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Production and extraction of pectinase from *Penicillum citrinum* using tomatoes peel as carbon source

Abstract

In recent years, there has been a growing interest in the potential use of agricultural waste in the production of enzymes that have monetary value. There are a wide variety of applications for pectinolytic enzymes, making them one of the most essential commercial enzymes presently available. The manufacture, partial purification, and characterization of pectinase were all aspects that were investigated in this study. Pectinase was produced via solid state fermentation (SSF) of *Penicilium citrinum*, with tomato peel powder serving as the sole source of carbon. Putrefying tomato was used to isolate the fungus known as *Penicilium citrinum*. As part of the fermentation process, the fungi that had been separated were subcultured and consumed. On day six of the fermentation process, the crude enzyme reached its highest level of activity (11.233U/ml) and protein content (8.216mg/ml), according to the preliminary studies that were carried out. A partial purification process was then carried out on the crude enzyme that was collected on the sixth day of fermentation. This process involved the use of various concentrations of ammonium sulphate. The optimal percentage of ammonium sulphate saturation was found to be 80%, and the polygalacturonase activity was found to be 315.07 units per millilitre. Both the pH and temperature of the partly purified pectinase enzyme were determined to be optimal, with the former being 4.0 and the latter being 40 degrees Celsius. It has been demonstrated through this research that tomato peel has the potential to serve as a valuable source for the manufacturing of pectinase.

Keywords: pectinase, tomatoes peel, *penicillum citrinum,* extraction, enzymes

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Introduction

Pectinases are a group of enzymes that can break down pectin and other pectic substances. Timothy et al.,[1] say that pectinases are defined by their ability to break down the galacturonan backbone of molecules of pectic substances. Most of these enzymes are in three groups: protopectinases, pectinesterases, and hydrolases (which are a mix of lyases and hydrolases).[2] It helps make pectic acid when pectinesterases remove the ester from the methoxyl group of pectin. Pectic substance depolymerases, on the other hand, can either get rid of the α(1-4) glycosidic links between galacturonic monomers entirely or break them down.

Submerged or solid state fermentation (SSF) are both viable options for producing pectinases, as stated by Biz et al.,[3] and Emochone et al.[4] Submerged fermentation is a method where microorganisms are cultured in a liquid environment. The process is quite wasteful, requires continual churning, and consumes a large amount of water. Microbes thrive in SSF conditions—an aerobic setting devoid of or with minimal free water, and typically without requiring aseptic procedures—as they develop and secrete their products on or within solid substrate particles.[5] It has been discovered that pectinase can be produced by bacteria, yeast, and fungus. According to Collares et al.,[6] most pectinases utilised in commercial preparations come from fungal sources. Degumming and retting plant fibres, pretreating pectic effluent from fruit and vegetable processing units, converting black pepper skin to white pepper, extracting vegetable oil, purifying viruses, and ennentate coffee and tea leaves are all examples of applications for pectinases.[7] Also, they're useful in plant pathology, wood preservation, making alcoholic beverages and foods, adding them to chicken feed, making paper and pulp, and using protoplast fusion technology.[8]

Tomato, also known as *Lycopersicon lycopersicum* in the scientific community, is a warm-season crop that is recognised for its mild-mannered nature. The distinctive glandular hairs, also known as trichomes that exist on this plant are responsible for producing a potent odour when they are crushed. When it comes to viny, prostrate tomato plants, the presence or lack of inflorescence at the base of the plant's stems defines whether the plant is a determinate, semi-determinate, or indeterminate plant. The research was carried out by Biz and colleagues in the year 2014. Numerous hues, including yellow, can be found on fruits; nevertheless, the most common colour is red. The fruit size and shape, the development of the placenta, and the thickness of the fleshy mesocarp can vary significantly from one variety to another.[9,10] Fruit typically has a dry weight foundation that is composed of thirty percent cellulose, thirty percent hemicellulose, and thirty-five percent pectin.[11] The structural protein in fruit is approximately one to five percent glycoprotein. According to Pierce[12] and Opena & Kyomo,[13] the yield of tomatoes is higher than that of any other vegetable crop—even in Africa. Naika et al. conducted research that indicated that in the year 2001, a total of approximately 3.9 million hectares of land was used to harvest 105 million metric tonnes of fresh fruits. According to Naika et al. (2005), this crop is a compelling prospect for increasing production due to its short growing season, high yield, and economic attractiveness.

