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Enzymatic Hydrolysis of Pectin and Some of Its Agro-industrial Applications.

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ABSTRACT

Polygalacturonase produced from *Streptomyces* sp GHB5 used for hydrolysis of banana fiber, clarification of orange and apple juice and in hydrolysis of agriculture wastes (orange peel, lemon peel, corn cob and wheat straw). *Streptomyces* sp. GHB5 isolated from Egyptian soil, screened for polygalacturonase production and molecular identified as *Streptomyces* sp. GHB5. Scanning electron micrographs stated separation of banana fiber cells after enzymatic treatment and reducing sugars were increased by increasing enzymatic treatment period to reach 95.68 µmol/ml after 60 minuts. In case of apple juice results showed increase in produced juice to 7.5 and 18.3 ml by using 10 and 20 ml of enzyme added to apple pieces respectively, this indicates juice volume increased by increasing treated enzyme volume. Orange juice become clearer by polygalacturonase treatment as compared to control. By enzymatic hydrolysis of agriculture wastes, orange peel was found to have highest reducing sugars followed by lemon peel 445 and 325 µmol/ml reducing sugars respectively.

Key words: *Streptomyces* sp. GHB5, polygalacturonase, applications.

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INTRODUCTION

Pectin is a major component found in plant cell walls; it present in the middle lamella, which is the 1st part of the wall that is formed during cytokinesis, following cell division. Hence pectinases are cell wall degrading enzymes that permits apple juice release from the apple fruit by making degradation to the pectin which are a large molecules of polysaccharide found in plant cell walls [1]. By destroying the cell walls, This will increase the quantity of produced juice (will increase the yield), lowers the viscosity of the juice (become more runny), reduces the cloudiness of the juice, resulted from suspended particales of cell wall by pectin degradation that found in cell walls of plants [1, 2].

Orange juice has a high pectin amount that responsible for turbidity and viscosity increase of juice. This leads to extraction and filtration difficulty to be done. Because pectin particle do not allow juice passing by blocking filtration machine. Addition of pectinase (specifically polygalacturonase) enhance fruit juice extraction via an easier process by decreasing viscosity of juice and destroying the gel structure, by this improved juice concentration capability. Clarification enzymatically may increase percent of yields by 90% or more as compared to juicing via conventional machines, in addition the organoleptic (color, flavor) and nutritional properties and technological efficiency (ease of filtering) were improved [3, 4].

Lignocellulose is a big source of biomass, although, the high efficiency of converting lignocellulose to fermentable sugars needs a big amount of enzyme [5]. Biomass enzymatic hydrolysis is more effective and go under ambient conditions without production of any toxic waste products and promised to get high yields of product [6]. In bio-products manufacturing, reduction in the costs of the used hydrolyzing enzymes of the raw material is a key factor and improve their efficiency to make the process economically feasible [5, 7]. On the same time bio-products cost decrease can be processed via efficient technologies for hydrolysis that includes the use of better "enzyme cocktails" and condition for hydrolysis [8].

Microorganisms as sources for industrial enzymes are favored rather than plants and animals, this is because of their low manufacturing cost, more controllability, predictability of enzyme contents and cheap raw materials can be found easily for its cultivation and enhanced growth rate. On the same time microorganisms are safe and an efficient host where enzymes may be expressed via cloning and expressing genes from another organism. Streptomycetes are known as plant debris active degraders therefor it had the ability for extracellular enzymes production as pectinase, cellulase and xylanase. A range of eighty percent of the produced metabolites by streptomycetes come from *streptomyces* sp. [9].

Application of pectinases in biotechnological process had increased significantly in latest years. In food and its related industries, its main import role was attached to the usage of enzymes in upgrading high quality, increasing amounts of yields, make stabilization to the product, and responsible for enhancement of flavor and by product usage [10, 11]. Alkaline pectinases represented as important industrial enzymes of great importance within the modern biotechnological field with a wide-range of applications in textile processing, degumming of plant bast fibers, treatment of pectic wastewaters, paper making, animal feed, oil extraction, purification of plant fibers and coffee and tea fermentations. [12, 11].
This study intends to explore the potential of new alkaline polygalacturonase produced from *Streptomyces* sp. GHB5 for industrial applications like clarification of orange and apple fruits, banana fiber processing and scarification of agriculture wastes.

