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MACERATION OF SOME PLANT PECTIC MATERIALS WITH *Aspergillus niger* PECTINASES PRODUCED ON ORANGE PEEL WITH SOLID-STATE FERMENTATION

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ABSTRACT

Different forms of pectinases are produced by the fungal isolates tested. *Aspergillus niger* No. 36 was found as the most active strains tested for PG, PGL, PMG, PMGL and PMGE biosynthesis. *Aspergillus achuleatus* was highest pectinases producers, but found in the second order. Enzymes productivity reached its maximum after 120, 96, 144, 120 and 144 hours of incubation for PG, PGL, PMG, PMGL and PMGE, respectively. The highest biosynthesis of pectinases were observed at 30°C and pH 4.0 in the medium containing 3% (W/V) sugar cane molasse as carbon and energy source. 0.07% and 0.105% as nitrogen content of corn steep liquor induced greatly PG, PMG, PMGE and PGL, PMGLase activities, respectively. *A. niger* cultures produced highest amount of pectinases between 0.5:1 to 0.7:1 (solid waste to liquid ratio of the fermentation media. PH 4, 7, 6, 8 and 6 were found as the optimum pH for PG, PGL, PMG, PMGL and PMGE, respectively. *A. niger* pectinases were found stable between pH 4.0 to pH 8.0. 30, 40, 50, 50 and 40°C were found as the optimum temperature for these enzymes, respectively. These enzymes were also highest stable up to 60°C, then decreased gradually. Some of metal ions such as Na^{+} induced greatly pectinases activity, but other such as Hg^{+2} reduced greatly these enzymes activities. *Aspergillus niger* pectinases system success with higher grade to hydrolysis some plant materials, this is due to its having pectate and pectin enzymes. Therefore, these enzymes systems well useful in the biotechnological process of fruit and vegetable tissues such as fruit and vegetable extraction and clarification.

Keywords: Fungi, *Aspergillus niger*, pectinase, PG, PGL, PMG, PMGL, PMGE, solid-state fermentation, maceration.

INTRODUCTION

Although the ability to degrade plant pectic substances is widespread amongst microorganisms. Degradation of the polysaccharide pectin requires the participation of different enzymes which compose the pectinolytic multi-enzymatic system. Pectinases are widely used in the industrial processing of fruits and vegetables, because of their capacity to degrade pectin and related substances. This results in greater yields of extracted juices and their clarification. Pectinases account for 10% of the total food enzyme trade, which, the two major classes of pectinase are esterases and depolymerases. Many attempts have been made to extract pectins from various sources using microbial pectinolytic enzymes. Protopectinase from various microorganisms was used to release pectin from mandarin orange and lemon peel and endo-polygalacturonase from *Kluyveromyces fragilis* to liberate pectin from carrot,

radish and citrus peel (Chesson and Codner, 1978; Panayotou *et al.*, 1993; Donaghy and Mckay, 1994; Devi and Rao, 1996; Solis *et al.*, 1997 and Shady *et al.*, 2001).

Current commercial pectic enzyme preparations are solely of fungal origin. Characteristically these exhibit high endo-polygalacturonase (endo-PG, EC 3.2.1.15) and variable pectin esterase (PE EC 3.1.1.11) activities. Fungal endo- PG's possess pH optima in the range pH 4-5 close to the natural pH of many fruits and it is this field as an aid to juice extraction and clarification. *Aspergillus* species are used for pectinase production on commercial scale, especially, *Aspergillus niger*, because it produces several enzymatic components such as endo- and exo-polygalacturonase (PG), pectin-lyase (PL), pectin esterase (PE) and oligogalacturonase (OG). Also, high levels of endo-polygalacturonase were produced by *Aureobasidium pullulans* and *Tubercularia vulgaris* on orange-peel waste and orange-pulp pellets, respectively (Chesson & Codner, 1978; Fonseca & Said, 1994 and Solis *et al.*, 1997). Also, these enzymes have been isolated from several fungal species, including the plant pathogen, *i.e.*, *Fusarium oxysporum*, *Trichoderma konningii* and *Aspergillus japonicus* (Arguelles *et al.*, 1995 and El-Sawah & Shady, 1999). Recently, *Aspergillus foetidus* pectinases are the most important enzymes used in fruit clarification, which contained several types of pectinases (Shady *et al.*, 2001).

The present study aimed to studying the maceration of some plant pectic materials with *Aspergillus niger* pectinase produced on citrus peel with solid-state fermentation. Some properties of these enzymes were also investigated.

