6. Summary

Pectins are the most structurally complex polysaccharides; mainly occur in plant cell walls; especially in the middle lamella of the primary cell wall of higher plants. Pectins contribute to many biological and physiological functions; especially during abscission and growth as they determine the size, shape of cells and consequently the integrity and rigidity of plant tissues.

Pectins are subjected to many pectinolytic enzymes of plant and microbial origin. These enzymes are composed of large number of different enzymes collectively called pectinases.

One hundred isolates of different bacteria and fungi had a pectinolytic activity. These isolates were used as representatives of the microbial flora of rotten fruits, vegetables and seeds. Pectinolytic activity was followed up resulted in selection of 21 isolates with high hydrolyses activity.

Characterization of pectinases secreted by 21 isolates was carried out through determination of the following enzymes: polygalacturonase (PG); polygalacturonase lyases (PGL); polymethylgalacturonase (PMG); polymethylgalacturonase lyase (PMGL) and pectinoesterase (PE). The obtained results showed that isolates No. 100, 98, and 94 were higher for both PG and PMG producers while isolates No. 97, 60 and 19, 38 were best producers of PG and PMG ;respectively.

These isolates were species of *Aspergillus* and were identified as follows:

- Three isolates (No. 19, 97 and 98) belong to *Aspergillus fumigatus.*
- Three isolates (No. 38, 94 and 100) belong to *Aspergillus niger.*
- One isolate (No. 60) belongs to *Aspergillus carbonarius.*

In this concern, different factors affecting production of PG and PMG were studied including: carbon, nitrogen sources, and initial pH of the enzyme production medium, agitation rate, and fermentation period and temperature.

In case of the effect of carbon source on production, data showed that soluble starch and sucrose resulted in highest production of PG in all the tested isolates while high PMG production was attained upon utilizing carboxymethylcellulose, and mixture of pectin and oxalic acid by *Asp. fumigatus* No.98.

The source of nitrogen incorporated in enzyme production medium greatly affected the production of both PG and PMG. The obtained results showed that gelatin, yeast extract, ammonium nitrate, ammonium sulphate and peptone promoted the highest production of PG by certain isolates, while alanine, glycine, sodium nitrate and ammonium sulphate were fairly good in production of PMG by certain isolates.

Concerning the influence of initial pH of the enzyme production medium, the obtained results showed that pH values of 4.0 and 7.0 seemed to be optimal for production of both PG and PMG enzymes; respectively .

In case of agitation, the data showed that there is a direct relation between the rate of agitation and the production of both enzymes. As productivity of both enzymes were increased as the rate of agitation reached 150rpm.

As regards the effect of fermentation period on the production of the two enzymes by *Asp. fumigatus* No.98, it was found that optimum production of PG was between 4-8 days while that of PMG was at 7 days.

The effect of fermentation temperature on the production of PG and PMG by the selected isolates was investigated. The highest level of PG was achieved at 30^{o}C in case of *Asp. fumigatus* No. 97 and No. 98 and *Asp. niger* No. 100. While other isolates of PG were at 25^{o}C except isolate No.100. However, PMG produced by *Asp. niger* isolates No.38 and 94 showed high levels at 25^{o}C. While *Asp. fumigatus* No. 19 and No. 98 produced highest levels at 35^{o}C.on the other hand production by *Asp. niger* No. 100 was optimum at 30^{o}C.

Some factors affecting the activity of extracellular crude PG and PMG enzymes including pH, temperature and the presence of cations have been studied:

On studying the influence of various pH values of the reaction mixture on the enzyme activity, the obtained results showed that the optimum pH of the activity of PG produced by *Asp. fumigatus* No.98 and *Asp. carbonarius* No.60 was 4.6, while the optimum pH of produced by *Asp. niger* No.100 was 7.0. A pH range of 4-8 was optimal for the activity of PMG produced by *Asp. fumigatus* No.98. However, a pH 6.6 was optimal for the activity of PMG produced by *Asp. niger* No. 94 and No.100 at pH 6.6.

Incubation temperature of reaction mixture affected greatly PG and PMG activities. It was found that a temperature of 45 ^{o}C was optimum for the activity of PG produced by *Asp. fumigatus* No.98 while the optimum temperature for the activity of PG produced by *Asp. carbonarius* No.60 and *Asp. niger* No. 100 was at 50 ^{o}C and 55^{o}C; respectively. However 45^{o}C was the optimum temperature for the activity of PMG produced by *Asp. fumigatus* No.98 and *Asp. niger* No. 100. While in case of PMG produced by *Asp. niger* No. 94 was 50^{o}C.

On studying the influence of different cations on the activities of PG and PMG crude enzymes, the obtained results showed that Fe^{+2} and Na^{+} ions caused activation in both enzymes in all the tested strains, while Ca^{+2}, Cu^{+2}, Hg^{+2}, Zn^{+2} had an inhibitory effect in case of all tested enzyme preparations, however Mg^{+2}, Mn^{+2}, Ni^{+2}, Al^{+3}, Li^{+} and Cr^{+2} showed activation for some isolates and inhibition for others.

The obtained data showed that *Asp. fumigatus* isolate No. 98 was the best isolate for the production of polygalacturonases so it was selected for further studies.

This isolate produced two exo-polygalacturonases (PGI, PGII).purification of these two enzymes was carried out using chromatography on Sephadex G-100 and diethyl amino ethyl cellulose (DEAE)-cellulose. The exo-PGI and exo-PGII were purified 19.3 and 21.5 folds; respectively. The two enzymes have the same molecular weight of 78.75 K.D. They have similar bimodal pH profiles with two pH optima at 5.6 and 8.3. Their optimum temperatures were 45°C and 55°C; respectively. Complete inactivation of the two enzymes was observed with $(NH_4)^{+}$, Zn^{2+}, Hg^{2+}, Cu^{2+}, Cr^{2+}, Ca^{2+} and Al^{3+}. Variable degrees of inactivation were observed with other metallic ions: Ni^{+}, Ba^{2+}, Mg^{2+}, Fe^{3+}, Na^{+}, Li^{+}, K^{+} and Mn^{2+}. On the other hand these enzymes were stimulated by Fe^{2+}. PG-I and PG-II hydrolyzed polygalacturonic acid and to a lesser extent citrus pectin. The thin layer chromatography (TLC) of the reaction products supported the exo-activity for PGI and PGII since galacturonic acid was identified as sole product of polygalacturonic acid breakdown by the enzyme.

REFRENCES