Fruit Pomace: A new substrate for Polygalacturonase Production through Solid State Fermentation

Dissertation submitted in partial fulfilment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

by

Shilpi Maheshwari

(Roll. No: 141817)

Under the Guidance of

Dr. Garlapati Vijay Kumar

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS

HP-173234,INDIA

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CERTIFICATE

This is to certify that the work reported in the B.Tech. thesis entitled "**Fruit Pomace: A new substrate for Polygalacturonase Production through Solid State Fermentation"** by **Shilpi Maheshwari (**Roll.No**:** 141817**)** at **Dept. of Biotechnology and Bioinformatics**, **Jaypee University of Information Technology, Waknaghat , India,** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(**DR. GARLAPATI VIJAY KUMAR**)

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JUIT. Waknaghat, HP-173234,India.

DECLARATION

I hereby declare that the work reported in the B-Tech thesis entitled **"Fruit Pomace: A new substrate for Polygalacturonase Production through Solid State Fermentation"** submitted at **Dept, of Biotechnology and Bioinformatics**, **Jaypee University of Information Technology, Waknaghat India,** is an authentic record of my work carried out under the supervision of **Dr. Garlapati Vijay Kumar** and have not submitted this work elsewhere for any other degree or diploma.

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My thanks and appreciations also go to all the people of Jaypee University of Information Technology who have willingly helped me out with their abilities.

(**SHILPI MAHESHWARI**)

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

There is a developing enthusiasm for utilization of food and agricultural wastes for the production of enzymes. Using the agro-industrial wastes reduces the cost of production. Enzyme, pectinase is an essential compounds utilized as a part of natural product processings. Pectinases are produced by both solid state or submerged fermentation from different plant and microorganisms. Fungal culture was used to produce pectinase by submerged fermentation using apple pomace as carbon source in the present study. The fungus was subcultured and grown on agar slants. Various optimizations were done to get the maximum production of the enzyme. Pectinase assay was performed according to the Dong and Wong method with a few modifications. In conclusion, this experiment portrays that apple pomace is a successful source of carbon, for the production of pectinase.

Keywords: Apple pomace, pectinase production, solid state fermentation, *Aspergillus awamori*

CHAPTER 1

INTRODUCTION

==========

Enzymes are responsible for catalyzing various reactions in the making of various food products. It is an important tools in food industry because while processing, intermediate processes are simplified by the use of enzymes. The most important group of enzyme used in fruit processing industry is pectinases. The first enzymes to be used for the first time at homes were Pectinases. Commercial application of Pectinase was in 1930 to prepare wines. As a result, pectinases are the most important enzymes used in the commercial sector for fruit juice factories and as pre-requisites for obtaining clear juices.The addition of pectinases diminishes the consistency of the natural product squeeze, the crush capacity of the mash turns out to be better, the jam structure separates and the juice is acquired with higher yields.

75% sale value of industrial enzymes is reported to be of pectinases. Pectinases have a range of applications such as- extraction of fruit juices and wine production, animal feed production, in paper and pulp industry. Pectinase enzymes are produced mainly from microbial sources. 50% of which is produced by filamentous fungi and yeast, bacterial sources and the remaining from plant or animal origin. Pectinase is a common term for enzymes. This broadly refers to pectin lyase, PGAL, and pectin methlyesterase. These all are known as pectic enzymes together. The function of all these enzymes is to break the compound found in cell walls of plants, called as Pectin.

Pectinase enzyme is responsible for the breakdown of a polysaccharide compound, pectin, present in plant cell walls. Pectinase enzyme hydrolyzes the nondigestible carbohydrates that comprise fiber. Pectinase enzyme consist of pectin methylesterase. This enzyme demethylates pectin and polygalacturonase, which hydrolyzes a D-1, 4-galacturonide. The breakdown of apple pomace is brought about by different enzymes which includes pectin methylesterase, exo and endo polygalacturonase, pectate lyase. The fermentation in this study was done using *Aspergillus awamori* as the inoculums and the substrate used was apple pomace. The parameter tested are size of inoculums, temperature, time of incubation.

CHAPTER 2

LITERATURE REVIEW

==========

2.1 Apple pomace

Sustenance industry when all is said in done, produces an expansive amount of waste (i.e peel, seed, pomace, clothes, bits and so forth.), which is biodegradable in nature. Such squanders have awesome potential as a substrate in maturation and include creation of valuable items because of their synthesis in starches, dietary strands.

The pomace of Apple is a build up after the extraction of sap, cider or puree. It contains peel, seeds and pulp. It consists of all the wastes and parts that are left after the extraction of sap from apple. The basic components of pomace also have different insoluble sugars. These are cellulose, hemicelluloses and lignin. The pomace of apple has rich components like starches, acidic components, vitamins and mineral(mainly essentially elements). Waste production of apple is approximately 5.7 million tones. This mainly comes from agro-industrial sector during processing of apple in different sectors with the major waste generated from Turkey.