MATERIALS AND METHODS

Microorganisms:

Aspergillus niger DSM 823, and *A. niger* No. 36 (local isolates), *Aspergillus achuleatus* DSM 63261, *Aspergillus wentii* 2001, *A. foetidus* NRRL 341, *A. terreus*, *Fusarium culmorum* NRRL 3288, *Geotrichum candidum* NRRL. Y-552 and *Trichoderma reesei* NRRL 3653, utilized in the present study were obtained from microbiol. Dept., Soil, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt. The culture was maintained on potato-dextrose agar (PDA) slants and subcultured monthly.

Media and culture conditions:

Cultivation of all fungal strains used were performed at 30°C for 168 hrs in 500 ml Erlenmeyer flasks each containing 50 ml of the cultivation medium (Tuttobello and Mill, 1961) containing 2% sucrose, 0.2% NH_4NO_3, 0.05% Na_2SO_4 and 2.5 g orange peel powder / flask. All of these ingredients were mixed and the pH was adjusted at 7.4, then sterilized at 121°C for 20 min. Spores obtained by incubation of the strains tested on PDA agar slants at 25 ± 1°C for 14 days were suspended in water at a density (0.8 – 1.2) x 10^5 / ml and used as a seed material. 1 ml of spore suspensions was used as suitable inocula. After cultivation for 7 days, the cultures were harvested

by filtration through glass wool filter and then centrifuged. The clear supernatants were used for enzyme assays.

Pectinases assays:

Polygalacturonase (PG):

Polygalacturonases are usually assayed by measuring reducing groups of liberated galacturonate monomers (Wang and Pinckard, 1971). One unit (U) of PG-ase activity is defined as that amount of enzyme under assay conditions that catalyzes the release of I μg of D–galacturonic acid / 1 hr.

Polygalacturonate lyase (PGL):

Enzyme activity of lyases (PGL) can be measured by following the increased absorption of the digest at 548 mμ (Moore and Couch, 1968). A unit of trans-eliminase (lyase) activity (PGL-ase) was arbitrarily defined as that amount of enzyme in 6 ml of reaction mixture that caused the absorption of light at 548 mμ to increase 0.005 units in 1 h.

Polymethylgalacturonase (PMG) and Polymethylgalacturonate Lyase (PMGL):

The activities of these enzymes were assayed according to the assay procedures described by Ayers *et al.* (1966). The absorbance of the solutions was determined at 515 mμ to detect PMG-ase activity and at 550 mμ for products of trans-eliminase (PMGL). One unit (U) of PMG-ase activity is defined as that amount of enzyme under assay conditions that release 1 μg of galacturonic acid / hr. One unit of PMGL-ase activity was defined as the amount of enzyme that caused the absorption of light at 550 mμ to increase 0.005 units in 1 hour.

Polymethylgalacturonate esterase (PMGE):

Pectinesterase activity (PMGE) was determined by measuring the amount of acid produced in 2 hours at 30°C according to the method described by Matta and Dimond (1963). One unit (U) of pectin methylesterase activity was defined as the activity that released 1 equivalent of carboxyl groups / 1 hours at 30°C.

Protein assay:

Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard (Fluka AG, CH – 9470 Buchs).

pH stability:

The effect of pH on enzyme stability was assayed with 20 mg enzyme protein using various buffers in the pH range 2.0 – 9.0 and incubating for 60 min at optimum temperature for each enzyme (Huang and Mahoney, 1999). The residual activity of different pectinases was assayed by the standard assay described above.

Thermal stability:

The effect of temperature on enzyme stability was assessed by incubating the enzyme in buffer at optimum pH for each enzyme at various temperatures in the range 20 – 100°C for 60 min (Huang and Mahoney, 1999). The residual enzyme activity was assayed as described above.

Effect of metal ions:

Different pectinases were incubated with 10 m mol/L metal ion solutions (Chloride salt) for 15 min at 4°C. After incubation, the remaining activity for each enzyme was assayed (Devi and Rao, 1996).

Maceration of different plant materials:

The maceration ability of *A. niger* pectinase was studied using tissue disks from yellow carrot, red apple, pear and potato (diameter of 0.5-0.6 cm, thickness of 0.2 – 0.25 cm). The disks were washed with distilled water, dried with filter paper, weighed, and incubated in the enzyme solution (25 mg protein in the universal buffers) at different pH level (4, 5, 6, 7 and 8) for 1 hour at 50C (medium temperature for different pectinases systems). The residual tissue was collected on a nylon filter and dried with filter paper, the extent of maceration was estimated from the losses in raw weight (Sapunova *et al.*, 1996).