There is a pressing worldwide need to find new uses for agro waste due to the rapid spoiling of certain agricultural commodities, such as tomatoes, and the increasing expense of commercially available enzymes. A lot of people in Nigeria love tomatoes. Much of this fruit goes bad and ends up as food waste due to a lack of storage space. This byproduct provides a less expensive and more convenient way to make enzymes, such as pectinase. The objective of this research was to find a way to isolate pectinase from *Penicillium citrinum* fermentation by using tomato peel powder as the only carbon source.

Materials and methods

Study area and sample collection

The present study was conducted at Federal University Wukari Central Research Laboratory, Wukari, Taraba state, Nigeria from December 2021 to March 2023. The geographical coordinates of Taraba State are: longitude 7.9994° N and latitude of 10.7740° E, whereas the geographical coordinates of Wukari is given as: 7.9303° N; 9.8125° E. Sufficient tomato samples were collected from Wukari new market, along Wukari-Jalingo Road, Taraba State, Nigeria, and brought to Central Research Laboratory in Federal University Wukari, Taraba state.

Sample preparation

The preserved tomato samples were washed, cut into small bits and dried room temperature for 16 days after which it, they were sundried for 7 days at approximately 30 degrees Celsius. The dried peels of the tomato samples were then ground to powder using a mill and packaged for further analysis.

Experimental design

Penicillium citrinum was isolated from decaying tomatoes and sub cultured on Potato Dextrose Agar (PDA) to obtain sufficient biomass for solid state fermentation. The fungi were harvested by washing the culture plate with acetate buffer of pH 5.0. This served as the inoculant. The fermentation media contained the tomato powder peels as the sole source of carbon. The medium was harvested on the day of maximum enzyme activity. The crude enzyme harvested was subjected to ammonium sulphate precipitation to obtain a partially purified enzyme. The precipitated enzyme was then characterized in terms of temperature and pH.

Fungi culture

Rotten tomato, were cut in tiny bits and inoculated into a PDA solid medium under the flame of Bunsen burner. The plates were incubated at (room temperature) till visible colonies were observed. A sub-culture was carried out using the same procedure on separate plates in order to obtain a pure culture.

Solid state fermentation for enzyme production

A 250 ml Erlenmeyer flask served as the containment for the solid state fermentation. 10 ml solution of NaCl (0.3 g), NH_4SO_4 (1.4 g), Na_3PO_4 (2.0 g) and urea (0.3 g) in distilled water were added to 10g of tomato powder which served as the sole source of carbon after which the mouth of the contaminant was sealed with foil paper. 5ml of the inoculum was introduced into the medium using a sterilized syringe. The medium was left to stand for 6 days for the production of pectinase.

Enzyme extraction

At the end of day 6 on which the highest enzyme activity was detected, acetate buffer 5.0 was added into the fermentation medium. The mixture was filtered using a sieve. The filtrate was centrifuged for 30 minutes at 3000rpm after which the supernatant was collected and used as the crude enzyme for further analysis.

Determination of protein concentration

The protein concentration of the crude enzyme was determined by using the method developed by Lowry et al.[14] In a test tube, an aliquant solution of E (5mi) (a freshly prepared alkaline solution) was added to 0.5mi of crude enzyme solution. The mixture was thoroughly mixed and allowed to stand for ten minutes. After that, 0.5mi of sample C (a folin-Ciocateau Phenol reagent) was added, and the solution was allowed to stand for thirty minutes. The absorbance of the solution was measured at 750nm utilising a spectrophotometer. The protein concentration was determined by utilising a calibration curve that was made using Bovine Serum Albumin (BSA) as the standard. The analysis was performed twice.

Polygalacturonase assay

The protein concentration of the crude enzyme was determined by using the method developed by Lowry et al.[14] In a test tube, an aliquant solution of E (5mi) (a freshly prepared alkaline solution) was added to 0.5mi of crude enzyme solution. The mixture was thoroughly mixed and allowed to stand for ten minutes. After that, 0.5mi of sample C (a folin-Ciocateau Phenol reagent) was added, and the solution was allowed to stand for thirty minutes. The absorbance of the solution was measured at 750 nm utilising a spectrophotometer. The protein concentration was determined by utilising a calibration curve that was made using Bovine Serum Albumin (BSA) as the standard. The analysis was performed twice.

