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تحديد عوامل مختلفة مؤثرة فى انتاج انزيم البكتينيز الخارجى بفطرالاسبرجيلس فلافس

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الملخص

تم دراسة انتاج انزيم البكتينيز بتقنية المزارع المغمورة بواسطة فطر الاسبرجيلس فلافس المعزول من ثمار التفاح والبرتقال الفاسدة .

تمت دراسة مختلف عوامل نمو الفطر المؤثرة على انتاج الانزيم باستخدام طريقة الاستجابة السطحية .

اولا تم استخدام تصميم لمستويين باسلوب "بلاكت بيورمان "لتحديد مدى تاثير سبعة عوامل وهى تركيز كبريتات الامونيوم ،تفل البرتقال، الاس الهيدروجينى ،حجم التلقيح ،معدل التهوية ،وفترة التحضين على انتاج انزيم البكتينيز وبناءا على النتائج المتحصل عليها فقد تم اختيار تركيز تفل البرتقال ،الاس الهيدروجينى وزمن التحضين لاجراء دراسات مكملة بهدف التوصل لتاثير ايجابى ومعنوى فى انتاج انزيم البولى جلاكتونيز الخارجى .

ثانيا فقد تم تطبيق تصميم "بوكس بنكن "على ثلاث مستويات للحصول على افضل ظروف للتخمير للبكتينيزللحصول على اعلى نشاط وهى الاس الهيدروجينى للبيئة ٨ ،تركيز تفل البرتقال عند ٣٠جم/لتر وفترة التحضين ٤٨ ساعة ليعطى اقصى تركيز ٤٠٧٣وحدة/مل والذى يتعدى ٢.٥ ضعف الانتاج المتحصل عليه من التجربة التمهيدية .

تمت تنقية الانزيم بواسطة السيفاديكس ج ١٠٠ ظهر افضل نشاط للانزيم عند اس هيدروجينى مقدارة ٧ واثبت الانزيم الثبات الحرارى العالى حيث فقد ٥٠% من نشاطة فقط عند التحضين على درجة ٥٠ م لمدة ٥٠ دقيقة وقد زاد النشاط الانزيمى فى وجود ايونات النحاس والكالسيوم حيث اعطى اعلى قيمة ٢٠٠٠ وحدة/مل واعلى قيمة ثبات تقدر بـ ٠.٨ مجم/مل .

DETERMINATION OF VARIOUS FACTORS AFFECTING OF PRODUCTION EXO-PECTINASES BY *ASPERGILLUS FLAVUS*

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ABSTRACT: *Pectinase production was studied in a submerged culture of Aspergillus flavus isolated from rotten oranges and apples fruits. Effects of the different growth factors on the enzyme production were studied using response surface methodology (RSM). A two levels "Plackett-Burman" design was used firstly for screening the effect of seven factors; ammonium sulfate concentration, orange pomace concentration, pH, temperature, inoculum size, aeration rate and incubation time on the pectinase production. Based on the obtained results; orange pomace concentration, pH and incubation time were selected for further studies due to their significant positive effect on the exo- polygalactouronase production. Secondly ,a three levels "Box-Behnken" design was applied to get the optimal fermentation conditions for pectinase as; medium pH of 8.0, orange pomace concentration of 30g/l and incubation time of 48 hr. we got a maximum activity of 4073 U/ml which is 2.5fold more than that obtained from the screening experiment. The enzyme was purified to homogeneity using Sephadex G-100. The optimal pectinase activity was detected in assays conducted at pH 7.0. The enzyme revealed a good thermal stability, as it lost 50% of its activity when it was incubated at 50°C for 50 minutes. The enzyme activity was enhanced in the presence of Cu^{+2} and Ca^{+2} and it gave a V_{max} value of 2000 U/ml and K_m value of 0.8mg/ml.*

Key words: *pectinase , response surface methodology, Aspergillus flavus, orange pomase and kinetics.*

INTRODUCTION

Pectins are a heterogenous group of high molecular weight complex, acidic structural polysaccharides consisting largely of D-galactopyranosyluronic acid that are α (1-4) glycosidically linked to polygalacturonic acid with small amounts of L- rhamnose (2-4%) and various side chains comprising L-arabinose, D- galactose and D- xylose (Esquivel *et al*., 1999& Singh *et al* ., 1999). Over the years, pectinases are being used in coffee and tea fermentations, oil extraction and treatment of industrial wastewater containing pectinacious material (Hoondal *et al*., 2002). Microbial pectinases account for almost 25% of the global food enzyme sales, and nearly all the commercial preparations of pectinases are produced from fungal sources, and mostly from *Aspergillus niger* (Jayani *et al*., 2005). Bacteria, yeast, actinomycetes and filamentous fungi have been reported to produce pectinases , which include exo and endo polygalacturonase, pectin esterase, pectinelyase and pectate lyase (Jayani *et al*., 2005 & Torres *et al* ., 2006).