RESULTS AND DISCUSSION

I. Evaluation of some fungal strains for their pectinases potentialities:

Screening of some fungal strains for their pectinolytic activities was carried out to select the most potent one. The results presented in Table (1) showed that *Asopergillus niger* No. 36 was the most active strain for all pectinases biosynthesis. *Aspergillus achuleatus* DSM 63261 was found in the second order, which also produced all pectinolytic enzymes with much more amount compared with other strains except of *A. niger* No. 36. Other organisms were produced enzymes, but with a moderate amounts. *Geotrichum candidum* NRRLY-552 was found as the lowest pectinases production. Thus, *Aspergillus niger* No. 36 was used for further studies. Also, it could be noticed from the results that all strains tested were produced all pectinases, which have constitutive in their nature of these enzymes biosynthesis. Therefore, these strains could be macerating or hydrolysis the pectino-wastes materials and their enzymes may be used for clarification and extraction of vegetable and fruit juices and other similar industries.

II. Enzyme production:

Time –course profile for pectinases production:

Pectinolytic activities are shown as a function of time in Table (2). The results obtained indicated that these enzymes were produced directly after cultivation and their production was increased steadily with time. This means that the tested organism were constitutive in its pectinase synthesis. Enzymes productivity reached its maximum after 120, 96, 144, 120 and 144

hours of incubation for PG, PGL, PMG, PMGL and PMGE enzymes, respectively, then these enzymes productivity decreased thereafter. El-Sawah & Shady (1999); Shady *et al.* (2001) found that pectinases production reached its maximum yield in the third day. Similar results were obtained by Panayotou *et al.* (1993) and Mansour (2001).

Table (1): Pectinolytic activities of some fungal strains.

Fungal strains	Pectinolytic activities				
	PG	PGL	PMG	PMGL	PMGE
Aspergillus niger No. 36	413.4	254.0	724.5	469.9	0.69
Aspergillus niger DSM 823.	190.6	210.1	118.9	265.3	0.54
Aspergillus Achuleatus DSM 63261	320.6	236.6	437.8	465.8	0.59
Aspergillus foetidus NRRL 341	161.3	140.5	115.7	197.6	0.18
Aspergillus wentii 2001	151.1	101.9	180.2	117.7	0.27
Aspergillus terreus	88.1	65.6	97.9	78.5	0.46
Fusarium culmorum NRRL 3288	77.3	51.3	92.3	67.7	0.23
Geotrichum candidum NRRL y-552	30.2	25.7	77.2	44.5	0.12
Trichoderma reesei NRRL 3656	61.0	30.8	66.5	55.9	0.15

PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Table (2): Evaluation of *A. niger* pectinases activities with incubation time.

Time hours	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE (ml)
24	32.3	54.6	90.0	110.3	0.22
48	100.5	103.9	190.5	200.5	0.41
72	270.9	297.1	352.2	314.9	0.62
96	348.7	498.1	520.6	420.1	0.78
120	516.0	352.4	798.0	553.6	0.91
144	478.5	311.3	867.6	493.2	0.98
168*	427.6	265.6	757.5	470.0	0.70
192	356.2	114.9	320.7	321.6	0.46
216	179.9	85.6	192.0	260.5	0.33
240	40.5	33.4	80.0	115.4	0.21

* Control.
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of incubation temperature:

Asperhillus niger in this experiments was grown at 20, 25, 30, 35, 40 and 45°C and the highest biosynthesis of pectinases were observed between 25 and 35°C. The maximum yield of all these enzymes was attained at 30°C. Above or below this optimum temperature, enzyme production was greatly decreased. Panayotou *et al.* (1993) found that 40°C was the optimum

temperature for *Aspergillus niger* polygalacturonase production. But, Mansour (2001) reported that 45°C was the optimum for pectinase production. Similar results were obtained by Shady *et al.* (2001).

Table (3): Effect of incubation temperature on enzyme production.

Temperature °C	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE (ml)
20	144.6	177.4	213.4	211.6	0.33
25	320.0	415.9	648.5	319.8	0.66
30*	520.1	502.7	873.0	559.7	0.99
35	480.9	457.4	757.4	533.8	0.77
40	261.3	313.6	413.9	339.4	0.54
45	164.5	137.2	277.0	266.5	0.29

* Control.
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of initial pH:

The effect of the pH of the growth medium on pectinase production was tested in a series of experiments, in which the pH was initially adjusted to various values from 3.0 to 8.0. The results are shown in Table (4). At pH 3.0 the enzymes activities were highest. But, these activities reached its maximum at pH 4.0. Marked repression of these enzymes synthesis occurred at higher pH values as well as at pH 2.0, but with a lesser degree. These results are in agreement with those obtained by Panayotou *et al.* (1993). Also, Mansour (2001) reported that the pH 4.0 was the best and suitable pH for pectinase biosynthesis. But, El-Sawah & Shady (1999) and Shady *et al.* (2001) found that pH 6.0 was the optimum pH for these enzymes secretion.

