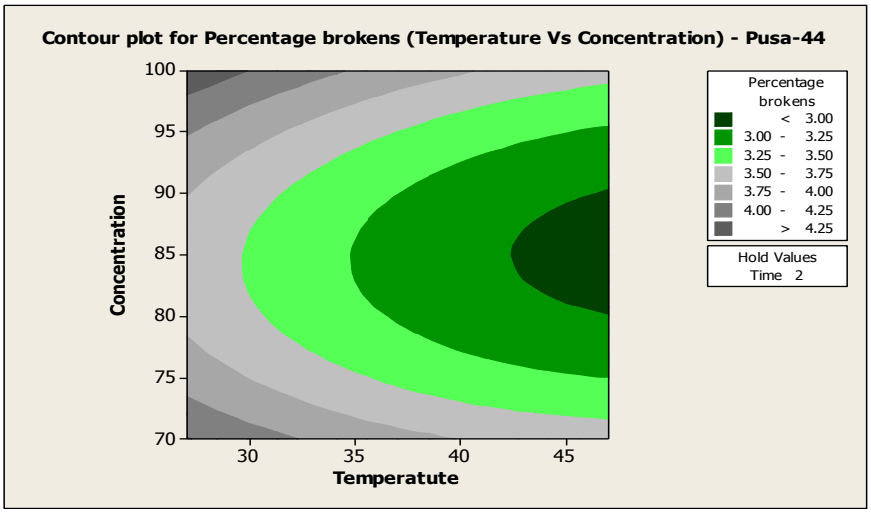
OPTIMIZATION OF THE CONDITIONS FOR THE ENZYMATIC PRETREATMENT OF BROWN RICE PRIOR TO POLISHING

Responses were optimized using Minitab 14 software (Trial Version). The optimum value for each dependent variable had different set of treatment conditions.

In response surface analysis, the selected model was used to locate the stationary point i.e a point at which the slope of the response is zeroed in all directions. The optimization was done with the following targets:

► Percentage brokens should be minimum
► Polishing time should be minimum
► Optimal cooking time should be minimum

To consider all the responses simultaneously for optimization contour plots were generated for each response for different interaction of any two variables while holding the third variable constant (Fig 2 , 4 and 6). To get optimum enzymatic pretreatment conditions contour plots were overlaid with the targets mentioned above.