The waste apple pomace could be bioconverted into various esteem included items, for example, Pectin, polyphenols, strands, catalysts, single cell protein, colors, smell mixes, alcohols, natural acids, polysaccharides, biohydrogen and cancer prevention agent substances [1].

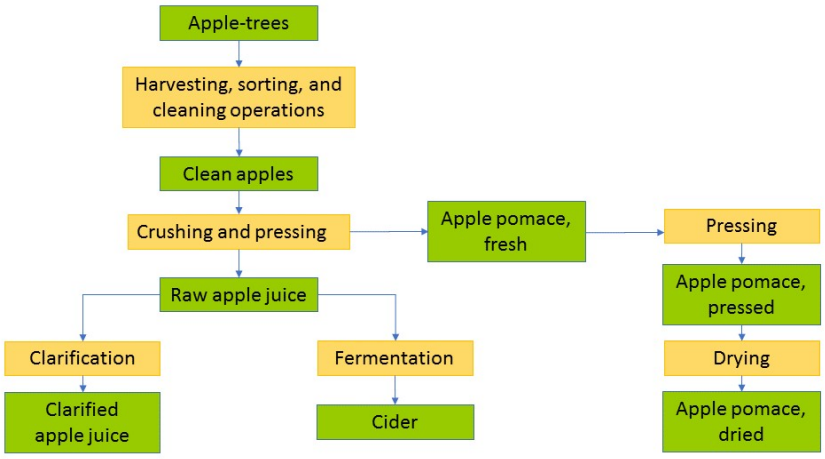

Figure 2.1: Apple pomace production process (culled apples).

2.1.1 Compostion: Apple Pomace

The pomace has 2-3%seed and seed core, less than 1% stems 30% as peel and the left over portion as apple substance.

2.1.2 Apple pomace applications

The extraordinary capability of apple pomace in biotechnology seeks scientists consideration on this buildup continuously increments. Primary application regions with apple pomace are generation of chemicals, natural acids, Pectin, protein-improved encourages, eatable mushrooms, ethanol, butanol, common cancer prevention agents, fragrance mixes, eatable filaments, vitality, phytochemicals and biofilms [2].