Table (4): Effect of initial pH on enzyme production.

Initial pH	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
2	413.0	237.5	517.9	433.7	0.06
3	715.4	567.6	914.5	885.5	1.00
4	901.5	703.5	1278.6	942.3	1.15
5	868.6	646.0	1125.3	857.6	1.13
6	614.3	569.4	991.4	773.5	1.05
7*	525.5	505.0	889.0	565.4	0.99
8	298.0	311.0	600.0	327.0	0.73

* Control.
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of different carbon sources:

Previous studies showed that *Aspergillus niger* produces all pectinases when cultivated in media containing orange peel powder and sucrose as carbon and energy source. But, of the carbon sources examined

instead of sucrose, they supported enzyme productivity (Table 5). Rates of secretion of these pectinolytic enzymes were increased greatly with the use of some carbon sources. This means that some carbon sources examined were greatly induced the biosynthesis of these enzymes. But, sugar cane molasse was the highest inducer for these enzymes biosynthesis, this may be due to its ingredients. Lactose, maltose, vinase and glucose syrup were highly stimulated these enzymes biosynthesis, but were found in the second order. Therefore, these enzymes were constitutive enzymes in their nature, but also induced ones with some substances. These results are in closed agreement with those obtained by Sapunova *et al.* (1997); El-Sawah & Shady (1999) and Mansour (2001).

Table (5): Effect of different carbon sources on enzymes production.

Carbon sources	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
Sucrose*	902	705	1280	945	1.15
Galactose	1023	905	1119	820	0.95
Glucose	1117	785	1050	978	0.83
Lactose	1320	915	1330	1111	0.97
Arabinose	735	311	640	763	0.56
Maltose	1250	668	1650	1257	1.23
Raffinose	911	960	1020	815	1.00
Fructose	863	433	934	720	0.96
Xylose	1270	715	865	600	0.81
Sorbitol	815	568	428	557	0.57
Manitol	1115	609	537	822	0.27
Soluble starch	989	515	643	653	0.48
Yellow dextrin	1139	739	735	609	0.32
Sugar cane molasse	1669	1169	1898	1463	1.49
Vinase	950	935	1650	1150	1.11
Glucose syrup	1511	988	1778	1390	1.27

*** Control.**
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of different concentration of sugar cane molasse:

The effect of sugar cane molasse on pectinases production was also investigated (Table 6). The enzyme productivity (PGL, PMG, PMGL and PMGE) consistently increased with the increasing of sugar cane molasse concentration up to 3% (W/V), thereafter enzymes biosynthesis decreased sharply. But, PGase activity reached its maximum at 2% conc. This means that, at this sugar cane molasse concentration, enzymes productivity enhanced greatly with its ingredients, and above or below, enzymes biosynthesis repressed sharply with feed back inhibition. These observation are similar to those obtained by Mansour (2001).

Table (6): Effect of different concentrations of sugar cane molasse.

Sugarcane (Comc. %)	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
1	698	640	758	857	0.97
2*	1670	1169	1898	1463	1.49
3	1400	1398	2105	1689	1.87
4	1036	915	1783	1569	1.22
5	505	561	643	687	0.86

*** Control.**
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of nitrogen sources:

Many authors described stimulatory effects of ammonium and nitrate sources of nitrogen on synthesis of pectinases (Sapunova *et al.*, 1997) by fungi. Many of the nitrogen sources (inorganic and organic) in this work stimulated with different extents the synthesis of either pectin enzymes or pectate ones by *Aspergillus niger* (Table 7). Corn steep liquor (CSL) was highly supported the biosynthesis of these enzymes, which gave highest yield of enzymes. Other sources such as asparagine, ammonium tartarate & sulphate, potassium nitrate and casein hydrolysate were also greatly stimulated the enzymes formation, but found in second order. In the other side, tryptone, beef extract inhibited the enzyme synthesis. Sapunova *et al.* (1997) reported that ammonium salts of sulphoric and hydrochloric acids were the best sources for the formation of pectinases by *Aspergillus alliaceus*. Similar results were reported by Mansour (2001) and Shady *et al.* (2001).

Effect of different concentrations of CSL:

The dependence of pectinases synthesis by *A. niger* on the concentration of CSL is very necessary, which it is one of the most suitable sources of nitrogen induced these enzymes formation.. At lower and higher concentration of this compound, the fungus produced significant amount of these enzymes (Table 8). However, at 0.07% as nitrogen content, the fungus produced highest amount of PG, pMG and PMGE ases, which the enzymes biosynthesis were induced greatly But, productivity of PGL and PMGL ases reached its maximum at 0.105% as nitrogen content of CSL. Generally, these results indicated that CSL is highly induced *Aspergillus niger* pectinases biosynthesis. Sapunova *et al.* (1997) reported similar results.