Materials and Methods

Organism:

Streptomyces sp. GHB5 isolated from Egyptian soil, screened for polygalacturonase in previous research [13] and molecular identified by 16S rRNA as *Streptomyces* sp. GHB5. *Streptomyces* sp. GHB5 was maintained on starch nitrate slants, used to prepare spore suspensions.

Cultivation media:

Lemon peel nitrate media containing (g/l): Lemon peel, 15g; KNO_3 2.8g; K_2HPO_4 (anhydrous), 1.0g; $MgSO_4$. $7H_2O$, 0.5g; NaCl, 0.5g; $CaCO_3$ 3.0g; $FeSO_4$. $7H_2O$, 0.01g; Distilled water 1000 ml, incubated on a rotary

shaker (NEW BRUNSWICK SCIENTIFIC, EDISON, N., J., USA) (180 r.p.m) at 30 ^{0}C for six days. After incubation process, the supernatant was separated via filtration process, the filtrate was used as crude enzyme source.

Analytical methods

Reducing sugar estimation by Dinitro Salicylic Acid method [14] against glucose standard.

Enzyme application in Banana fiber hydrolysis

Banana fibers were collected by hand stripping, dried and then treated with polygalacturonase enzyme obtained from the novel identified strain *Streptomyces SP.* GHB5 for 60 min at 45°C and 150 rpm in a rotary shaker. The reducing sugar level was checked at regular intervals [14]. Finally, scanning electron micrographs of both treated and untreated fibers were taken to observe the effect of enzyme treatment [15].

Enzymatic clarification of Orange Juice

Clarification of orange juice was carried by the following treatment [16**,** 17]. The mature orange fruits were obtained from market in Egypt. The orange bagasse were sorted, washed and peeled. The juice was extracted using a domestic juice extractor. The extracted juice was pasteurized at 85°C for 3 minutes to inactivate the natural fruit enzymes or microbes present and then cooled down to 40°C before the addition of polygalacturonase enzyme. The samples were incubated at 45°C for 1 hour. After incubation, the samples were filtered to filtrate the juice from the remaining that settle down.

Enzymatic clarification of apple Juice

Chop the apples into cubes that are roughly 5 mm on a side (Use care with the knife!). It is important to chop the apple into very small pieces added surface area helps the enzyme break down the pectin in the plant cell walls, releasing more juice. Weigh equal amounts of chopped yellow apple (about 25g) into different four beakers. Add 10 ml and 20 ml of polygalacturonase enzyme obtained from novel identified strain *Streptomyces SP.* GHB5 to two different beakers and equivalent volume of water into the other two beakers. Stir the chopped apple pieces in each beaker with a separate plastic spoon. Be sure to wet all of the pieces. Cover the beakers with plastic wrap. Put beakers into water bath at 45 ^{0}C for 1 hrs. The water should come up to the level of the chopped apples, the beakers float and tip over. The juice from the preparation was filtered using a Whatmanns No.1 filter paper in funnels into a measuring cylinder. The cylinders were appropriately labeled and the amount of juice in each cylinder was measured at regular intervals of 5 mint intervals for 30 min.

Enzymatic hydrolysis of agriculture waste

Orange peel (Op) and lemon peels (Lp) were selected for hydrolysis, obtained from local market in Egypt, cut into small pieces (~2 mm), washed with tap water several times in order to remove all water soluble compound then dried and grinded. Corn cobs (Cc) and wheat straw (Ws) were collected from Giza farm, allowed to dry in the oven at 70 ^{0}C for 72 h. These samples ground well before use. Raw materials (Op, Lp, Cc and Ws) were pretreated by boiling (cooking) with distilled water using a solid to liquid ratio of 5:100 (w/v) in the 250-ml Erlenmeyer flask, and cooked for 1 h. After cooling, the residues were washed with distilled water, subsequently, followed by centrifugation (10,000 x g, for 15 min). The residues were dried in an oven at 50 ºC for 24 hr.