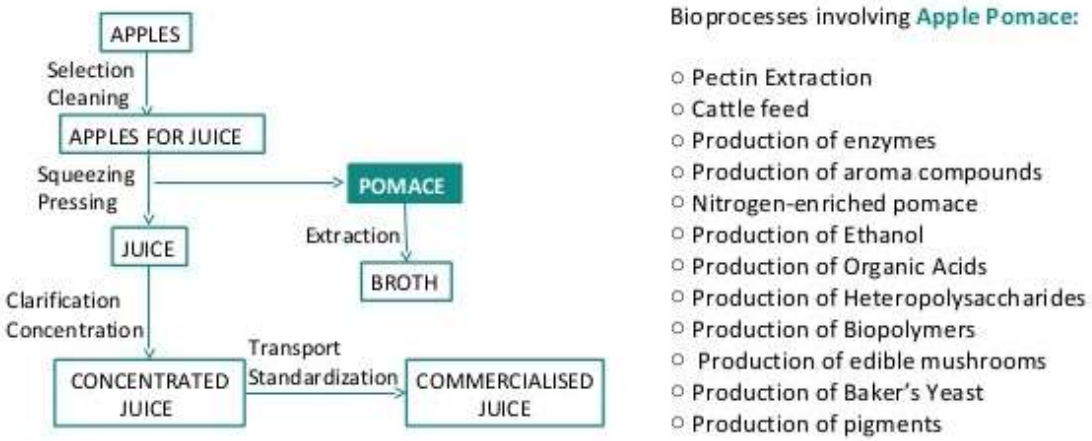

Figure 2.2: Applications of apple pomace and its production

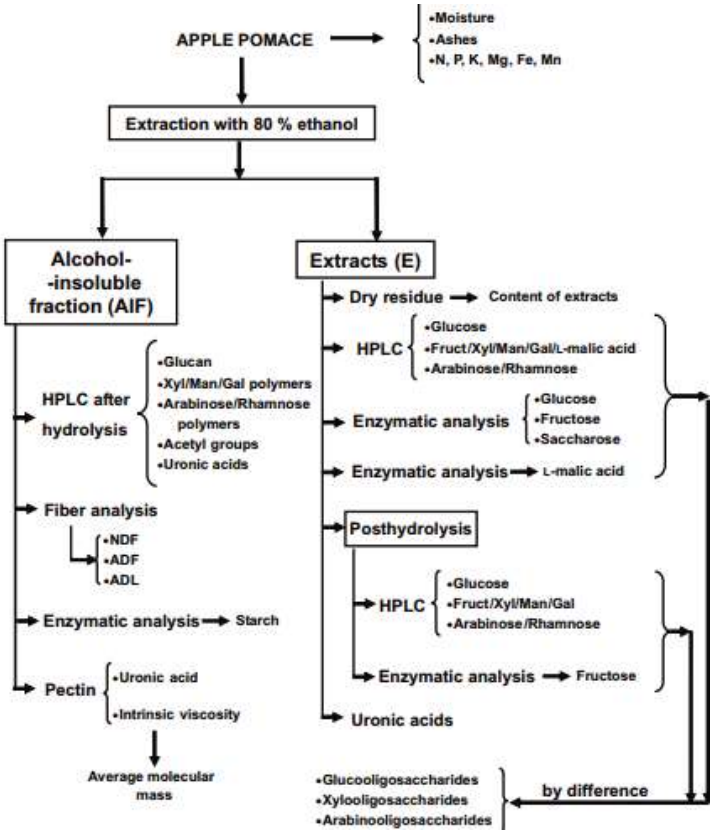

Figure 2.3: Procedure used to determine the composition of apple pomace.

2.2 Pectin

Pectin, in local frame is situated inside cell divider which is synced with other auxiliary polysaccharides and proteins to form insoluble protopectin. In an immature organic product, Pectin is held to cellulose small scale fibrils in the cell divider. Such Pectin is not soluble and thus presents unbending nature to cell dividers. At the point when the tissue is ground, the Pectin is found in the fluid stage (dissolvable Pectin) causing an expansion in consistency and the mash particles, though other Pectin atoms stay bound to cellulose fibrils by methods for side chains of hemicellulose and, in this manner, encourage water maintenance. Every single earthbound plant contain Pectin, which ties with cellulose, and makes protopectin, a substance that gives plants their structure. Given this part, it ought not amaze that Pectin is discovered essentially in the plant cell dividers, and in the district between cell dividers, called the lamella, where it aids the authoritative of one cell divider to another. Notwithstanding giving plants their structure, Pectin has other critical parts, for example, deciding how permeable the cell is, and its pH [3].

2.2.2 Structure of Pectin

Pectin is a component of cell wall of plants being a heterosaccharide. The chain length of different pectins vary. The complexity of structure and order of the monosaccharide units is also different for different types of pectin. The structure of pectin has a straight line chain composed of alpha (1-4) linked D-galacturonic acid. This mainly forms the backbone of the chain.

There is esterification with methoxy groups of the acids that are present in the chain. On the hydroxyl groups, these is presence of acetyl groups. The chain of galacturonic acid has rhamnose groups present. This disturbs the formation of helix chain. In the side chains, sugars are present. Most common being arabinose, xylulose and galactose [4].

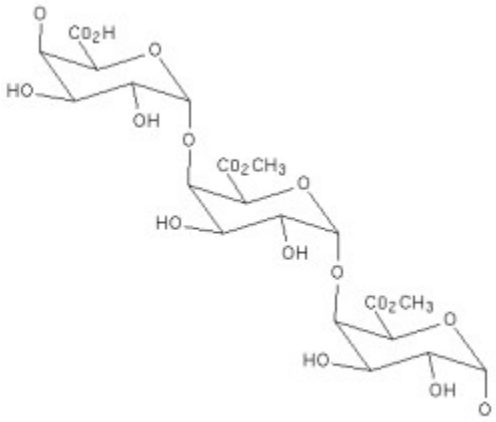

Figure 2.4: Structure of Pectin

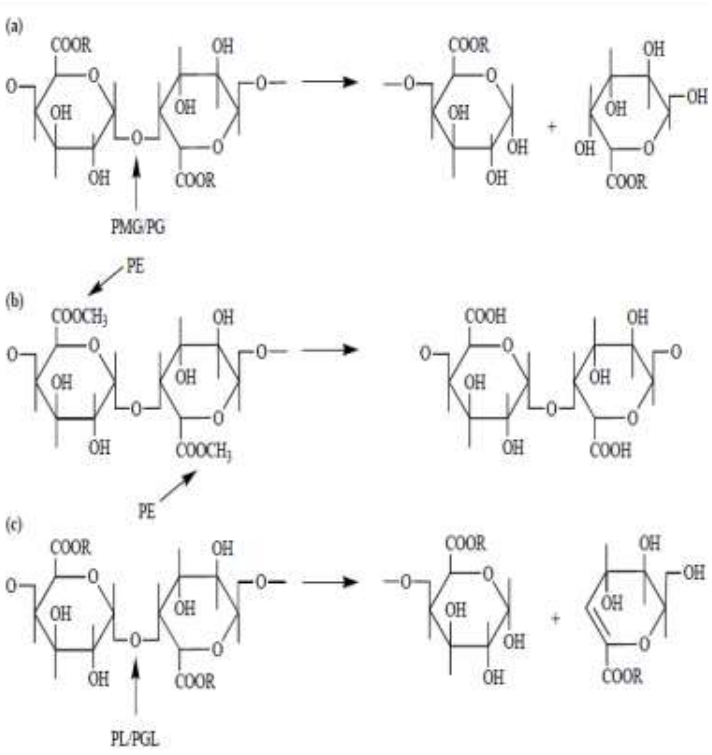

Figure 2.5: Mode of action of enzyme Pectinase

2.2.3 Application of pectinase:

Pectinase enzyme has varied uses in the commercial sector. In biotechnology, it can be used in extraction of sap, fermentation of coffee, treatment of waste water, organic production of paper in paper and pulp industry, etc.