Table (7): Effect of different nitrogen sources on enzyme production.

Nitrogen source	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
Ammonium nitrate*	1670	1398	2110	1670	1.87
Ammonium sulphate	1885	1691	2151	1768	1.36
Ammonium chloride	1515	1415	1387	1121	1.05
Ammonium dihydrogen phosphate	1647	1323	1126	993	0.95
Ammonium oxalate	1515	1111	1743	1651	1.47
Ammonium tartarate	1721	1765	1981	1711	1.73
Potassium nitrate	1867	1497	1321	1493	1.17
Sodium nitrate	1351	905	799	1115	0.82
Yeast extract	1211	996	1221	1257	0.93
Beef extract	893	66	683	738	0.55
Casein hydrolysate	1856	397	1151	1495	1.15
Peptone	1173	758	982	1320	0.97
Tryptone	985	463	853	1119	0.83
Asparagine	1893	1514	2214	2253	2.15
Corn steep liquor	2140	1956	2543	2323	2.43
Zea glutein	1580	1117	997	1267	1.57

* Control.
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Table (8): Effect of different concentration of CSL on enzyme production.

CSL conc. N%	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
0.035	1795	1643	2011	1950	1.19
0.070*	2140	1956	2543	2323	2.43
0.105	2017	2005	2493	2605	2.36
0.140	1656	1781	1635	2051	1.91
0.175	1421	1514	1267	1641	1.15

* Control.
PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.
PMGE equivalent NaOH (0.01 N)/h.

Effect of substrate concentration:

The effect of varying substrate concentration (orange peel powder) on *Aspergillus niger* pectinases production was investigated (Table 9). For lower substrate concentrations (up to 0.4:1, solid to liquid ratio), *Aspergillus niger* cultures produced highest amount of pectinases. Highest concentrations of solidfied substrate give similar results. However at medium concentration (0.5:1 and 0.7:1), much more amount of enzymes were obtained. This means that, this substrate concentration is the optimal and suitable concentration for the highest stimulating enzymes biosynthesis. Also, indicated that the synthesis of *A. niger* pectinases is constitutive in their nature, but depended strongly in its induction on the substrate concentration. Therefore the production of these enzymes induced greatly at 0.5 to 0.7 : 1

as the best concentration of substrate. Similar observation were obtained by Schwan & Rose (1994); Arguelles *et al.* (1995); Sapunova *et al.* (1997) and Mansour (2001).

III. Enzyme properties:

Effect of pH on enzyme activity and stability:

The effect of pH on enzyme activities is shown in Fig. (1). The maximum enzymes activities was observed at pH's 4.0, 7.0, 5.0, 6.0, 8.0 and 6.0 for PG, PGL, PMG, PMGL and PMGE ase, respectively. Results are shown in Fig. (2) indicated that, maximal residual PGase activity was obtained between pH 3.0 and 6.0. But, the maximum residual enzyme activity of PGL, PMG and PMGE ase was observed between pH 5.0 and 7.0. However, the maximum residual PMGL ase activity was present between pH 7.0 and 9.0. Outside, this pH's ranges, sharp decreasing in enzymes activities were found. Also, the results indicated that pectinases were most stable at these pH's ranges. However, the pH activity profile varied according to the type of enzyme. This may reflect differences in ionic strength to which the enzyme is very sensitive. Also, it is clear that the fall-off in activity of each enzyme on each side of the maximum is due to the effect of pH on the ionization of critical groups involved in substrate binding and/or catalysis, rather than to stability. These results and observations are similar to those obtained by Arguelles *et al.* (1995), Sapunova *et al.* (1995), Devi & Rao (1996), Astapovich and Ryabaya (1997), Huang and Mahoney (1999) and Shady *et al.* (2001).

Table (9): Effect of substrate concentration (orange peel powder) on enzyme production.

Substrate Conc. Solid: Liquid ratio	Pectinase activities				
	PG	PGL	PMG	PMGL	PMGE
0.2 : 1	867	922	1259	1245	1.17
0.3 : 1	1170	1358	1811	1698	1.57
0.4 : 1	1893	1730	2117	2137	2.16
0.5 : 1	2140	2005	2543	2605	2.43
0.6 : 1	1674	2215	2657	2837	2.57
0.7 : 1	1223	1973	2923	3105	1.97
0.8 : 1	930	1489	2430	2517	1.05
0.9 : 1	650	905	1915	1927	0.86
1 : 1	440	611	1227	1397	0.43

*** Control.**

PG, PGL, PME and PMGL = units/g dry weight of the substrate / h.

