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COMPARISON OF APPLICATION RESOPNSE SURFACE METHODOLOGY AND TAGUCHI METHOD, IN THE OPTIMIZATION OF EXTRACTION OF NOVEL PECTINASE ENZYME, DISCOVERED IN RED PITAYA (*HYLOCEREUS POLYRHIZUS*) PEEL

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ABSTRACT

Peels from plant might be a likely origin of novel pectinases for use in industrial applications, due to their broad substrate specificity, with high stability under extreme conditions. Here, different plan was used to check whether the optimization of pectinase enzyme is achieved or not. Comparison was made on Response Surface Method and Taguchi method, the effect of extraction variables, namely buffer to sample ratio(2:1 to 8:1, X_1), extraction temperature (−15 to +25 °C, X_2) and buffer pH (4.0 to 12.0, X_3) on specific activity, temperature stability, storage stability and surfactant agent stability of pectinase from pitaya peel was investigated. The study demonstrated that, the optimum conditions for the enzyme extraction of pectinase were more stable, with Response Surface Method than Taguchi method. The extraction from pectinase, to achieve high temperature stability (78%), specific activity (15.31 U/mg), storage stability (88%) and surfactant agent stability (83%) were done. Hence, best action to get the highest activity and stability of pectinase enzyme from pitaya peel was 5:1 buffer to sample ratio, at pH 8.0 and 5 °C.

KEYWORDS: Fruit Enzyme; Specific Activity; Temperature Stability; Storage Stability & Surfactant Agent Stability

INTRODUCTION

Pectin enzymes or pectin as esperiodically accounts half of the World's food enzyme production and one among the important industrial enzymes for future demand for pectinases increasing in commercial industry. Red Pitaya peel account about 33% of whole fruit weight. The great significance is seen in the field of fruit and beverage and textile processing industries, pulp and paper making, and for coffee and tea fermentation. The parameters that have to optimize are extraction variables (buffer to sample ratio), extraction temperature, buffer pH (specific activity, temperature stability, and storage stability), and surfactant agent stability. The purpose of this study is to find a way to utilize waste material from agriculture, to produce commercial fruit enzyme and the most to meet the increasing growing demand of pectinases in commercial industry. The extraction of the pectinase is not produced till now.

METHODOLOGY

In the study, the most apt condition for extraction of the pectinase is considered the optimum point, if the extraction of the enzyme results in the enzyme specific activity, temperature stability, surfactant agent stability and storage stability. Overall, optimum extraction condition was obtained by running multiple graphical and numerical optimizations. Multiple graphical optimizations were done by drawing the overlaid counter plot, to determine the optimum region of the pectinase extraction conditions. The extraction conditions under the recommended optimum condition yields in the extraction of the pectinase, from pitaya peel with desirable enzymatic properties. For the purpose of the graphical optimization process, the 3D response surface plotting is followed, to achieve the optimum conditions in Response Surface methodology, where Taguchi OA method optimizes by numerical and graphical method. In order to determine the response surface equations, a comparison was made between the experimental data and predicted values, from the reduced response regression. The results shows that, the extraction using B/S ratio at a concentration of 5:1, at pH 8.0 and at 5 °C for 4 min, provided the overall optimum region in terms of all pectinase properties.

According to Response Surface Methodology we use,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Where, Y represents response function, β_0 is an intercept, β_1, β_2 and β_3 are the regression coefficients for linear terms, β_{11}, $\beta_{22}$and β_{33} are quadratic effects, and β_{12}, β_{13} and β_{23} are the interaction terms. X_1, X_2, X_3 and X_4 represent the independent variables.

Accordingly to Taguchi methodology:

[Intercept]=Intercept

[A]=A+BC

[B]=B+AC

[C]=C+AB

Where, [A, B, C] are factors like temperature, Ratio, pH.