The pectinolytic enzymes are of great significance, especially, in fruit processing, textile and paper industries (Reid and Ricard, 2000). The major industrial applications of pectinases include extraction and clarification of fruit juices and grape musts, citrus fruit juice and wine technology, maceration of vegetables and fruits and extraction of olive oil (Fogarty &Kelly, 1983). The most upcoming application of pectinase is their use in the de-gumming of plant fibers without any damage to the end products (Henriksson *et al*., 1997; Kapoor *et al*., 2001). Several pectin rich substrates such as wheat bran (Taragano *et al*., 1997), sugar cane bagasse (Solis-Pereyra *et al*., 1993and 1996) coffee pulp (Boccas *et al*., 1994), lemon peel (Larios *et al*., 1989), apple pomace (Hours *et al*., 1988) and deseeded sun flower head (Patil, 2004) have been used for the production of microbial pectinases in both submerged and solid-state conditions.

The selection of an appropriate micro-organism, physico-chemical factors for fermentation process (pH, temperature, and moisture) significantly affects microbial growth , product formation and ultimately determines the overall success or failure of the fermentation process. In this respect, optimization that carried out by response surface method (RSM), not only allowed quick screening of large experimental domain but also reflected the role of each factor. This optimization process involves three major steps; performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model (Gupta *et al*., 2008). Enzyme production by fungi differs according to the type of the fungus, culture medium composition and fermentation conditions like temperature, pH, and aeration rate and incubation time. So, Objective of the present study was to engineer a growth medium using statistical experimental designs with low cost carbon and nitrogen sources in a relatively short period to optimize the production of exo pectinase in a submerged culture of *Aspergillus flavus* .

MATERIALS AND METHODS

1. Isolation of the pectinolytic fungus

Pectinase- producing fungi were isolated from rotten fruits (orange and apple) that obtained from the local markets. The pectinolytic fungal isolates were selected on the basis of their utilization of pectin as a sole carbon source using a selective medium contained (g/l dist. water): Ca Cl2. 2H2O , 0.05; KH2 PO4, 0.2; MgSO4.7H2 O,0.8; NH4 SO4 ,1.0; yeast extract,1.0; Agar,15; citrus pectin, 0.05(Moyo *et al*.,2003). The fungal spores were taken directly from the infected material on to the selective medium and the plates were incubated at 25^{o}C for 48 hr. One fungal colony, surrounded by the widest transparent zone diameter was selected as a potential producer of pectinase and was identified according to its growth and microscopic morphology using different universal manuals (De Hoog & Guarro, 1995 and Kendrick, 2000).

2. Inoculum preparation

For preparing inoculums suspension, 48 h grown *Aspergillus flavus* spores were used in saline solution. Spore suspension diluted to adjust the spores amounts. Spore suspension was regulated to 10^4 spores ml^{-1} using spectrophotometer at 530nm wave length.

3. Pectinase assay

Enzyme activity was assayed by mixing and incubating 0.2 ml of the culture filtrate and 0.8 ml of the pectin solution (1% in 0.2 M sodium acetate buffer pH 5.5) for 10 min at 50 °C. Number of the reducing groups expressed as galacturonic acid released was quantified by the Di Nitro Salicylic (DNS) method (Miller,1959). One unit of the enzyme activity is defined as the amount of enzyme releasing one µmol of galacturonic acid per minute (Silva *et al*., 2005).

4.Experimental statistical analysis design

4.1 Placket- Burman design

Cultivation parameters were evaluated based on a Plackett-Burman factorial design, each factor was cored at 2 levels: (-1) for the low level, and (+1) for the high level. Seven assigned variables (concentration of each of ammonium sulfate and orange pomase, pH , medium volume, inoculum size, temperature and incubation time) were screened in 12 experimental trials (Table1), based on the following first-order model:$Y=\beta 0+\Sigma\beta ixi$; where Y is the response (pectinase activity), β0 is the model intercept, βi is the linear coefficient, and xi is the level of the independent variable. All the experiments were carried out in triplicates and averages of the pectinase yields were taken as a response. The main effect of each variable was calculated as the difference between the average of measurements made at the high value (+) and at the low value (-) using SAS JMP 8 NULL program (SAS, 2006).

Table. 1: Response of the exopolygalacturonase yield as influenced to different environmental and nutritional conditions.

Factors / Trial no.	Temperature (^{o}C)	Incubation Time (h)	Aeration (medium volume /flask)	Ammonium Sulfate (g/l)	pH	Inoculum size (spores/ml)	Substrate (Orange pomace) (g/l)	Enzyme activity (U/ml)
1	30	48	0.4	3	6	10000	80	1624
2	25	48	0.4	3	4	1000	40	1580
3	30	24	0.2	1	6	1000	40	1362
4	25	24	0.4	1	6	10000	80	399
5	30	48	0.4	1	4	1000	80	258
6	30	24	0.2	3	4	10000	80	453
7	25	48	0.2	3	6	10000	40	1642
8	25	48	0.2	1	6	1000	80	1991
9	30	24	0.4	3	6	1000	40	1127
10	30	48	0.2	1	4	10000	40	1169
11	25	24	0.2	3	4	1000	80	396
12	25	24	0.4	1	4	10000	40	1634

4.2 Box-Behnken design

To describe the nature of the response surface in the experimental region, a Box-Behnken design was applied. Factors with the highest confidence levels were prescribed at three levels, coded -1, 0, and +1 for the low, middle, and high concentrations (or values), respectively. Table, (2) represented the design matrix for 15 trials, along with the natural values for the three factors (g/l). To predict the optimal point, a second order polynomial function was fitted to correlate the relationship between the independent variables and the response (pectinase yield). The equation for the three tested factors as follow:

$$Y=\beta0+\beta1\ X1+\beta2\ X2+\beta3\ X3+\beta12\ X1X2+\beta13\ X1X3+\beta23\ X2X3+\beta11\ X12+\beta22\ X22+\beta33\ X3$$

Where Y is the predicted response; β0 is the model constant; X1, X2, and X3 are the independent variables; β1, β2, and β3 are the linear coefficients; β12, β13, and β23 are the cross product coefficients; and β11, β22, and β33 are the quadratic coefficients. SAS JMP 8 NULL program was used for the regression analysis of the obtained experimental data. Quality of fit of the polynomial model equation was expressed by the coefficient of determination "R^2". All the experiments were performed in triplicate and the mean values were given.