Ammonium sulphate precipitation

The crude enzyme was placed in nine (9) test tubes, each of which contained ten millilitres. For the purpose of precipitating the enzyme, a solution containing solid ammonium sulphate at a concentration ranging from 20% to 100% was used in each test tube at a 10% interval. After completely combining the contents of the tubes and allowing them to stand for twenty-one hours, the test tubes were centrifuged at a speed of three thousand five hundred revolutions per minute for thirty minutes. In the meantime, the pellets were re-dissolved in an equal amount of sodium acetate buffer with a pH of 5.0, which served as the partially purified enzyme. The filtrates were then decanted. In order to determine the pectinase activity, the contents of each tube were examined.

Effect of temperature on pectinase activity

The effect of temperature on pectinase activity was determined using the method of Tobechukwu et al. Different temperatures range from 25-50ºC at 5ºC intervals, taking 1 mL of the crude enzyme and 1 mL of tomato powder solution as substrate into 12 test tubes (each temperature with a test tube and an enzyme-blank test tube) in a water bath and the temperature at which the enzyme expressed maximum activity was taken to be its optimum temperature.

Effect of pH on pectinase activity

The effect of pH on enzyme activity was determined using 0.05M sodium acetate buffer pH 3.5-5.5, at intervals of 0.5. 0.1% tomato powder solution was prepared by dissolving 0.1g tomato powder solution in 100ml of the respective buffers. 0.5 ml of the partially purified enzymes was added to 0.5 ml of each of the buffers. 0.5ml of each of the enzyme-buffer solution was then mixed with 0.5ml tomato powder solution at the corresponding pH for pectinase assays.

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Results and discussion

Enzyme activity and protein concentration of crude enzyme.

There was a steady increase of enzyme activity and protein concentration which peaked on day 6 of fermentation period (Figure 1).

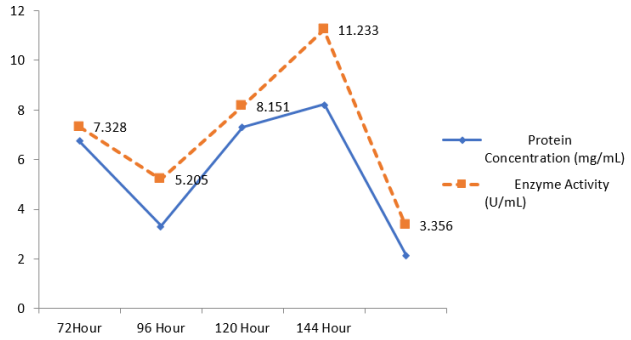

Figure 1 A combined graph of enzyme (pectinase) activity and the protein concentration against fermentation period.

Ammonium sulphate precipitation of the crude enzyme

Ammonium sulphate Precipitation profiling of Pectinases (Polygalacuronase) obtained from *Penicillium citrinum*. Figure 2 shows the results of ammonium sulphate precipitation of the crude enzyme. The highest activity (315.07U/ml) was obtained using 80% ammonium sulphate precipitate. Whereas the lowest activity of pectinase was obtained using 20% ammonium sulphate precipitate

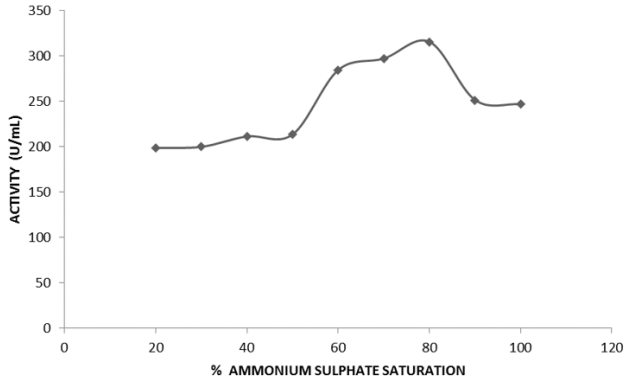

Figure 2 Ammonium sulphate precipitation graph curve.

Effect of temperature on the activity of pectinase

Figure 3 shows the results of the effect of temperature on pectinase activity. The present study revealed that temperature change displayed an effect on pectinase activity. At 20°C, pectinase activity was observed to be 200 U/ml. At 40°C, pectinase activity was observed to be 200.92U/ml. A sharp increase in pectinase activity was seen at 50-65°C, producing an activity of 280 U/ml at 65°C. The highest pectinase activity (325U/ml) was obtained at 80^{0}C, after which, there gradual decrease in pectinase activity (Figure 3).