RESULTS AND DISCUSSIONS

Introduction

The study was done by the Taguchi method. The Taguchi method is a orthogonal array design from Taguchi's textbook Explore Regular Two-Level Factorial and Optical and Optical effect is needed. The factors are needed to be categorised in three fundamental factors such as Temperature, Ratio and pH with their units and ranges. The design matrix evaluation for the factorial main effect model is calculated by

Factorial Effects Alliases

[Est. Terms] Allased Terms

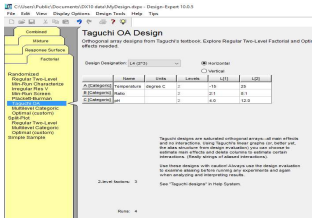
[Intercept]=Intercept

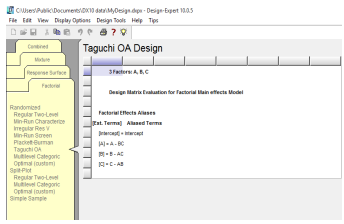
[A]=A+BC

[B]=B~AC

[C]=C~AB

Where, [A, B, C] are factors like temperature, Ratio, Ph





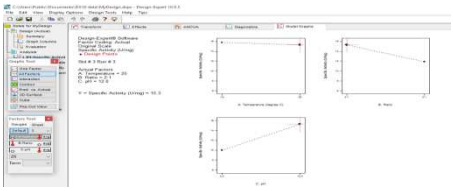
ANALYSIS

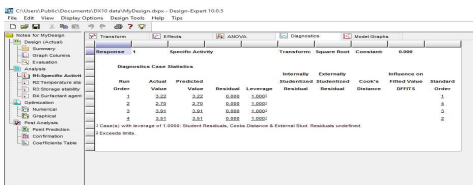
In the analysis of the optimization of the pectinases enzyme, we need to feed with specific Activity, Temperature stability, Storage Stability and Surfactant Agent Stability.

Specific Activity of Pectinase

The main aim of all independent variables (*based on* B/S ratio, temperature and pH of buffer), as well as all square root of variables indicated a good (α = 0.5) effect on the specific activity of the enzyme. The main effect of temperature is the most (α = 0.5) significant effect, based on the B-ratio (15.2) of this independent variable. It confirms the enzyme is heat sensitive and the activity is less at high or low temperature, due to de-naturation of the tertiary structure of pectinase.

The graph also shows the graphical view of specific activity at different Temperature, Ratio and pH. The highest specific activity of pectinase (15.31 U/mg) was obtained, when the pectinase was extracted at -15 °C temperature, B/S ratio 2:1 and pH of buffer 12.0, whereas, in Respone Surface method the highest specific activity of pectinase (15.31 U/mg) was obtained, when the pectinase was extracted at 5 °C temperature, B/S ratio 3:1 and pH of buffer 5.0, which is more stable in nature.

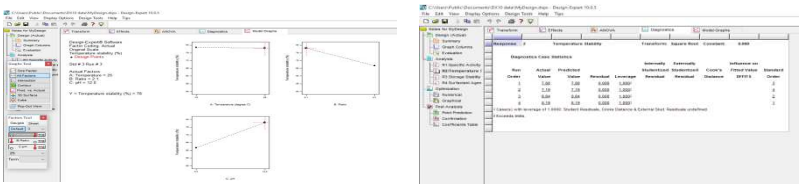




Temperature Stability of Pectinase

The effect of all independent variables except temperature significantly (α = 0.5), was carried out by the extraction of pectinase from pitaya peel. It's important note the temperature stability of the pectinase is one of the good characteristics of the enzyme. The advantages of most able pectinases, especially in industrial processes include less risk of contaminants and also, cost of external cooling is less substrate solubility and a lower viscosity and allowance for accelerated mixing.