5. Enzyme purification

Precipitation

A .flavus fermentation broth filtrate was subjected to ammonium sulphate precipitation (50% saturation) to remove undesirable proteins, and further increased to 95% saturation to precipitate most of the exo pectinase enzyme . The enzyme precipitate was suspended in 10 ml of sodium- acetate buffer (50mM, pH 4) and dialyzed against the same buffer. The concentrate was loaded on a sephadex G-100 (Pharmacia) column (1.0cm x 30cm) equilibrated with 40 mM acetate buffer (pH 5.0) and eluted with the same buffer at a flow rate of 0.5 ml min^{-1}. Fractions (4 ml each) were collected and assayed for protein and exo-poly galactouronase activity (Silva *et al.,* 2007). Protein content of the enzyme fractions was determined by measuring the absorbance at 280 nm.

Table 2. Box – Behnken factorial experimental design representing response of the pectinase yield as influenced by elapsed fermentation time, substrate concentration and pH

Factors Trial no.	Time (h)	pH	Substrate (Orange pomace g/l)	Enzyme Activity (U/ml)	
				Actual	Predicted
1	60	6	40	3322	3422
2	60	8	20	3392	3293
3	48	8	30	4073	4139
4	60	7	30	3392	3415
5	60	7	30	3466	3415
6	60	6	20	2907	3005
7	72	7	20	3496	3464
8	48	7	20	3627	3660
9	60	8	40	3620	3522
10	48	6	30	3637	3506
11	72	8	30	3417	3548
12	72	6	30	3860	3794
13	72	7	40	3865	3832
14	60	7	30	3387	3415
15	48	7	40	3907	3938.75

6. Enzyme Characterization:

6.1 Effect of different metal ions on the enzyme activity

Effects of some metal ions on the enzyme activity were investigated by its incubation with 1 mM of each of Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, K^{+}, Cu^{2+} and Fe^{2+} in 0.2 M Sod. acetate buffer (0.2 M ,pH 5.5) for 30 min at 30°C. The residual activity was measured at the standard assay conditions and compared with that of the control (without metal ions).

6.2 Effect of different pH values on the enzyme activity

Effect of the pH values on the enzyme activity was determined by incubating 1% pectin with 0.2 M sod. acetate buffer ranged from pH 4.0 to pH 10.0. The residual activity was measured at the standard assay conditions and compared with that of the control .

6.3 Thermal stability

The influence of temperature on the stability of the enzyme was carried out by subjecting an aliquot of the purified enzyme to a temperature of 50°C in water bath for period ranging from 0. 5 to 45 min at interval of 5 min and the enzyme activity of the heated enzyme was measured. The residual activity was measured at the standard assay conditions and compared with that of the control (100% positive).

6.4 Kinetic parameters

K_m (Michaelis constant) and V_{max} (maximum reaction velocity) values were determined by incubating 50 µl of the purified exo- polygalactouronase enzyme in 1 ml of sodium phosphate buffer (0.2M, pH 5.5) at various pectin concentrations (1-10 mg/ml) at 30°C for 30 min then the enzyme activity was measured. K_m and V_{max} values were then calculated by plotting the substrate concentration divided by velocity (S/V) versus the substrate concentration (S) (Hanes woolf plot).

The equation is shown as follow: $(S)/v = (K_m / V_m) + (1/V_m)(S)$
Where:
(S)= concentration of pectin V= rate of galactouronic acid formation
K_m= Michaelis- Menten constant
V_m= maximum forward velocity

RESULTS AND DISCUSSION

1. Identification of the fungal isolate

The fungal colonies which surrounded by the widest clear zone indicates its pectinolytic activity. Morphologically the colonies are lime green in colour, wooly to cottony in texture with septated hyphae and globose to sub globose vesicle. Matulae nearly covered the entire surface in bisriate with globose conidia . On the basis of its macroscopic and microscopic characteristics, the fungal isolate was identified as a variety of *Aspergillus flavus*

2.Enzyme production (Experimental statistical analysis design)

2.1 placket- Burman

By applying the placket Burman factorial design, a wide variation of the exo-polygalacturonase activity ranged from 258 to 1991 U/ml was achieved (Table 1),thereby reflecting the importance of studying the medium composition for attaining a higher productivity. On analyzing the regression coefficients for the seven studied variables, it was found that; both the incubation time and the pH values had a positive effect on the exo-polygalacturonase production, whereas orange pomace concentration, temperature and aeration had negative effects. The other two factors (inoculum size and ammonium sulfate concentration) nearly had no effect on the enzyme production (Fig..1).