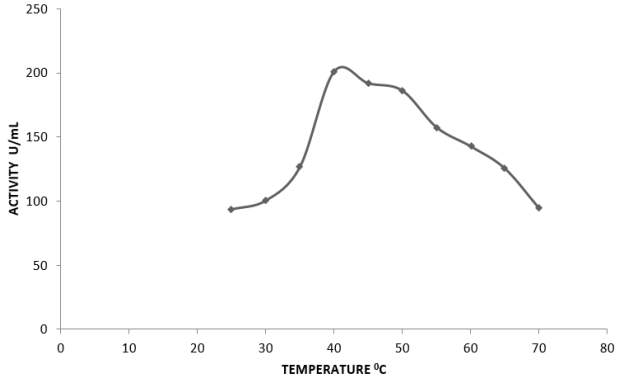

Figure 3 Graph curve on the enzyme activities of pectinase on varying temperatures.

Effect of pH on the activity of pectinase

Figure 4 shows the results of the effect of pH on pectinase activity. This study investigated the effect of various pH values on pectinase activity. At pH 3.5, pectinase activity was observed to be 40 U/ml. At pH 4.0, pectinase activity was 48 U/ml. Exceeding pH 4.0, a decrease in pectinase activity was observed. At pH 4.5, pectinase activity was seen to fall below 40 U/ml. Beyond pH 4.5, pectinase activity was again seen to increase. The highest activity 47.95U/ml was obtained at pH 5.5 (Figure 4).

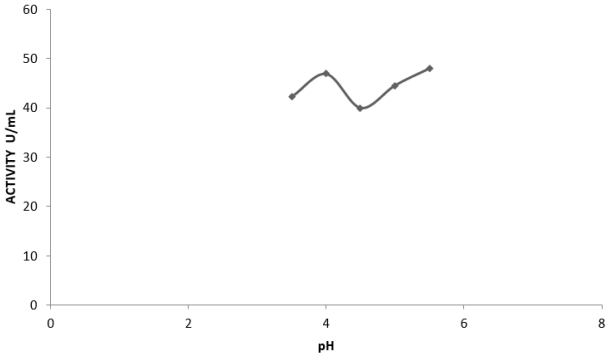

Figure 4 Graph curve of pectinase activity on varying Ph.

Discussion

Plants, nematodes, insects, yeasts, fungus, and bacteria are only some of the creatures that naturally produce pectinolytic enzymes.[15] Pectinolytic enzymes are found in a wide variety of animals. There has been a significant amount of attention over the course of the past few decades in the search for methods that can enhance enzyme synthesis by making use of agricultural waste. For the purpose of this work, the synthesis, extraction, and characterisation of pectinase from *Penicillium citrinum* were investigated. The powdered tomato peel was used as the carbon source in the process of fermentation that took place in stable conditions.

Penicillium citrinum was isolated from rotting tomatoes and then grown on PDA solid medium after it was isolated by isolation. This culture was utilised in the process of solid state fermentation

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in order to produce pectinase because it was necessary. A full week had elapsed by the time the fermentation process was complete. The medium was harvested on the sixth day of fermentation, which coincided with the greatest amount of enzyme activity that was present during that time period. The crude enzyme was precipitated using ammonium sulphate, which resulted in a partial purification of the enzyme. After the enzyme had been precipitated, its properties were analyzed by determining its pH and temperature following the precipitation process.

Continuous monitoring of enzyme activity and protein content was carried out throughout the fermentation process. The results showed a gradual increase that reached its highest point on day 6. The enzyme activity was 11.233 U/mL at the end of the sixth day of fermentation, and the protein content was 8.216 g/mL with the fermentation process having been completed. The researchers Amsal et al.,[16] found that the highest concentration of proteins and enzyme activity was found on the sixth day of their experiment when banana peel was utilized as the only source of carbon. The results of their study seem to be in agreement with those of this one. On the other hand, Daniel et al.,[17] found that the maximum levels of protein and enzyme activity were detected on day 5 due to the utilization of mango peel as the sole source of carbon. On the other hand, Patil & Dayanand,[18] assert that it is possible to increase the yield as well as the amount of pectinase by incorporating additional carbon sources into strawberry powder.