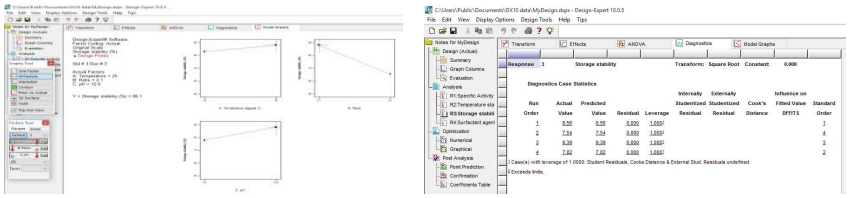
The highest Temperature stability of pectinase (78%) was obtained, when the pectinase was extracted at 25 °C temperature, B/S ratio 2:1 and pH of buffer 12.0, whereas, in Response Surface method the highest specific activity of pectinase (78%) was obtained, when the pectinase was extracted at 5 °C temperature, B/S ratio 2:1-5:1 and pH of buffer 8.0, which is more stable in nature.



Storage Stability of Pectinase

High storage stability is one of the most important parameters, which should be considered in the extraction procedure. Note that, the enzyme stability is significantly decreased, at acidic pH and pH 9.0.

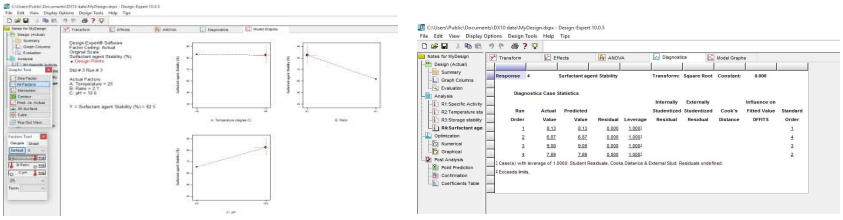
The highest storage stability of pectinase (88.5%) was obtained, when the pectinase was extracted at 25 °C temperature, B/S ratio 2:1 and pH of buffer 12.0, whereas in Respone Surface method, the highest specific activity of pectinase (88%) was obtained, when the pectinase was extracted at 5 °C temperature, B/S ratio 5:1 and pH of buffer 4.0-12.0, which is more stable in nature.



Surfactant Agent Stability

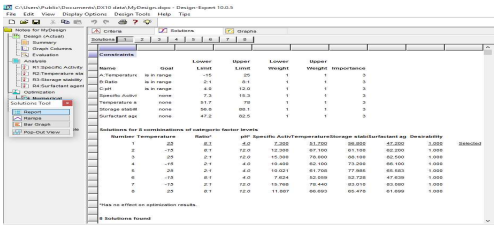
Surfactant agent stability of the enzyme is the important fabric enabling enzymes, to be used in different types of industries, especially the detergent industry. The surfactants which contact with proteins cause distinct electrostatic and hydrophobic regions, and alter the secondary or tertiary structure of enzyme.

The highest Surfactant Agent stability of pectinase (82.5%) was obtained, when the pectinase was extracted at 25 °C temperature, B/S ratio 2:1 and pH of buffer 12.0, whereas in Response Surface method the highest surfactant agent stability of pectinase (83%) was obtained, when the pectinase was extracted at 5 °C temperature, B/S ratio 5:1 and pH of buffer 8.0, which is more stable in nature.



CONCLUSIONS

The respective study of optimization of pectinases shows that, the design achieved by Taguchi OA design is not optimized, since the interaction factors are very high and the product tends to be decomposed very fast through this method, since the reading of Taguchi OA Design. The study demonstrates the desirable condition, for the extraction of pectinases from Red Pitaya Peel is B/S 2:1, at temperature of 25°C, and pH 12.0, whereas, in the Response Surface methodology study is that, the desirable action for extraction of pectinase from pitaya peel was using a B/S ratio 5:1, at pH and 8.05 °C temperature. This is more stable for long shelf life of the product uses.



CONCLUSIONS

The present study shows that, the enzymatic properties of pectinases from Red Pitaya Peel were more stable with Response Surface method, than that of the Taguchi OA deigns, as the study states that, the extraction from the Red Pitaya Peel using B/s ratio 5:1, at 5° C Temperature and pH 8.0 is more stable than B/S 2:1, at temperature of 25°C, and pH 12.0, which is obtained by Taguchi OA design, there was no optimization result from Taguchi method.

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