2.2 Box benken

Studying the effect of the three selected factors out of the Plackett- Burman experiment , each at three levels, revealed an optimal fermentation conditions for the exo-polygalacturonase production as medium pH 8.0, orange pomace concentration of 3.0% and incubation time of 48 hr. These conditions yield a maximum activity of 4073 U/ml , which is a 2.5 fold more than that obtained from the screening experiment (Table 2). Patil and Dayanand (2006a) reported an increase in the level of pectinase production at pH 5.0 ,temperature 34 ^{0}C, and inoculums size of $1x10^5 ml^{-1}$. When box- Benken applied for optimizing the tested factors, the predicted and experimental values are very close, reflecting the accuracy and applicability of the Box- Benken design (Table, 2). Moyo *et al*., (2003) reported a 1.5 fold increase in pectinolytic enzymes secretion by *Kluyveromyces wickerhamii*, when pH, temperature and incubation period were optimized by RSM(response surface methodology). Results of the Pareto chart of the Box- Behnken design (Fig..2) , displayed the magnitude of each estimate and showed the ranking of the factor estimates. It was found that the separate effect of each of the substrate or the pH on the exo-polygalacturonase production is higher than their effect when each was examined with time.

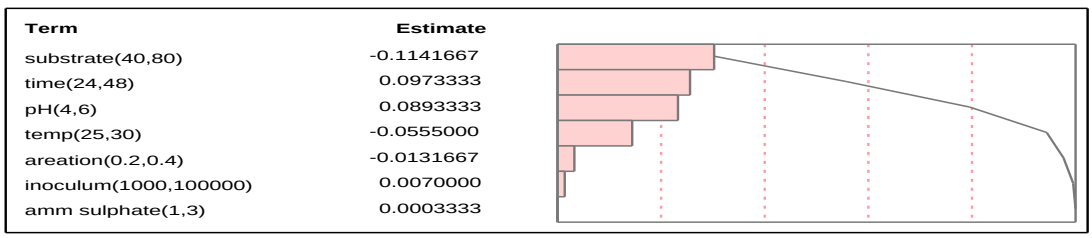


Fig. 1: Pareto chart rationalizing the effect of each of the seven used variables on the pectinase production by *Aspergillus flavus* using Plackett-Burman design.

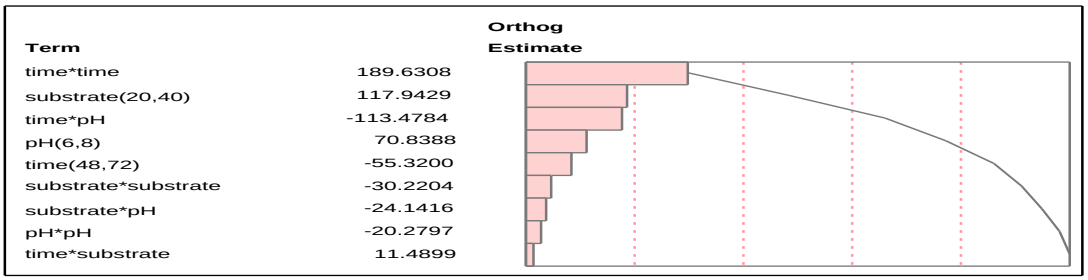


Fig .2: Pareto chart rationalizing the effect of each variable on the pectinase production by *Aspergillus flavus* using Box-Behnken design.

Interaction Profiles

The results in Fig. (3) revealed the interactive effect between the examined factors " incubation time, pH and pomace concentration". Increasing the pomace concentration from 20 to 40g/l has showed a positive effect on the enzyme activity (Fig.. 3a and 3f),while decreasing the incubation time from 72hr to 48 hr, led to an increase in the pectinase yield (Fig..3c,3e). Maximum enzyme activity was achieved, when both incubation time and pomace concentration set at their optimum levels of 48hr and 40g/l, respectively (Fig.., 3c).These results are in contrast to that obtained by khalaf (2000)who reported that the exopectinase activity was reached to the maximum value after 4 days.

The interaction between incubation time and pH revealed that pectinase yields were increased gradually with increasing the incubation time up to 72hr with a final pH value of 6.0 (Fig..3b). The pectinase activity reached the highest value of 4000U/ml , when the fermentation medium incubated for 48hr at pH value of 8.0. The effect of both pH values (6, 8) intersect in a point coincide with enzyme activity of 3600U/ml after 65 hr.

The interaction between substrate concentration and pH values revealed that pectinases yield is increased gradually by increasing substrate concentration from 20 to 40g/L when the medium pH reached "8" (Fig.,3d). Increasing the incubation time from 48hr to 72hr at pH of "8" led to a decrease in the enzyme activity. (Fig..,3e).

Cube plot was a convenient means for assessing quantitative effects of the three tested factors on a response and to visualize the limits (maximums and minimums) of the response for various combinations of levels of factors. Each corner of the cube is the average response value for each combination of the factors levels. The following plot (Fig.. 4) confirmed that, the maximum enzyme activity (4167.88 U/ml) was obtained at pH "8" , substrate concentration of 40g/l and incubation time of 48hr. followed by 3982.87U/l that obtained at pH8,substrate concentration of 20g/l and incubation time of 48hr. The lowest enzyme activity (3255.87 U/l) was obtained after incubation time of 48 hr, pH 6 and substrate concentration of 20g/l.