At a saturation level of 80%, the ammonium sulphate precipitation profile displayed the maximum enzyme activity, which was measured at 315.07U/ml under the conditions. It was discovered by Ibeawachi et al.,[19] that the pectinase activity reached its highest point at a saturation level of 80% ammonium sulphate. This finding is in good agreement with theirs. On the other hand, they discovered that the enzyme activity was reduced when pineapple peel was used as a source of carbon. According to the findings of Chowdhury et al.,[20] the optimal degree of ammonium sulphate saturation for pectinase activity was found to be 80%. On the other hand, they discovered that the enzyme activity was decreased when *Bacillus subtilis* was removed from the soil sample. On the other hand, El Enshassy et al.,[21] discovered that the pectinase activity was at its peak at a saturation level of 65% ammonium sulphate when *Penicillium* Sp was isolated from soil.

It was established that the optimal temperature for the isolated pectinase was 400 degrees Celsius, with an activity of 200.92 units per millilitre. This was determined by studies that investigated the impact that temperature has on the activity of pectinase. Ezugwu et al.,[22] also observed that optimal temperatures occur at a temperature of 400 degrees Celsius. Their findings are comparable to those of these researchers. Ibeawachi et al.,[19] found that the optimal temperature was 700 degrees Celsius, which is a higher temperature than the actual temperature.

A pH of 5.5 was found to be optimal for the enzyme that was isolated, and its activity was found to be 47.95 Uml for optimal performance. In spite of the fact that Ezugwu et al.,[22] investigated the possibility of mango pectin being utilised as a carbon source, we found that the pectinase activity was greater and that the optimal pH was 5.5. The results that they obtained are in agreement with this finding. Using *Bacillus* species that were isolated from soil samples, Jansirani and colleagues conducted a different experiment in which they determined that the optimal pH level is 7.0. According to Ramachandran et al.,[23] the utilisation of waste from oranges and vegetables as a source of carbon resulted in a pH number that was lower than the desired value of 5.0. There is a possibility that the bacteria that are utilized throughout the fermentation process could induce alterations to the optimal pH.[24–29]

Conclusion

This research considered production, extraction and characterization of pectinase from *Penicillum citriunm* using tomato peel as carbon source. Results from this study indicated that tomato peel can be successfully used to induce the production of pectinase under solid state fermentation system. Maximum enzyme precipitation was achieved at 80% ammonium sulphate saturation with an activity of 315.07U/ml. Studies on the characterization of pectinase showed that the optimum pH was 5.5 with an activity of 47.95U/ml while the optimum temperature was 40^{o}C with an activity of 200.92Uml. This work has shown that agro waste such as decaying tomato can be harnessed as a source of carbon in the fermentation process for the production of enzymes like pectinase which have a wide range of applications.

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Conflict of interest

The authors declared that there are no conflicts of interest.

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References

1. Timothy M, Mayel MH, Yohanna ER, et al. Pectinase production from a local isolate of *Aspergillus niger* using orange bagasse as a carbon source. *Asia J Nat Prod Biochem*. 2022;19(2):81–86.
2. Shaibu CO, Dinshiya J, Shaibu VE. Short communication: extraction and characterization of pectin from ripe and unripe mango (*Mangifera indica*) peel. *Asia J Nat Prod Biochem*. 2022;20(1):16–20.
3. Biz A, Farias FC, Motter FA, et al. Pectinase activity determination: an early deceleration in the release of reducing sugars throws a spanner in the works. *PLoS One*. 2014;9(10):e109529.
4. Emochone RY, Christopher S, Moses AA, et al. Isolation, partial purification and characterization of proteases from *Aspergillus niger* under solid-state fermentation. *Asian J Res Bios*. 2023;5(1):27–34.
5. Garg G, Singh A, Kaur A, et al. Microbial pectinases: An ecofriendly tool of nature for industries. *3 Biotech*. 2016;6(1):47.
6. Collares RM, Miklasevicius LVS, Bassaco MM, et al. Optimization of enzymatic hydrolysis of cassava to obtain fermentable sugars. *J Zhejiang Univ Sci*. 2012;13(7):579–586.
7. Raveendran S, Parameswaran B, Ummalyma SB, et al. Applications of microbial enzymes in food industry. *Food Technol Biotechnol*. 2018;56(1):16–30.
8. Kaur G, Kumar S, Satyanarayana T. Production, characterization and application of a thermostable polygalacturonase of a thermophilic mould sporotrichum thermophile apinis. *Bioresour Technol*. 2004;94(3):239–243.
9. Prashanth SJ. Genetic variability and divergence studies in tomato (*Lycopersicon esculentum Mill)*. M.Sc (Agri.) Thesis, Uni. Agric. Sci., Dharwad (India). 2003.