2.3.1 Production of enzyme

Microbes are the main source of enzymes production, but a lot of enzymes are also obtained from other sources like animal or plant. It represents approximately 90% of total market. Micro-organisms are good for the production of enzymes because they are versatile and easier to culture. The necessary nutritional requirements of micro organisms are easy to supply; and their genetic makeup can also be altered. Enzymes can be produced by these four stages [5].

Enzyme synthesis: Fermentation of extract or juice.

Enzyme recuperation: Involving strong fluid divisions, cell extraction or focus ventures to remove chemical from delivering cell framework.

Enzyme decontamination: Aiming to expel undesirable materials from unrefined protein.

Enzyme item definition: Involving last cleaning tasks, adjustment and institutionalization to give last item.

Chemical tests are research facility techniques for estimating action of a compound. An information of the action of proteins is required as a result of the underneath reasons:

• To seek generation and detachment of proteins

• To decide and settle the properties of business arrangements

• To set up the best possible measure of enzyme

2.4 Solid- State Fermentation

Solid-state fermentation (SSF) is characterized as a fermentation process in which the growth of micro-organisms is on the moist solid substrates. The fermenatation process also has addition of different sources like carbon. The main advantage of this technique is that nutrient rich waste materials can be used as solid materials. Other points of interest canbe recorded as tall volumetric efficiency, low equipment cost, superior abdicate of item and low waste generation. However, solid-state is constrained on mass and warm exchanges conjointly it is difficult to set factors since of dealing with solids.
It coordinates flawlessly with aging methods including parasites and other microorganisms that require less dampness substance, since organisms can develop in nature on strong substrates. Solid state fermentation cannot be performed on microbial strains that require high water content [6].

2.5.1 Challenges and advantages of Solid State Fermentation

There are different critical variables that deliver colossal effect on the achievement of a specific innovation, thus, should have been considered for the improvement of any bioprocesses and so are with the SSF. It incorporates choice of microorganism and substrate, ideal process parameters and furthermore cleansing of the final result, which has been a test for this innovation. Organisms and yeast were named as appropriate microorganisms for SSF as indicated by the hypothetical idea of water movement. The foundation of the connections between the physiology of the microorganisms and the physico-compound components is the go for the advancement of legitimate models. These variables incorporate temperature, pH, air circulation, water movement and dampness, bed properties, nature of strong substrate utilized, and so forth. Among a few basic components, dampness and nature of strong substrate utilized are the most vital factor influencing SSF forms. Determination of dampness relies upon microorganism utilized and furthermore the idea of substrate. Growth brings down dampness, 40– 60 for every penny dampness could be adequate. High dampness rate brings about low substrate porosity which, thus, avoids oxygen entrance, while low dampness substance may prompt poor openness of supplements coming about in hampered microbial development. The results of these challenges for bioreactor execution have been all

around investigated. Warmth age due to metabolic exercises of the micro-organisms, is frequently deadly for biotechnological forms in light of the fact that a substantial piece of the compounds delivered amid the maturation can be warm denatured toward the finish of the process [7].

2.6 Inoculum type

The type of inoculums is one in every of the foremost vital issue of fermentation. Fermentation needs solely one species of organism or mixture of 2 or more organisms. For single culture studies; the substrate ought to be decontaminated and autoclaved, to get rid of unwanted microorganisms before immunization with the specified microbe. Typically in food processing; mixture of two or more micro-organisms is employed to succeed in fascinating final product. It needs involvement of many microbic species. These species act at the same time or consecutive to administer fascinating product of selection [8].

2.7 *Aspergillus awamori*

Species of *Aspergillus* square measure vital medically and commercially. Some species will cause infection in humans and different animals. Some infections found in animals are studied for years, whereas different species found in animals are delineated as new and specific to the investigated illness, are called names already in use for organisms like saprophytes. quite sixty Aspergillus species square measure medically relevant pathogens.Members of the genus also are sources of natural product which will be employed in the event of medicines to treat human illness [9].

Classification of *Apergillus awamori*

Fungi, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiomycetidae, Eurotiales, As pergillaceae, Aspergillus

Aspergillus awamori is used to make a famous drink of Japan, awamori. This strain is a sub-species of *Aspergillus.* It can convert the starch into sugar and can also produce the citric acid [10].

Figure 2.6: Growth of *Aspergillus awamori* on petri plate.

Figure 2.7 : Spores of Aspergillus awamori being subcultured in Potato dextrose broth.