Prediction Expression

A high degree of correlation between the experimental and predicted values of the pectinases production was expressed by a high R^2 value of 0.92 (Fig..5). These results are coincide with that obtained by Gupta *et al*., (2008) ,where they reported R^2 value of o.988 .

Verification of Model

The optimal conditions realized from the optimization experiment were verified experimentally and compared with the data calculated from the model (Fig..5). Obtained pectinase yield is 4073 U/ml and the polynomial model predicted value is 4139.25 U/ml. Thereby this confirming the high accuracy of the model resulted at more than 92% under the investigated conditions. Analysis of the results indicated that the experimental values are in a good agreement with the predicted ones, and also suggested that the models are satisfactory and accurate.

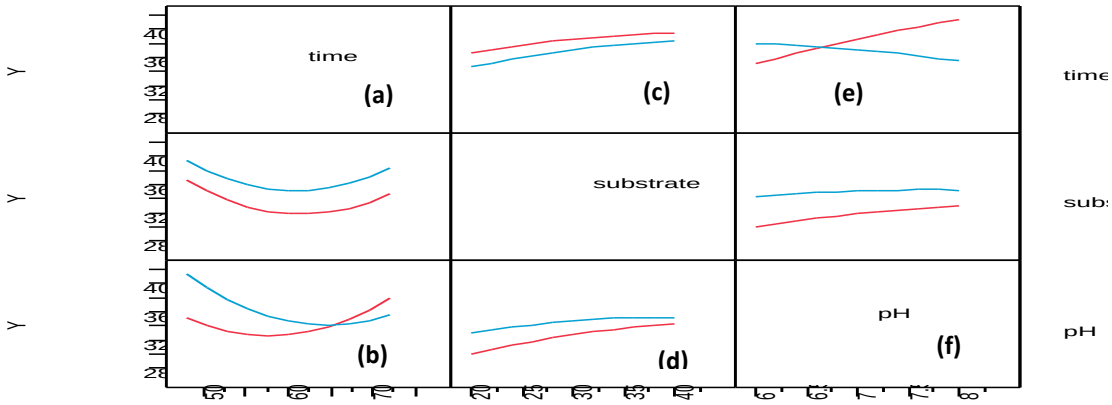


Fig .3: Response surface plot of the pectinase yield from *Aspergillus flavus* as a function of substrate concentration , pH and incubation time under optimal conditions.

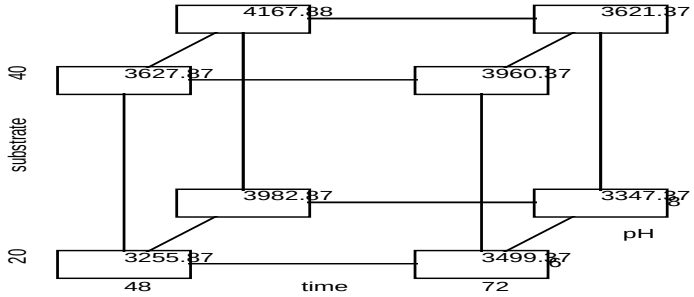


Fig . 4: Cube Plot of pectinase yield.

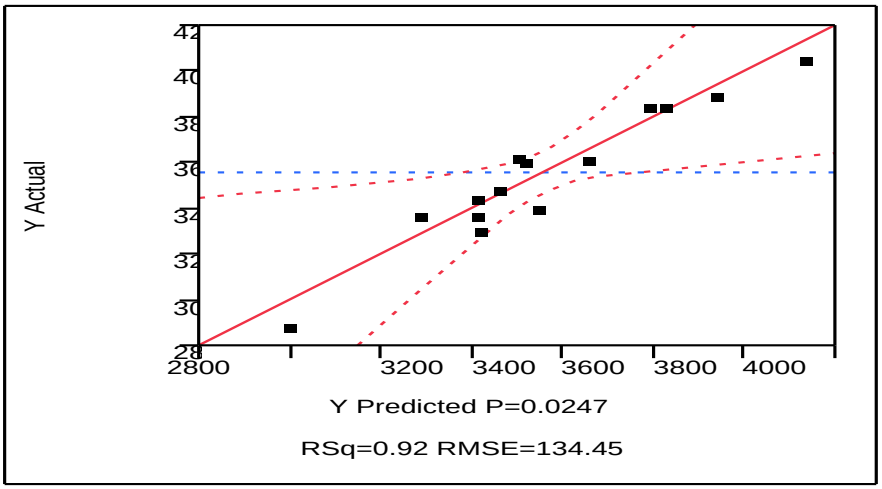


Fig. (5): Experimental values vs. predicted values for the pectinase production by *Aspergillus flavus*

Y = 3415 +- 75.750 * ((T- 60) / 12) + 161.5 * ((S - 30) / 10) + 97 * (pH - 7) + ((T- 60) / 12) * (((S - 30) / 10) * 22.25) + ((T- 60) / 12) * ((pH - 7) * -219.75) + ((S - 30) / 10) * ((pH - 7) * -46.75) + ((T - 60) / 12) * (((T - 60) / 12) * 372.625) + ((S- 30) / 10) * (((S - 30) / 10) * -63.875) + (pH - 7) * ((pH - 7) * -40.875).

Purification

Elution profile of the enzyme from the sephadex G-100 gel filtration column is shown in Fig. (6). Most of the enzyme activity is shown in three well separated protein peaks, indicating a relatively pure enzyme preparation.