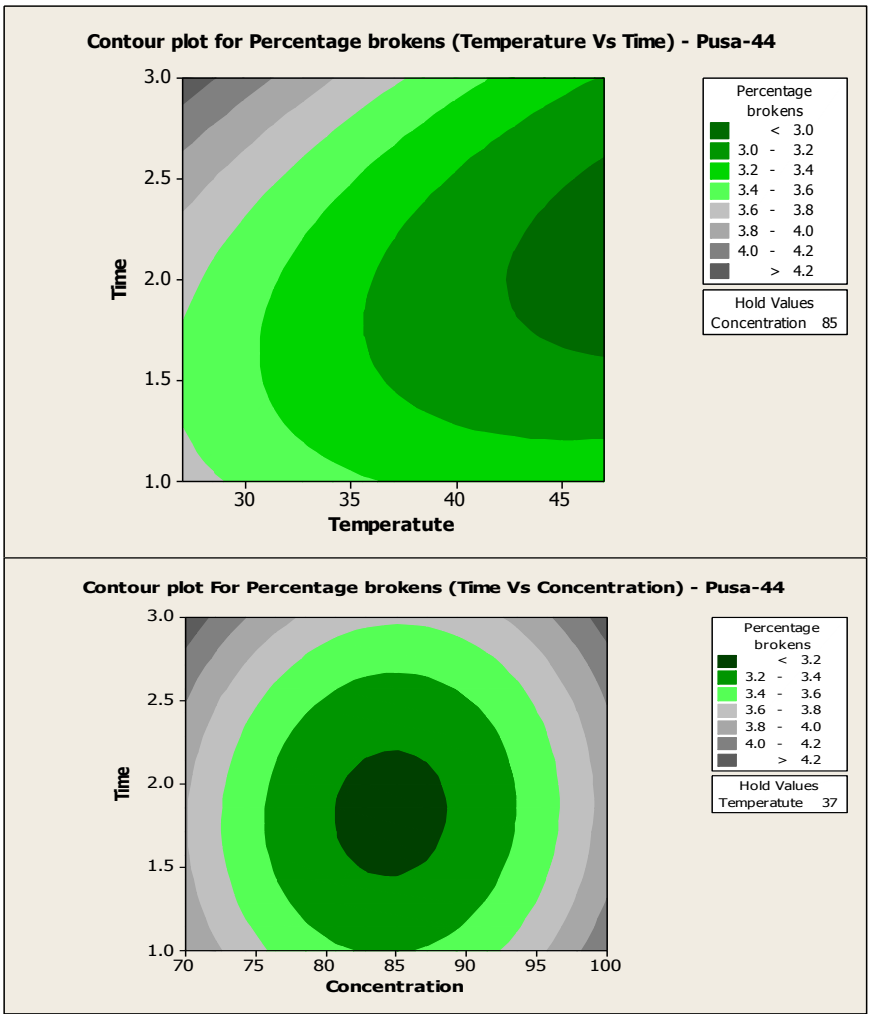
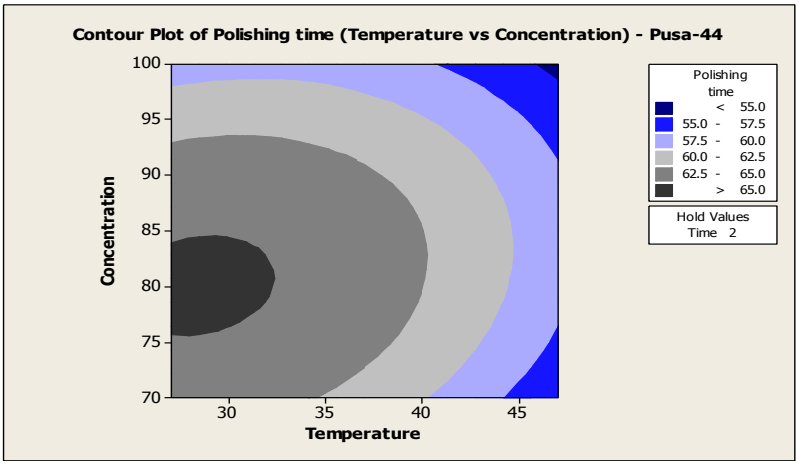