The mixture of agriculture waste and polygalacturonase enzyme were incubated at 45 ºC in a shaker incubator for various incubation times (half-24 hrs.). At indicated intervals of time, samples were withdrawn and heated in a boiling water bath for 5 min to inactivate the enzymes. Then centrifuged for 15 min at 10,000 x *g*, the supernatant was used to analysis of total reducing sugars by [14].

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RESULTS AND DISSCUSION

Enzymatic application in Banana fiber hydrolysis

The first application of enzymatic hydrolysis of pectin that present in banana fiber. It was interesting to notice that the level of released reducing sugar increased gradually to reach 95.68 µmol/ml, this an indication of the effectiveness of the used polygalacturonase produced by *Streptomyces* sp. GHB5 in hydrolysis of banana fiber (Figure 1). In the same time, scanning electron micrographs were taken to show the effect of enzyme in hydrolysis of the fibers (Figure 2). It was noticed from the photographs that the cells were separated after enzymatic treatment this due to hydrolysis of pectin.

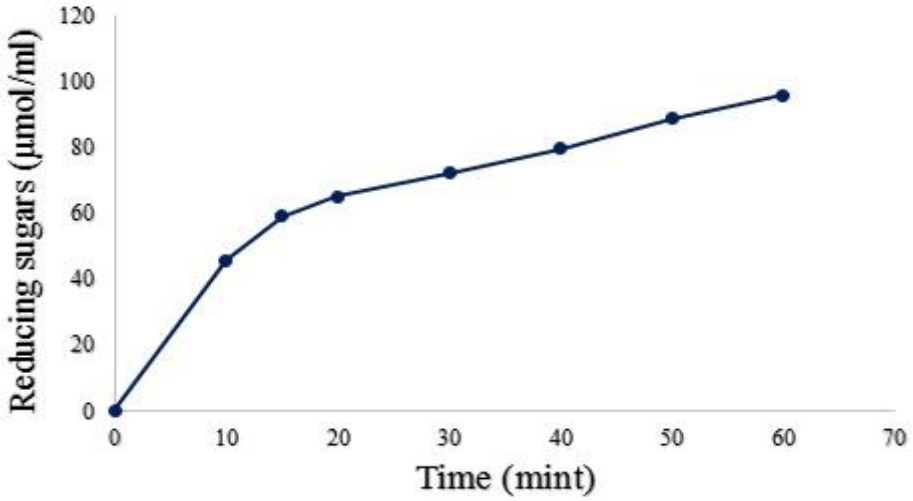

Figure 1: The reducing sugar level of the fiber treatment with polygalacturonase produced by *Streptomyces* sp. GHB5 for 60 min.