Enzyme characterization

Several polygalactouronases (PGS) showed a temperature optima between 40–60˚C (Urbanek and Zelewska-Sobczak 1975.

Results shown in Fig (7A) showed an enzyme activity maintained without loss for 5 min., after which there is a gradual decrease in the enzyme activity for the first thirty minutes and after 45 min , a 43% loss of the enzyme activity was recorded.

For temperatures greater than 50˚C ,inactivation was notable after a short period of heating (Sakai *et al.,* 1993) . Silva *et al*., (2002) reported that, after 1 hr of incubation at 55˚C, the PG produced by *Penicillium viridicatum* RFC3 lost approximately 90% of its initial activity

The enzyme activity reached the maximum at pH 7 (Fig.. 7B), while the incubation at pHs 4,5,6,8,9 and 10 result in 36%,23%, 9%, 13% ,17% and 64% loss of PG enzyme activity respectively. Nearly the same results were obtained with PG from *Thermoascus aurantiacus,* which retained more than 90% of its activity when incubated in pH between 7.0 and 8.0 (Martins *et al*., 2002). while, PG from *P.viridicatum* maintained 90% of its activity after incubation in pHs from 5.0 to 8.5 (Silva *et al*., 2002).

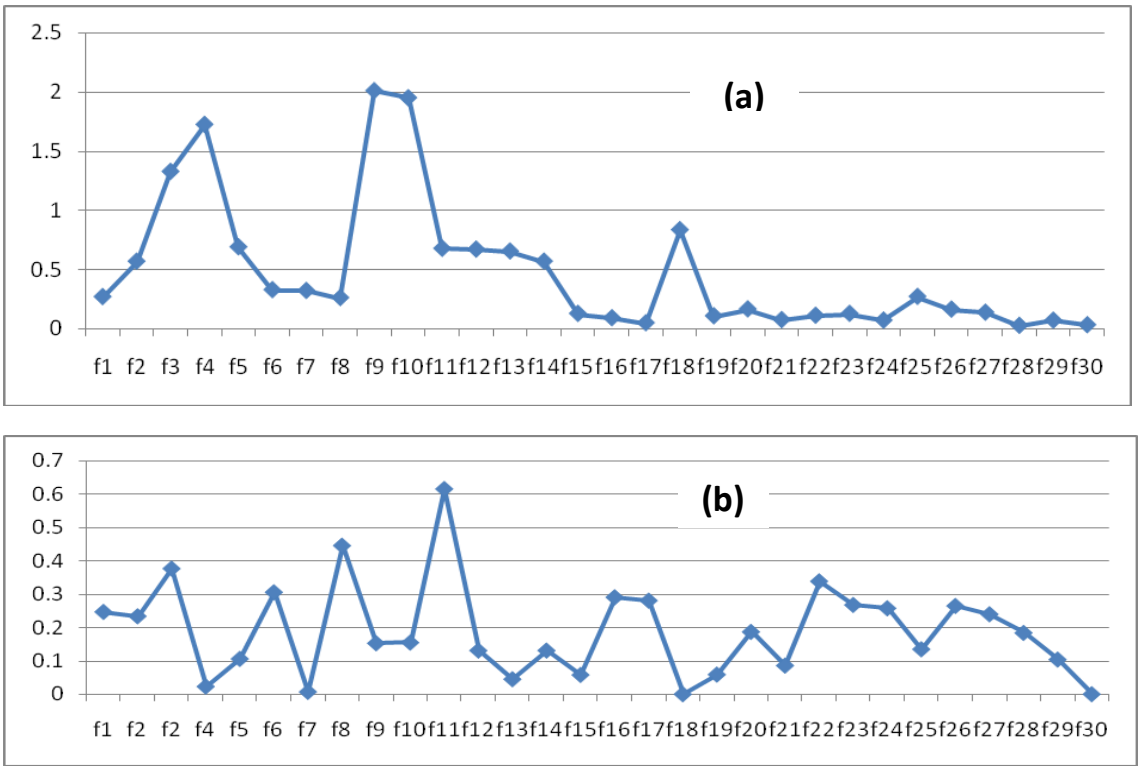
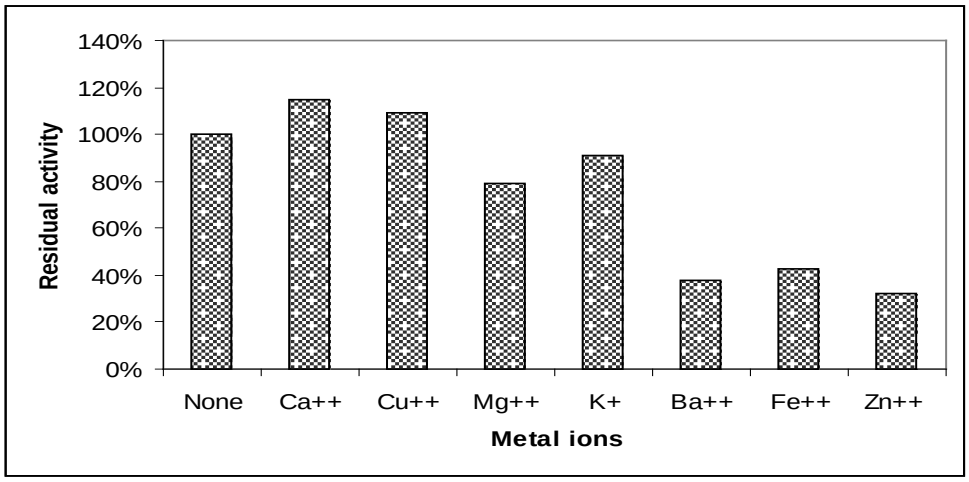
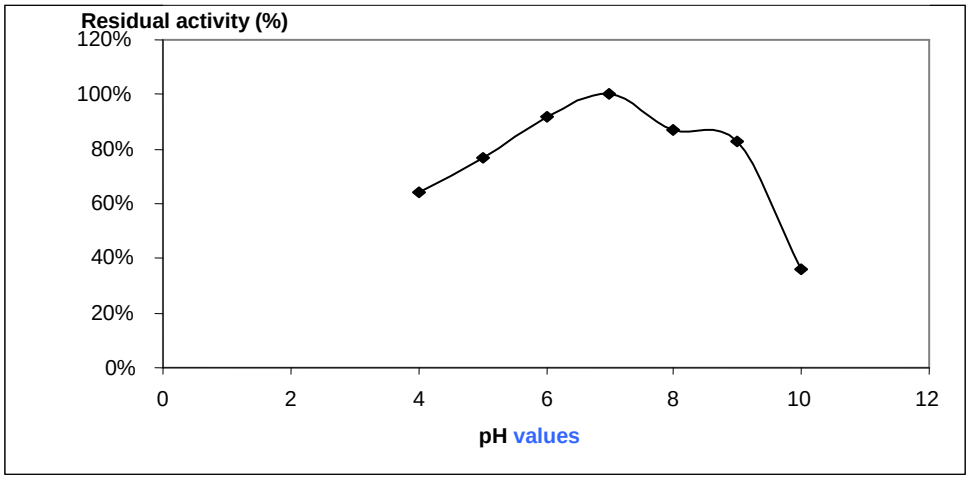