2.8 Czapek-dox Media

CZAPEK-DOX BROTH is usually used for the cultivation of fungi. It is just like Czapek-Dox Agar, while not the agar, and is employed to grow bacterium and fungi that are capable of exploitation of sodium nitrate as a sole supply of nitrogen. Czapek-Dox Broth is a medium that contains sodium nitrate as the

only supplier of nitrogen, that is inorganic. The medium is detailed with inorganic sources of nitrogen. It's helpful during microbiological procedures, as well as soil biology, and fungi and mold resistance tests. This medium can yield moderately sensible growth of most saprophytic *Aspergilli*. Sucrose is that the only sugar providing carbon and energy. Nitrogen comes from NaNo3. Potassium salts act as a buffer system. Potassium cloride contains essential ions. Czapek-Dox media should appear slightly opalescent, and white to light amber in color. Times and temperatures of incubation vary significantly in step with the fungi. As a general rule, incubate from 7-14 days at temperature (approximately 30°C). Most *Penicillium* grow best between 20-30°C; fungus genus species grow well at around 30°C [11].

Figure 2.8 : Czapek-dox media is transparent in appearance.

OBJECTIVES:

Based on the literature review, the specific objectives of the present research work are summarized as follows:

- To check the effectiveness of apple pomace as substrate
- To find out the optimum condition of fermentation for solid state fermentation
- To determine the enzymatic activity of pectinase produced by solid state fermentation by *Aspergillus awamori*

CHAPTER 3

MATERIALS AND METHODS

==========

3.1 Raw materials:-

Substrate:- For the Solid state fermentation processes, agro-industrial residues are used as substrates for the production of enzymes. Apple pomace was obtained from Minchy's food products, Kandaghat. It is the leftover from apple concentrate production. The apple pomace was pre-treated before using in the experiment. It was dried in hot air over at 90 °C for a period of 24 hours. The dried pomace was coarsely grinded, sieved and kept in sterile containers until used. The wet Pomace was kept at 4°c in a sealed and autoclaved plastic bag to avoid any spoilage and contamination.

3.2 Microorganism:-

Fungal strain *Aspergillus awamori* MTCC 9644 was from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Culture was maintained on Czapek's Agar Medium. After three days incubation at 30°C the agar slants were stored at 4°C.

3. 3 Chemicals:-

The strain, MTCC 9644 was revived on Potato dextrose broth and maintained on Agar slants of Potato dextrose agar.

Table 3.1: Composition of Potato dextrose agar to make agar slants

Component	Amount(g/100l)
Potato Dextrose Agar	3.9
Water	100

For the setting of solid state fermentation, czapek dox media was used along with the dried apple pomace in an Erlenmeyer flask.

Table 3.2: Composition of czapek dox media used for solid state fermentation

Sodium Nitrate	2.5 g/L
Mono-potassium phosphate	1g/L
Potassium chloride	0.5g/L
$MgSo_4.2H_2o$	0.5g/L

3.2 METHODS

3.2.1 Revival of the spores

The spores were revived by sub culturing them on autoclaved Potato dextrose broth. An Erlenmeyer flask was used and the broth with the spore was kept in an incubator shaker at 30 °C **for 5-6 days.**

Figure 3.1: Fungal spores of *Aspergillus awamori* revived on Potato dextrose broth

3.2.2 Agar slants

After the growth of spores on Potato dextrose broth, the spores were maintained on Agar Slants. From the Erlenmeyer flask, one spore was taken for one particular slant. The Agar slants were kept in the incubator at 30 °C for 4 days.

Figure 3.2: Growth of fungus on Agar Slants

3.2.3 Substrate preparation

The apple pomace was carefully kept in a sterilized glass plate and kept in hot air oven at 90°C for 24 hours to get completely dried. After the pomace gets dried, it is crushed using liquid nitrogen to make the crushing easy. It is to be kept in mind that the pomace is coarsely crushed and a fine powder is not made. After the crushing, the ground peels were sieved using mesh size of 60 BSS.

Figure 3.3 Wet Pomace

Figure 3.4: Dried Pomace

Figure 3.5: Using liquid nitogen to crush the dried pomace

Figure 3.6: Coarsely grinded pomace

3.2.4 Set up of Solid State Fermentation

The coarsely grinded Apple pomace was measured 4gm per flask. In total, 4 solid state fermentations were set up.The flask along with dried apple pomace had czapek dox media. The culture was autoclaved at 103 KPa pressure and 121°C temperature for 20 min. It was then cooled and kept at in an incubator for a day to check for contamination.In the case of lack of contamination, further steps were carried out.

Figure 3.7: Adding equal amount of substrate to set solid state fermentation

3.2.5 Inoculum preparation

The autoclavable items like the tips, eppendorfs, inoculation loop, 100 ml water, were autoclaved. The entire work of preparing the inoculum was done in the Laminar Air flow. These autoclaved items were kept in the UV radiation for 15 minutes to kill off any bacteria or microbe sticking to it. This is an additional step to keep away contamination.