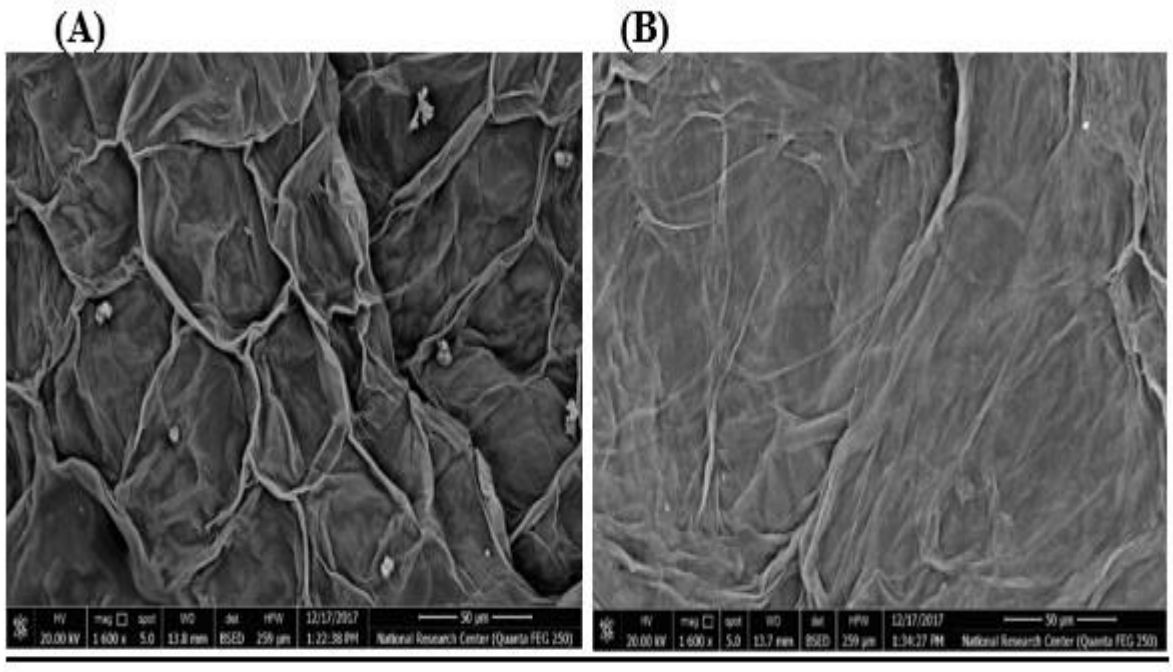

Figure 2: Scanning electron micrographs of banana fibers. (A) Control before treatment with enzyme, (B) after one hour of treatment with polygalacturonase from *Streptomyces* sp. GHB5.

Banana fibers are light weight, soft fibers. After fruit harvesting, banana stem discarded as a waste substance. Banana fiber may be utilized for the manufacturing of textiles, handicrafts or banana paper. Banana

paper had several advantages over conventional paper as light weight, water repulsion capability, luster, etc. When fibers treated with pectinase, the middle lamella destroyed and this leads to the separation of fibers. Pectinase manufactured from *Bacillus pumilus* dcsr1 had been used for the treatment of dried and decorticated ramie fibers [18]. In our study the treatment of banana fibers with polygalacturonase obtained from *Streptomyces* sp. GHB5 was found to be successful. Examination of reducing sugar levels and also scanning electron microscopic studies revealed the effectiveness of the process (Fig. 1, 2). Fiber cells had been intact within the control without any treatment although, the cells had been separated in the treated samples. Banana fiber treatment was reported with pectinase enzyme from *Streptomyces lydicus* and he found that in the treated samples banana fiber cells were separated [15].

Enzymatic clarification of orange juice

The second enzymatic hydrolysis of pectin that found in orange juice and thus result inturbidity reduction and by thus juice clarification. Results mentioned after enzymatic treatment of polygalacturonase produced from *Streptomyces sp*. GHB5 to orange juice, the juice become clearer. This result is an indication that pectin content of the orange juice was degraded enzymatically by the action of polygalacturonase (Figure 3).

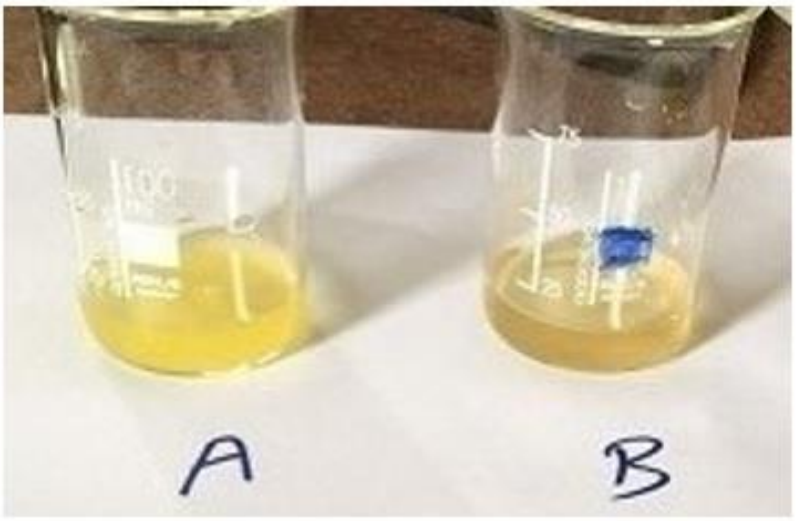

Figure: 3 Polygalacturonase treated orange juice (A) control and (B) after incubating with polygalacturonase produced from *Streptomyces sp*. GHB5 for 1 hour.