Fig. (6): Gel filtration chromatography of exopoly galactouronase produced by *Aspergillus flavus.* Proteins (a) and enzyme activity (b).

(A)



(B)



(C)

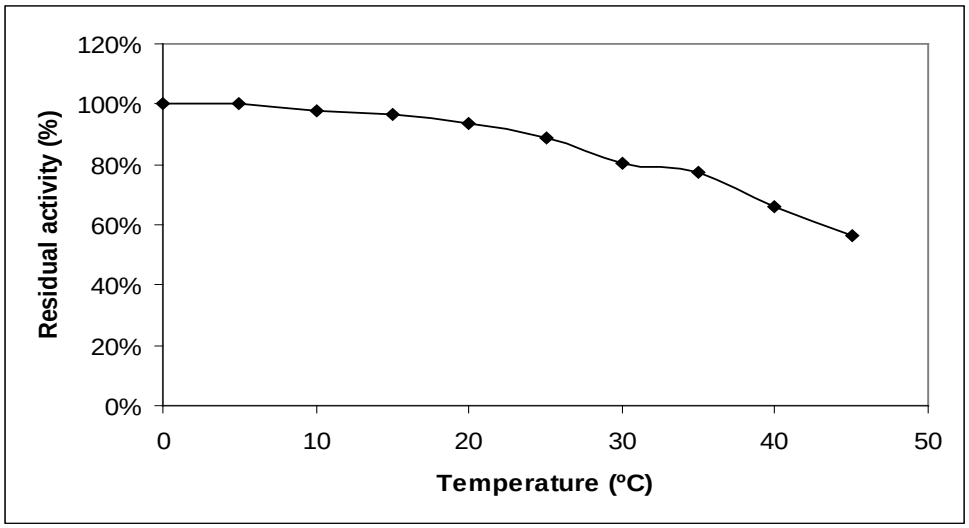


Fig.7: (A) Effect of different metal ions on the stability of pectinase produced by *Aspergillus flavus*. (B) pH stability of the pectinase produced by *Aspergillus flavus*.(C) Thermal stability of the pectinase produced by *Aspergillus flavus*.

Several studies showed that the *in vitro* fungal PG activities were inhibited by metal ions, such as Ba^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+} and Hg^{2+} (Cebanne and Doneche 2002; Mohamed *et al*., 2006; Martins *et al*., 2007).In the present study , there is a big loss in the enzyme activity especially with Ba^{2+}, Fe^{2+} and Zn^{2+} (62 % for Ba^{2+} , 68% for Zn^{2+}) ,whereas an increase in the residual activity in case of Ca^{2+} and Cu^{2+}(115% and 109%) was achieved . These results were in contrast with that obtained by Pedrolli and Carmona (2010), who reported that, Ca^{2+} failed to exhibit any significant effect on poly galactouronase activity, and a slight inhibition of purified PG activity was observed in the presence of Zn^{2+}, Ba^{2+}. Also, the purified PG activity was inhibited by Cu^{2+} and completely lost in the presence of Hg^{2+}. The cations may affect protein stability by electrostatic interaction with a negatively charged protein surface, by induction of dipoles, changes in the inter-stand dispersion forces and by their ability to modify the water structure in the vicinity of the protein and thus influence its hydration environment (Zhao, 2005 & Bauduim *et al*., 2006).