PMGE equivalent NaOH (0.01 N)/h.

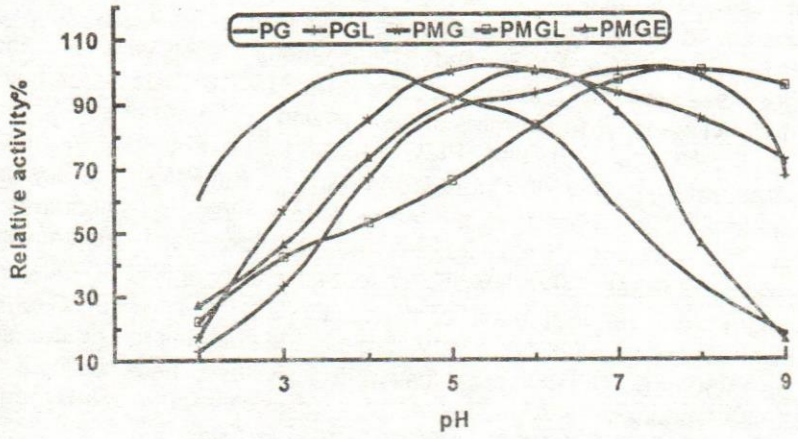

Fig. 1: Effect of pH on *A. niger* pectinases activities

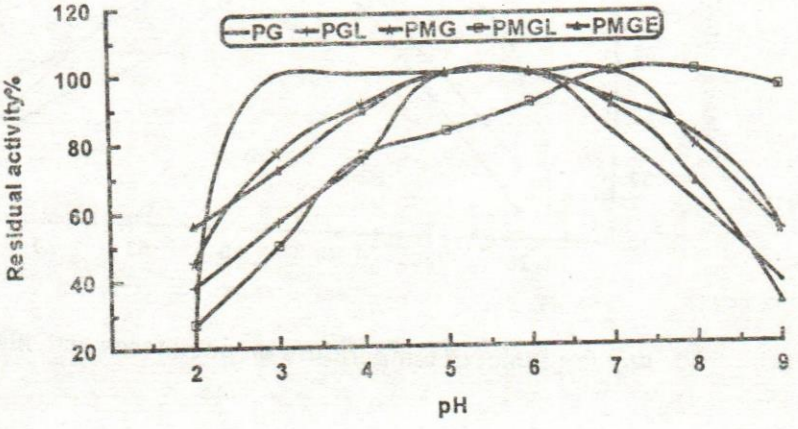

Fig. (2): pH stability of *A. niger* pectinases

Effect of incubation temperature on enzymes activities and stability:

The effect of temperature on enzyme activities is shown in Fig. (3). It could be observed from the results that temperature was affected greatly on all pectinases activity, but with different extent. Also, the results showed that, maximum enzymes activities were observed at 30, 40, 50, 50 and 40°C for PG, PGL, PMG, PMGL and PMGE ases, respectively. An increase or decrease in the reaction temperature by 10°C decreased sharply the hydrolytic and lyase activities of pectate and pectin enzymes.

PGase, PGL ase, PMG ase and PMGE ase were most stable at temperature below 50°C for 60 min (Fig. 4). But PMGL ase was most stable up to 60°C. Thereafter, the temperature degree had deleterious effects on enzyme proteins as well as enzyme activities were present with different extents. PGase and PMGE ase lost all activity at 90°C. PGLase, PMGase and PMGL ase lost most of all activities after boiling at 90°C for 60 min. In contrast, pectinases were very stable in the cold, but raising the temperature up to 50 or 60°C, led to rapid loss of its activities. These results are similar to those obtained by Arguelles *et al.* (1995); Astapovich & Ryabaya (1997); Huang & Mahoney (1999) and Shady *et al.* (2001).