Orange peel have large amounts of soluble carbohydrates, especially fructose, glucose, sucrose, pectins also insoluble cellulose had been used as fermentation feed stock for the enzymes production [19] The pectinase may able to work on pectin and degrade it wholly. Pectinase attack pectin and de-polymerize it by hydrolysis and trans-elimination as well as by de-esterification reaction that make hydrolysis to the ester bond between the carboxyl and methyl of pectin [20]. The results mentioned that clarity of orange juice enhanced in comparison to control one which contained the same volume of enzyme added just before keeping the reaction mixture in boiling water. Clarity of the enzyme treated juice enhanced due to take off the colloidal and suspended particles in the juice specifically pectin.

Enzymatic clarification of apple juice

The third application of enzymatic hydrolysis of pectin present in the chopped apple fruit pieces. By addition 10 ml and 20 ml of polygalacturonase produced by *Streptomyces* sp. GHB5 to the chopped apple pieces resulted in 7.5 ml and 18.3 ml apple juice respectively, as final volume of obtained apple juice after 30 mints of filtration process. These results indicates the increasing of the produced volume of apple juice with increasing volume of used enzyme as compared to control 10 ml and 20 ml water that give no or little amount of apple juice (Figure 4). This result an evident for the role of enzymatic hydrolysis of pectin by polygalacturonase from *Streptomyces* sp. GHB5. By thus polygalacturonase from *Streptomyces* sp. GHB5 was effective in clarifying juice from apple fruits (Figure 4).

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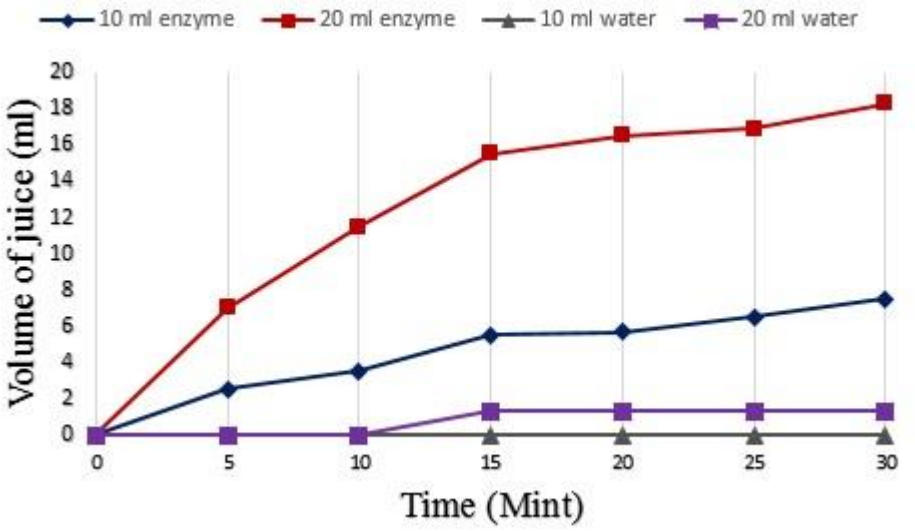

Figure 4: Clarification of Yellow apple juice with 10 ml and 20 ml of polygalacturonase from *Streptomyces* sp. GHB5 and water used as control.

The juice in the cylinders with polygalacturonase become clearer and the clouded particles decreased with the enzymatic treatment rather than the cylinders that contain water. This result mentions the key role of polygalacturonase enzyme produced from *Streptomyces* sp. GHB5 in the clarification process. Apple juice clarification was mentioned by enzymatic treatment of pectinase [1, 21 and 22]. A number of previous researchers had mentioned a reduction in the viscosity and turbidity of fruits, additionally the increasing in yield percent and clarity in juices of apple fruit in means of using hydrolytic enzymes [21].

Enzymatic hydrolysis of agriculture by-products

The fourth enzymatic hydrolysis for pectin present agriculture wastes. The experimental results and observations for enzymatic hydrolysis of different cellulosic substrates (orange peel, lemon peel, wheat straw and corn cob) at 45ºC. The highest reducing sugars were obtained from enzymatic hydrolysis of orange peels (445 µmol/ml) followed by lemon peel (325 µmol/ml), respectively after 24 hrs. (Figure 5).