The enzyme revealed a comparable affinity to the pectin substrate as indicated by its low K_m value (0.8mg/ml)(Fig..8). As K_m is an inverse measure of the affinity between the enzyme and substrate, most fungal PGs have K_m values between 0.19–20 mg/ml (Wang and Keen ,1970; Manachini *et al*., 1987; Devies & AppuRao 1996; Rao *et al*., 1996). However, other authors have described PGs with lower substrate affinities: 2mg/ml for *Aspergillus niger* CH4 (Acuna-Arguellles *et al*., 1995) and 1.8 mg/ml for Rohapect P (Pifferi *et al*.,,1989) The enzyme exhibited a Vmax value of 2000 U/ml and similarly the endo-PG produced by *Fusarium moniliforme* revealed V_{max} 2080 nkat/mg on homogalacturonan, which is 4–folds higher than the value of the endo-PG of *Neurospora crassa* (V_{max} 500 nkat/mg) (Zhang *et al*., 1999). From a commercial point of view, the best enzymes for biotechnological applications will be those with lower K_m and high V_{max}/ specific activity (Niture ,2008).

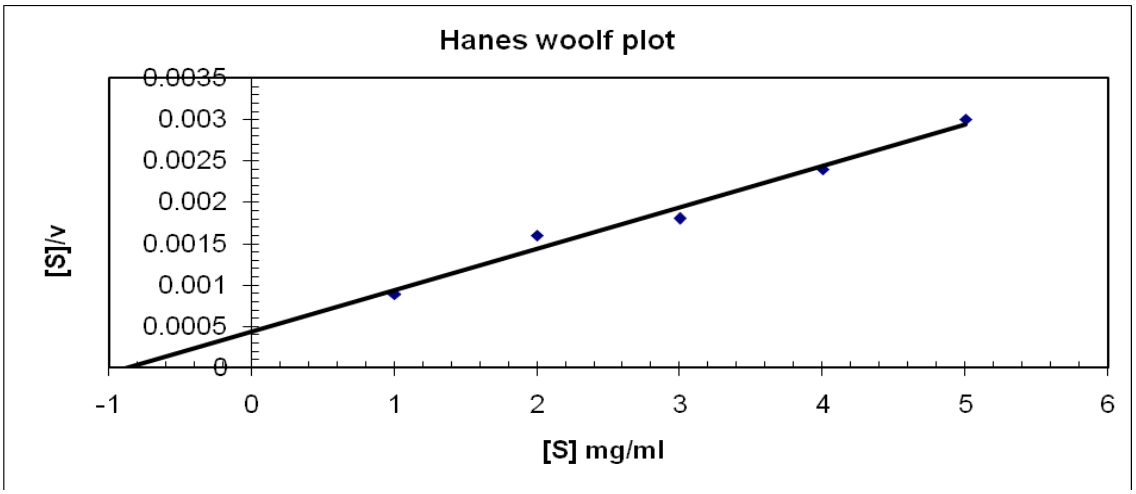


Fig.(8): Hanes woolf plot of the pectinase produced by *Aspergillus flavus*.

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تحديد عوامل مختلفة مؤثرة فى انتاج انزيم البكتينيز الخارجى بفطرالاسبرجيلس فلافس

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الملخص

تم دراسة انتاج انزيم البكتينيز بتقنية المزارع المغمورة بواسطة فطر الاسبرجيلس فلافس المعزول من ثمار التفاح والبرتقال الفاسدة .

تمت دراسة مختلف عوامل نمو الفطر المؤثرة على انتاج الانزيم باستخدام طريقة الاستجابة السطحية .

اولا تم استخدام تصميم لمستويين باسلوب "بلاكت بيورمان "لتحديد مدى تاثير سبعة عوامل وهى تركيز كبريتات الامونيوم ،تفل البرتقال، الاس الهيدروجينى ،حجم التلقيح ،معدل التهوية ،وفترة التحضين على انتاج انزيم البكتينيز وبناءا على النتائج المتحصل عليها فقد تم اختيار تركيز تفل البرتقال ،الاس الهيدروجينى وزمن التحضين لاجراء دراسات مكملة بهدف التوصل لتاثير ايجابى ومعنوى فى انتاج انزيم البولى جلاكتونيز الخارجى

ثانيا فقد تم تطبيق تصميم "بوكس بنكن "على ثلاث مستويات للحصول على افضل ظروف للتخمير للبكتينيزللحصول على اعلى نشاط وهى الاس الهيدروجينى للبيئة ٨ ،تركيز تفل البرتقال عند ٣٠جم/لتر وفترة التحضين ٤٨ ساعة ليعطى اقصى تركيز ٤٠٧٣وحدة/مل والذى يتعدى ٢.٥ ضعف الانتاج المتحصل عليه من التجربة التمهيدية

تمت تنقية الانزيم بواسطة السيفاديكس ج ١٠٠ ظهر افضل نشاط للانزيم عند اس هيدروجينى مقدارة ٧ واثبت الانزيم الثبات الحرارى العالى حيث فقد ٥٠% من نشاطة فقط عند التحضين على درجة ٥٠ م لمدة ٥٠ دقيقة وقد زاد النشاط الانزيمى فى وجود ايونات النحاس والكالسيوم حيث اعطى اعلى قيمة ٢٠٠٠ وحدة/مل واعلى قيمة ثبات تقدر بـ ٠.٨ مجم/مل

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