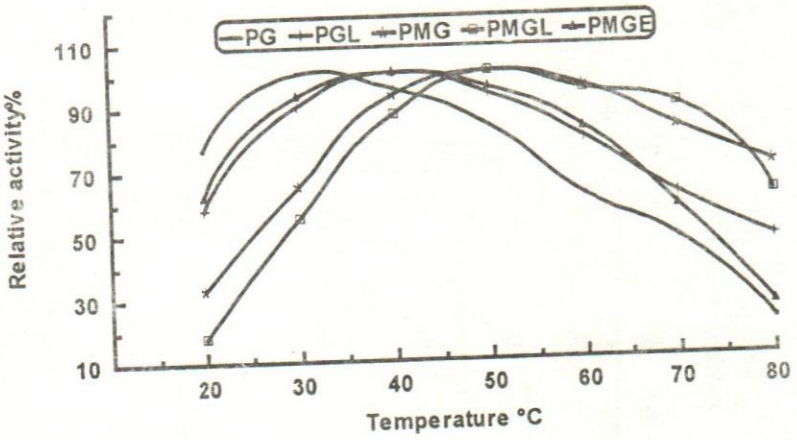

Fig. (3): Effect of temperature on pectinases activities

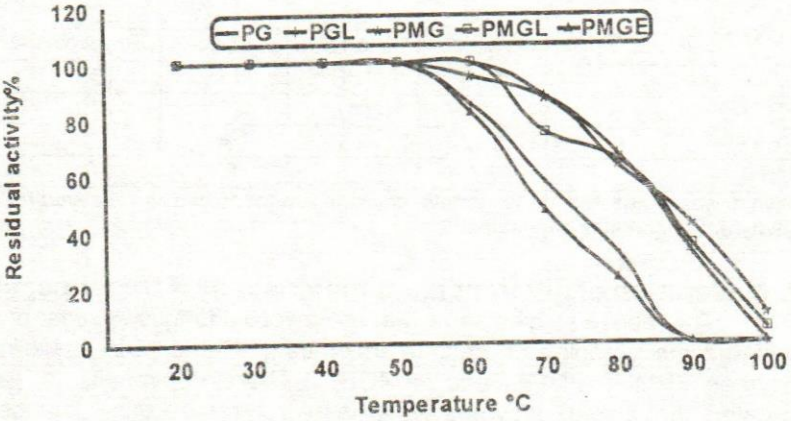

Fig. (4): Thermal stability of pectinases.

Effect of metal ions:

The sensitivity of pectate and pectin enzymes activities toward metal ions is shown in Table (10). Generally, these enzymes were sensitive toward most of the metal ions. Hg^{+2} ions was found as the most inhibited all pectinases activities. PMG ase was more sensitive than other enzymes toward Hg^{+2} ions inhibition. Na^{+} was found as the strong activated and induced pectate and pectin enzymes activities. Other metal ions such as Cu^{+2}, Zn^{+2} and Ca^{+2} induced PGase activity. K^{+2} also induced PGL and PMG ases activities. These results are also indicated that these enzymes were expressed as metalo-activated enzymes in some cases. Similar observations were reported by Devi & Rao (1996) and Huang & Mahoney (1999).

Table (10): Sensitivity of pectinases toward metal ions.

Metal ion	Relative maximum enzymes activities				
	PG	PGL	PMG	PMGL	PMGE
Control (without salt)	100	100	100	100	100
Ca^{+2}	105	100	103	98	107
Co^{+2}	11	95	60	65	93
Cu^{+2}	126	91	82	87	66
Fe^{+2}	92	88	91	61	75
Mg^{+2}	103	95	107	103	91
Hg^{+2}	73	41	14	23	33
K^{+}	109	106	115	103	87
Na^{+}	134	117	123	139	104
Zn^{2}	112	93	96	98	70
Mn^{+2}	87	77	83	86	76

Enzyme was incubated in 10 m mol/L chloride salt for 15 min and assayed for remaining activity under optimum conditions.

IV. Maceration of different plant materials by *A. niger* pectinases:

The above studies show that the mycelial fungus *A. niger* produces a multi-enzyme complex of pectate enzymes ((PG and PGL) as well as pectin enzymes (PMG, PMGL and PMGE). Therefore, results in Table (11) indicated that these enzymes systems hydrolyzed pectic substances by two mechanisms (hydrolases, PG and PMG) and lyases or transeleminases (PGL and PMGL). Also, the results revealed that the macerating activity of the constitutively pectinases systems distructed all the disks of substrates, but with differ extent (Table 11). At pH 4 (the optimum pH of PG ase), the hydrolysis or macerating activity was little than those obtained at other pH's values. Highest maceration activity was observed at pH 8.0, except, the highly maceration extent of potato was observed at pH 6.0. These results indicated that, when the pH of the reaction mixture go to neutral or alkaline side, the degree of hydrolysis was increased generally. Also, the results show that *A. niger*, which produced pectate and pectin enzymes which had different pH optimum may be important and development of biotechnological process of fruits and vegetables. Sapunova *et al.* (1996) and El-Sawah & Shady (1999) showed similar results and observations.

Table (11): Maceration of different plant materials with pectinases.