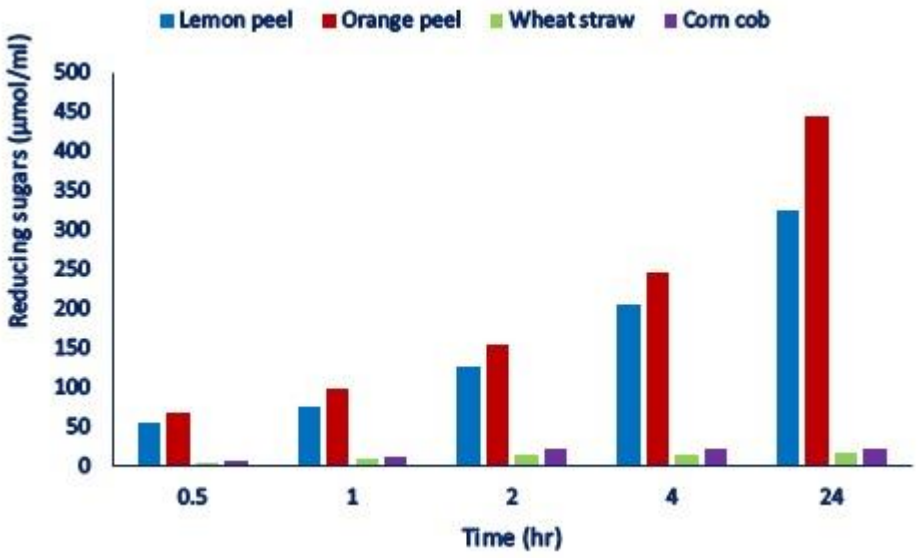

Figure 5: Increasing reducing sugars by enzymatic treatment of Lemon peel, Orange peel, Corn cob and wheat straw by polygalacturonase produced from *Streptomyces* sp. GHB5.

Hydrolysis process of cellulosic biomass had increased attention in the research field due to its global availability and substantial capacity for its transformation to sugars. By thus enables to relieve worldwide environmental pollution. Biomass hydrolysis enzymatically is more effective and proceeds under ambient conditions without generating any toxic waste and promising approach to have maximum product yields. Using low cost residues as cheap substrates in sugar manufacturing is mainly interesting for countries that have agro-industrial residues abundantly [23].

Boiling agriculture wastes with H_2O is an ideal pretreatment method would reduce the lignin content and crystallinity of the cellulose and increase the surface area for enzymatic reactions [24]. The principle advantage of this pretreatment method is the absence of any chemicals, no requirement of corrosion resistant materials for hydrolysis and no need of chemicals for neutralization of produced pretreated sample [25]. The experimental results and observations for enzymatic hydrolysis of different cellulosic substrates (orange peel, lemon peel, wheat straw and corn cob) at 45ºC. The highest reducing sugars were obtained after twenty four hrs. from orange peels followed by lemon peel, respectively (Figure 5). The high percentage of hydrolysis in orange peel and lemon peel may be attributed to the presence of high level from pectic substance which hydrolysis easily by polygalacturonase enzyme. On the other side, this result may be due to the low lignin content in lemon peel and in orange peel. Orange peel composition: Crude fat 3.9± 0.1 %, Water soluble materials 41.1±1.2 %, Pectin 14.4±0.3 %, Protein 7.9±0.1 %, Cellulose 16.2±0.5 %, Hemicellulose 13.8±0.3 %, Ash 1.7±0.1 % and Lignin 1.0±0.02 % [26]. In nature, it is known that lignin physically encrusts cellulose that makes it resistant to enzymatic degradation and impedes hydrolysis of the carbohydrates [6, 27]. The hydrolysis of different agro-wastes had been mentioned by other researchers using enzymes from a variety of microorganisms as *Aspergillus oryzae* ITCC-4857.01, *Trichoderma reesei* NCIM 992 and *Dioscorea zingiberensis* [6, 27, 28 and 29].

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