Figure 3.8: Use of UV to kill all the microbes

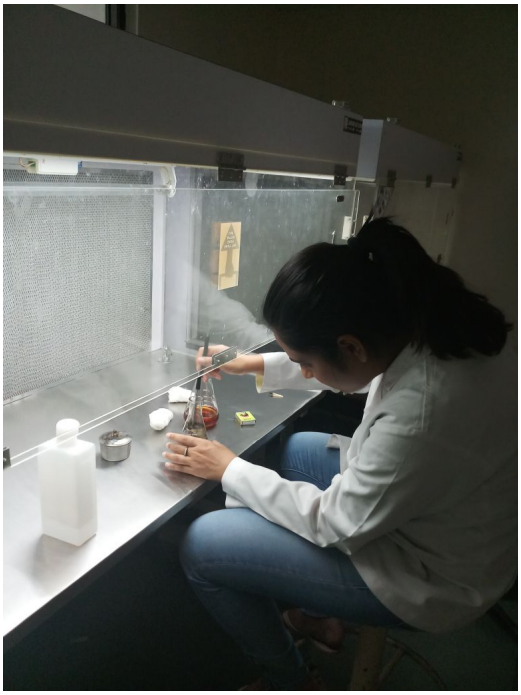

Figure 3.9: Laminar air flow hood

Using a 1 ml Pipette, 2ml of autoclaved water was added to the heavily sporulated agar slants. The agar slant was rubbed by inoculation loop, keeping in mind to not disturb the agar. This suspension was mixed with 8ml distilled autoclaved water. The total mixture is 10ml, out of which 1ml is added to all the Erlenmeyer flask having the apple pomace and czapek dox media. The solid state fermentation was kept at 30 ºC for 4 days in an incubator.

3.2.6 Extracting the crude enzyme

For the extraction of the enzyme from the solid state fermentation after 72 hrs, these flasks were incubated at 30°C for an hour after adding 20 ml of distilled autoclaved water. After incubating the enzyme was harvested, the fermented slurry was squeezed through muslin cloth and the extract was collected. Centrifugation was carried out at 2200g for 10 min. The supernatant obtained after centrifugation was used as crude enzyme extract and in the pectinase assay.

Figure3.10: Crude enzyme extract

Figure 3.11: Crude enzyme extract was filtered from the slurry after incubation with water at 30°C. The picture on left shows the growth in white(incubation for 3 days) and the picture on right shows the growth as black(incubation for 4 days).

3.2.7 Polygalacturonase Assay

Polygalacturonase (PG) causes softening of the fruits like tomatoes, peaches, pears, avacados and apples. The enzyme is a hydrolase and has its action similar to enzymes exo Polygalacturonase and endo Polygalacturonade. The action of Exo Polygalacturonase starts by the removal of galacturonic acid units from the non-reducing ends of polygalacturonic acid whereas endo PG hydrolyses Polygalacturonase acid haphazardly, giving rise to short chain oligogalacturonic acids which could final be hydrolyzed to a combination of tri-, di-, and mono galacturonic acids. In assessing fruit ripening method and period of time of fruits, assay of polygalacturonase is an honest indicator. PG activity is additionally measured in pathologic plants too, since an outsized variety of pathogens are reported to provide pectinase enzymes to macerate the plant tissues. Polygalacturonase activity is assessed by either reductometric or viscometric methods. Since measuring is not specific by viscometric method for PG, the reductometric technique is used. The reducing sugar of the extra cellular enzyme was determined according to the Dong and Wong method with a few modifications. The polygalacturonase activity was estimated by assaying the amount of reducing sugar released under assay conditions. The principle of the enzymatic assay is to hydrolyse the pectins in the pomace during ripening of the fruit, which leads to softening of the fruit.

The enzyme assay was performed with the crude enzyme extract from the solid state fermentation setups of Apple pomace that had been setup using strain MTCC 9644.. The reaction mixture (1ml) was prepared by putting equal amounts of polygalacturonic acid as substrate (1%) prepared in acetate buffer (ph 4.5) and suitably diluted enzyme. The mixture was given incubation time in water bath for 50c for 10 min. Followed by incubation, 3ml DNS solution was put to stop the reaction and the tubes were kept for incubation in boiling water (100c) for 15 min then 1 ml of 40% Sodium- Potassium tartarte solution was added and tubes were then left for cooling. The standard was prepared by the volume of sodium acetate buffer and polygalacturonic acid in the ratio 1:1. After cooling the developed color was read spectrophotometrically at 575 nm .The amount of sugar released was quantified using galacturonic acid as a standard. The action of enzyme was figured as the measure of chemical required to discharge a micromole of galacturonic corrosive per min under examine condition.