Figure 2 Contour plots for percentage brokens - Pusa-44



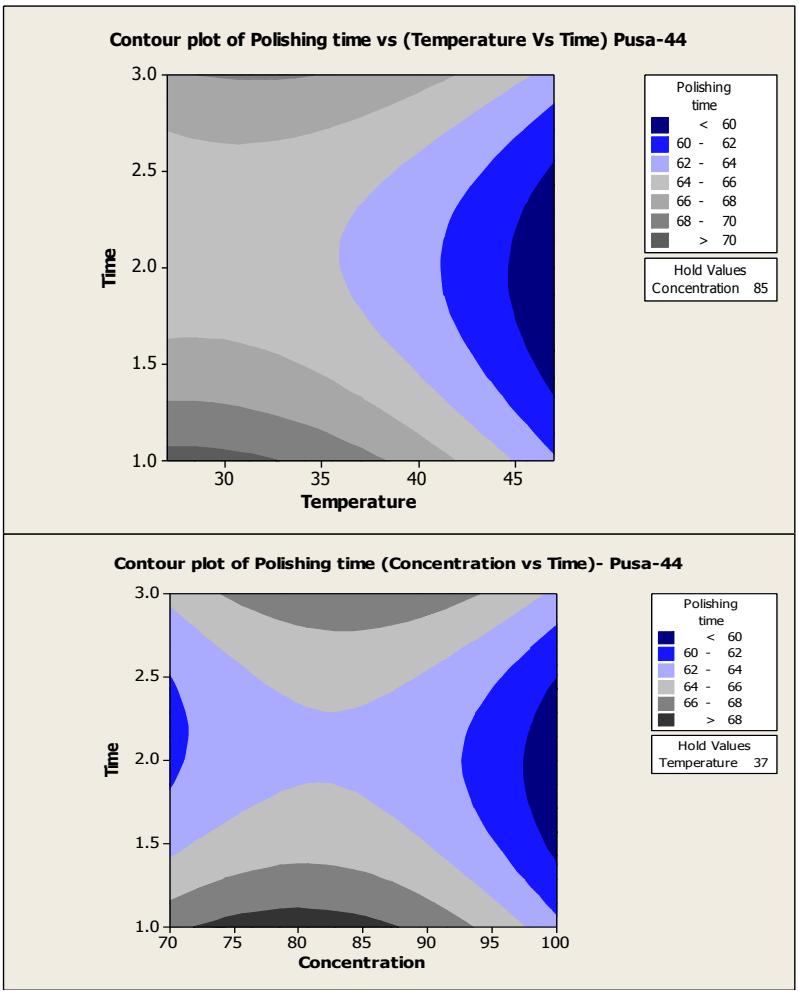
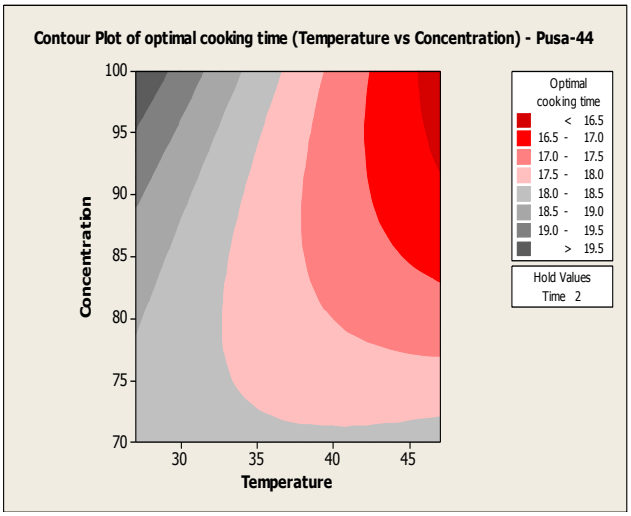


Figure 4 Contour plots for polishing time - Pusa-44



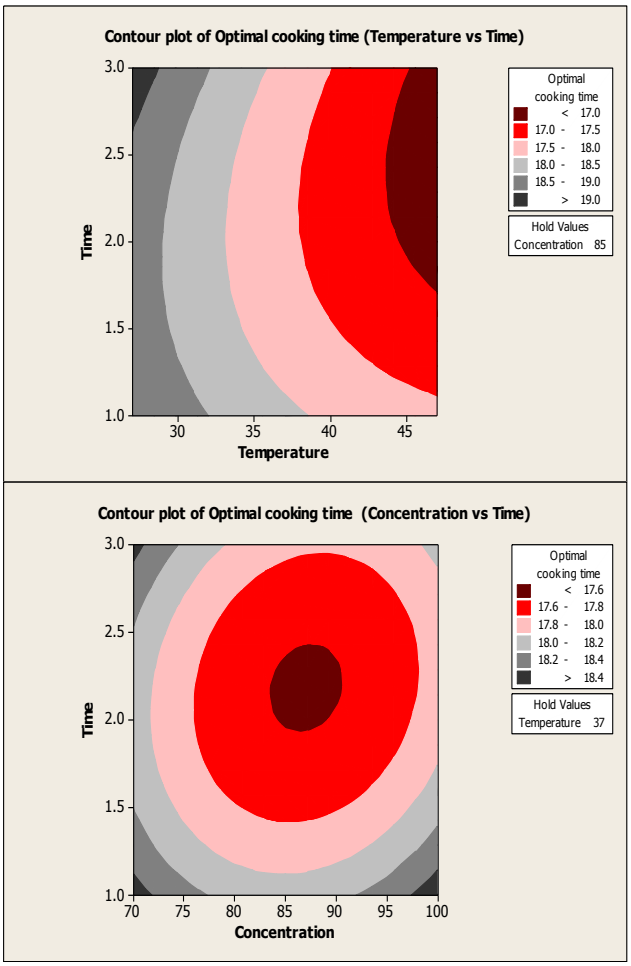


Figure 6 Contour plots for optimal cooking time - Pusa-44

References

Adinarayana K, Ellaiah P. 2002. Response surface optimization of the critical medium components for production of alkaline protease by a newly isolated Bacillus sp. J Pharmaceut Sci 5: 1152-1158.