Plant materials	Maceration as % weight loss				
	PH 4	PH 5	PH 6	PH 7	PH 8
Yellow carrot	16	22	27	30	32
Red Apple	23	29	31	35	38
Pear	21	26	29	30	31
Potato	27	33	39	37	35

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تحليل بعض المواد البكتينية بإستخدام إنزيمات البكتينيز من فطر الأسبرجلس نيجر المنتجة على قشر البرتقال بطريقة التخمير فى وسط صلب solid-state fermentation

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نظراً للعديد من الإستخدامات البيوتكنولوجية الهامة والحديثة لإنزيمات البكتينيز بصفة عامة والفطرية منها بصفة خاصة نظراً لإنتاج الفطريات للعديد من إنزيمات البكتينيز والتى تحلل البكتين سواء المنخفضة فى محتواه من مجموعات الميثوكسيل أو المرتفع منها لها القدرة أيضاً على تحليل مشتقات المواد البكتينية مما يجعلها ذات أهمية خاصة فى ترويق عصائر الفاكهة والخضروات وإستخلاص العصائر وكذلك صناعات عديدة منها صناعة المنسوجات ، ولذلك فقد تركزت الدراسة فى هذا البحث فى إنتاج هذه الإنزيمات الهامة غذائياً وبيوتكنولوجياً من فطر الأسبرجلس نيجر وكذلك إستخدامه فى تحليل بعض المواد النباتية البكتينية مثل الجزر والتفاح الأحمر والكمثرى والبطاطس ، وقد إنتهت الدراسة للنتائج التالية :

١. إستطاعت الفطريات المستخدمة فى هذه الدراسة لإنتاج العديد من إنزيمات البكتينيز وكان أفضلها فى الإنتاج هو فطر الأسبرجلس نيجر حيث إستطاع إنتاج إنزيمات تحليل البكتين (PMG و PMGL و PMGE) وإنزيمات تحليل البكتات وهى (PG و PGL) وقد وجد فطر الأسبرجلس أكيوليتس فى المرتبة الثانية من حيث كفاءة الإنتاج .

٢. وصل أعلى معدل لإنتاج البكتينيز من فطر الأسبرجلس نيجر بعد ١٢٠ و ٩٦ و ١٤٤ و ١٢٠ و ١٤٤ ساعة تحضين لإنتاج كل من PG و PGL و PMG و PMGL و PMGE ، على الترتيب .

٣. كانت ٣٠°م و pH ٤ هى أحسن درجة حرارة تحضين ودرجة تركيز أيون الأيدروجين لإنتاج هذه الإنزيمات فى بيئة تحتوى على ٣% (وزن / حجم) من مولاس قصب السكر كمصدر للكربون والطاقة .

٤. كان لإستخدام ٠,٠٧% و ٠,١٠٥% كنسبة نيتروجين من منقوع الذرة فى بيئة الإنتاج أثر حثى جيد فى إنتاج هذه الإنزيمات ، على الترتيب .

٥. بإستخدام ٠,٥ : ١ و ٠,٧ : ١ من قشر البرتقال كمادة مصلبة فى البيئة إلى حجم محلول البيئة أدى إلى إرتفاع معدل إنتاج هذه الإنزيمات .

٦. كانت درجات pH ٤ و ٧ و ٦ و ٨ و ٦ هى المثلى لنشاط إنزيمات PG و PGL و PMG و PMGL و PMGE ، على الترتيب ، وقد أظهرت هذه الإنزيمات ثبات عالى تجاه درجات الـ pH فى المدى من ٤ – ٨ على حسب نوع الإنزيم .

٧. كانت درجة الحرارة ٣٠ و ٤٠ و ٥٠ و ٥٠ و ٤٠ هى المثلى لنشاط هذه الإنزيمات ، على الترتيب ، وقد أظهرت هذه الإنزيمات ثبات شبه كامل حتى ٦٠°م ثم تناقص نشاط هذه الإنزيمات تدريجياً .

٨. كان لتواجد بعض المعادن فى مخلوط التفاعل الإنزيمى مثل كلوريد الصوديوم أثر حثى جيد لنشاط هذه الإنزيمات فى حين كان لكلوريد الزئبقيق على الجانب الآخر أثر تثبيطى واضح لنشاط هذه الإنزيمات .

٩. نجح مخلوط إنزيمات البكتينيز المستخلص من فطر الأسبرجلس نيجر فى تحليل بعض المواد النباتية البكتينية كخضروات وفاكهة بدرجة كفاءة عالية وذلك نظراً لإمتلاكه إنزيمات تحليل البكتين التى تحلل المواد البكتينية المحتوية على مجموعات الميثوكسيل والبكتات مما يعنى إمكانية إستخدام هذا المخلوط الإنزيمى فى صناعات بيوتكنولوجية هامة فى مجال الصناعات القائمة على الفاكهة والخضروات المحتوية على المواد البكتينية مثل إستخلاص العصائر وترويقها وغير ذلك .