10. Veershetty V. Studies on variability, character association and genetic diversity in tomato (*Lycopersicon esculentum Mill*). M.Sc. (Agric.) Thesis, Uni Agric Sci Dharwad (India). 2004.

11. Cosgrove DJ. Assembly and enlargement of the primary cell wall in plants. *Annu Rev Cell Dev Biol*. 1997;13:171–201.

12. Peirce LC. Vegetables characteristics, production and marketing. John Wileyand Sons. Inc. Toronto. Canada. 1987.

13. Opena RT, Kyomo ML. Vegetable research and development in SADCC countries: proceedings of workshop held at Arusha, Tanzania N AVRDC.1990;90–328.

14. Lowry OH, Rosenbrough PJ, Farr AL, et al. Protein measurement with folin-phenol reagent. *J Biol Chem*. 1951;193:265–275.

15. Abdel-Moshen SI, Heba IA, Manal MH. A safe potential juice clarifying pectinase for *Trichoderma viride* utilizing Egyptian onion skins. *J Genet Eng Biotechnol*. 2016;14(1):153–159.

16. Abdul Ghani AB. Production of bacterial pectinase using banana peels agricultural waste as substrate. *Resource Biotechnol*. 2009;15948.

17. Udenwobele DI, Nsude CA, Ezugwu AL, Et al. Extraction, partial purification and characterization of pectinase isolated from *Aspergiluus Species* cultuled on mango (*Mangifera Indica*) peels. *Afican J Biotecnol*. 2014;13(24):2445–2454.

18. Patil SR, Dayanand A. Production of pectinase from deseeded sunflower head by *Aspergillus niger* in submerged and solid-state conditions. *Bioresources Technology*. 2006;97(16):2054–2058.

1. Ibeawachi AN. *Production and characterization of pectinase induced from Aspergiluus niger pectin extracted from pineapple peels*. University of Nigeria Nsuka. 2012.

19. Chowdhury TI, Jubayer F, Uddin B, et al. Production and characterization of pectinase enzyme from *Rhizopus oryzae. Potr S J F Sci*. 2017;11(1):641–651.

20. El Enshasy HA, Elsayed EA, Suhaimi N, et al. Bioprocess optimization for pectinase production using *Aspergillus niger* in a submerged cultivation system. *BMC Biotechnol*. 2018;18(1):71.

21. Ezugwu AL, Eze SOO, Chilaka FC, et al. Production and characterization of pectinases obtained from *Aspergillus fumigatus* in submerged fermentation system using pectin extracted from mango peels as carbon source. *Plant Prod Res J.* 2012;16:30–36.

22. Ramachandran S, Kurup G. Screening and isolation of pectinase from fruit and vegetable wastes and the use of orange waste as a substrate for pectinase production. *Int Res J Biol Sci*. 2013;2(9):34–39.

23. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analyt Chem*. 1959;31:426–428.

24. Mohandas A, Raveendran S, Parameswaran B, et al. Production of Pectinase from *Bacillus sonorensis* MPTD1. *Food Technol Biotechnol*. 2018;56(1):110–116.

25. Mukesh K, Saranya GM, Suresh K, et al. Production and optimization of pectinase from bacillus sp. MFW7 using cassava waste. *Asia J Plant Sci Res*. 2012;2(3):369–375.

26. Dam B, Marja G, Joep LG, et al. Cultivation of tomato: *production, processing and marketing*. *CTA*. 2005;17.

27. Stutzenberger FT. Pectinase production. In: *Encyclopedia of Microbiology*, Lederberg J. 3rd Ed. Academic Press, New York. 2005:27–337.

28. Yadav S, Yadav PK, Yadav D, et al. Purification and characterization of alkaline pectin lyase from *Aspergillus flavus*. *Process Biochem*. 2008;43(5):547–552.

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