Aggarwal YC, Kashyap MC, Sarkar BC, Singh BPN. 1997. Response surface analysis of enzyme aided extraction of soybean. J Food Sci Technol 34 (5): 386-390.

Anonymous. 2007 Statistical abstracts of India, Agriculture. Website: htpp://www.indiastat.com

Arora G, Sehgal VK, Arora M. 2007. Optimization of process parameters for milling of enzymatically pretreated Basmati rice. J Food Engg 82 (2): 153-159.

AOAC .1965. Official methods of analysis. 10th Ed. USA. pp. 191, 431.

Box GE, Behnken DW. 1960.Some new three level designs for the study of quantitative variables. Technometrics 2 (4): 455-475.

Cox GM, Cochran WG. 1964. Experimental designs. John Wiley and Sons, Inc. New York. pp: 35-375.

Choct M.1997. Feed non–starch polysaccharides. Chemical structure and nutritional significance. Feed Milling Int (June): 13 – 26.

Hoseney RC .1986. Principles of Cereal Science and Technology. American Association of Cereal Chemicals,Inc., U.S.A. pp. 19-20.

Houston DF. 1972. Rice Chemistry and Technology. Pub. AACC, U.S.A., Vol.IV. pp. 166-67.

Li Y, Lin J, Meng D, Lu J, Gu G, Mao Z. 2006. Effect of pH, cultivation time and substrate concentration on the Endoxylanase production by Aspergillus awamori ZH-26 under submerged fermentation using central composite rotary design. Food Technol Biotechnol 44: 473-477.

Mahindru SN. 1995. Manual of Basmati Rice. 1st edn. Metropolitan Publishers, New Delhi, pp 70 -72.

Minitab 14 Software Trail Version .2007. Available at Website: http://www.minitab.com/products/minitab/14/

Montgomery DC. 2004. Designs and analysis of experiments. New York: John Wiley and Sons.

Morris PC, Bryce JH. 2004. Cereal Biotechnology. Woodhead Publishing Limited, CRC Press, England.

Owens G . 2001. Cereal Processing Technology. Woodhead Publishing Limited, CRC Press, England. pp. 1-3.

Park YS , Kang SW, Lee JS, Hong SI, Kim SW. 2002. Xylanase production in solid state fermentation by Aspergillus niger mutant using statistical designs. App Microbiol Biotechnol 58: 761-766.

Nilsang S, Lertsiri S, Suphantharika M, Assavannig A. 2005. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial protease. J Food Engg 70 (4): 571-578.

Rezzoug S A, Boutekedijiret C, Allaf K .2005. Optimization of operating conditions of rosemary essential oil extraction by a fast controlled pressure drop process using response surface methodology.J Food Engg 71(1):9-17.

Sarkar BC, Singh BPN, Agarwal YC.1995. Effect of enzymatic pretreatment on dehulling of rapeseed. J Food Sci Technol 32: 426-28.

Silva RD, Lago ES, Merheb CW, Macchione MM, Park YK, Gomes E. 2005 Production of xylanase and CMCase on solid state fermentation in different residues by Thermoascus aurantiacus MIEHE. Brazilian J Microbiol 36: 235-241.

Smith WC. 2000. Sorghum: Origin, History, Technology, and Production.Published . John Wiley and Sons.Inc New York.

Thapar VK, Sehgal VK, Chhuneja PK, Arora M, Paul S .1998. Technical Bulletin. Department of Processing and Agricultural Structures, PAU, pp 32-38.