3.3 Selection of SSF Variables

In this experiment, 3 different fermentation variables were screened for the effects on the Polygalacturonase production. The variables are temperature, number of days and inoculum ratio of the fungus.

3.3.1 Selection of inoculum size

The inoculum ratio was varied in the 4 solid state fermentation that was done. The variation in inoculums size was 0.5ml, 1ml, 1.5ml, 2ml.

3.3.2 Selection of Incubation time

Another parameter varied was the number of days. The solid state fermentation was done for 3 days, 4days, 5 days and 6 days.

3.3.3 Selection of Incubation Tempearture

Variation in temperature was done with incubation temperatures being 25 °C, 30 °C, 35 °C, 37 °C.

CHAPTER 4

RESULTS AND DISCUSSION

==========

4.1 Production of Crude Enzyme extract

Crude enzyme extract was obtained by the process of solid state fermentation. The 1ml inoculum of *A. awamori* was inoculated in 4gm of substrate and 8ml of czapek-dox media. The fermentation procedure was carried out in controlled conditions. The pH condition was maintained at 4.5. The temperature condition was maintained at 30-32 °C. The incubation period was given for 120 hours. At the end of the solid state fermentation process, the substrate was filtered through Whatman filter paper. The culture filtrate was used as the enzyme extract.

4.2 Selection of Inoculum quantity

Every time before setting of solid state fermentation, sub culturing was done. Freshly prepared agar slants were made and used after 72 hours exactly to nullify the variation in inoculum. The concentrations taken for inoculum were 0.5 ml, 1 ml, 1.5 ml, 2 ml. The Optical density was calculated at the end of 72 hours of incubation. The same procedure for crude enzyme extraction and assay was followed. Optical density was calculated at 570nm. Maximum production of enzyme was in set 2, which had 1ml of inoculum.

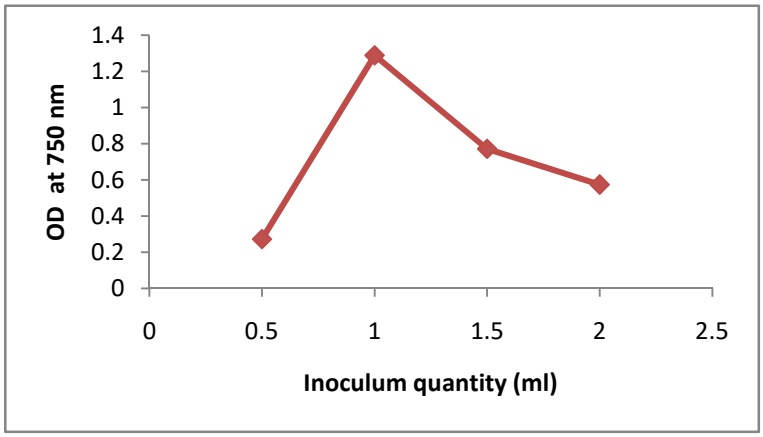

Figure 4.1: Selection of inoculum quantity

4.3 Selection of Incubation time

The optimised inoculum quantity, 1ml, was used as a factor for optimisation of number of days the maximum enzyme activity occurs. The Erlenmeyer flasks were properly labelled as 3, 4, 5, 6 days. Inoculation was done with freshly prepared agar slants that had a growth of 72 hours. Optical density was taken on different days and the readings were recorded. The maximum activity was found on 5^{th} day.

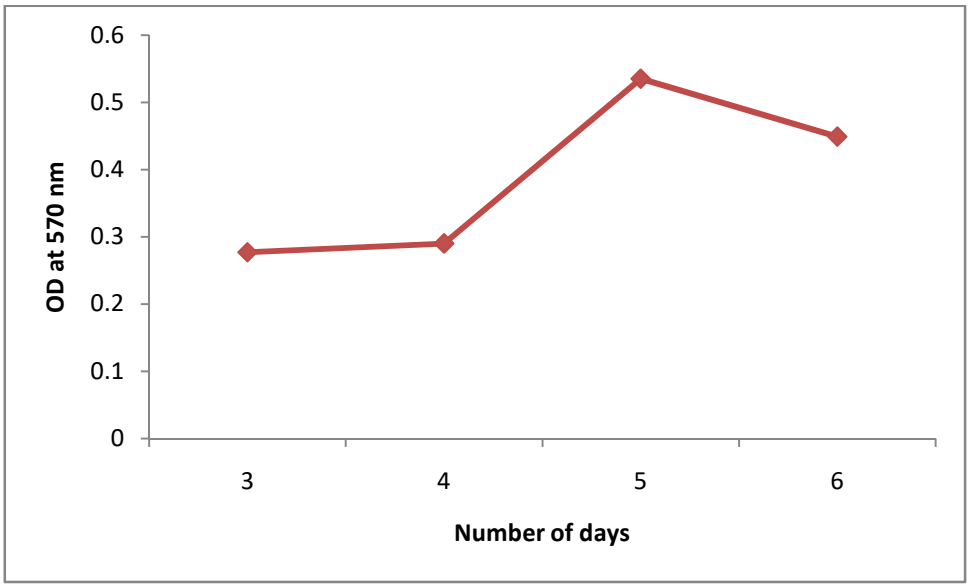

Figure 4.2 : Selection of Incubation time

4.4 Selection of Incubation temperature

The optimum temperature of the enzyme was determined by incubating the enzyme with apple pomace at 25°C, 30°C, 35°C, 37°C by Dong and Wong method. The activity was then assayed by calculating the Optical Density, spectrophotometrically at 575nm. Temperature versus optical density graph was plotted to obtain the optimum temperature at which the enzyme production is maximum.

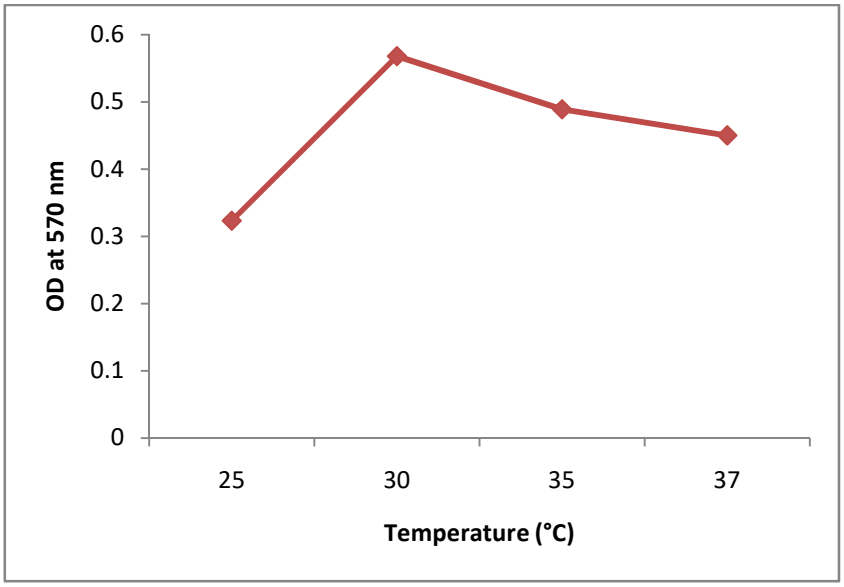

Figure 4.3 : Selection of Incubation Tempearture

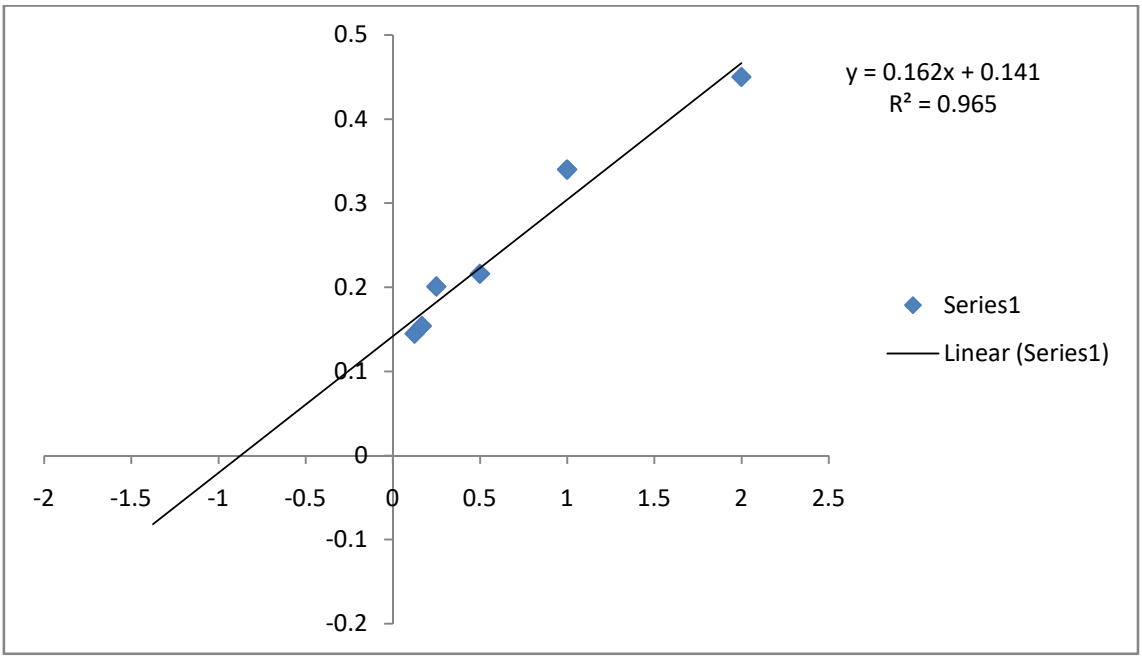

Figure 4.4: Standard curve for enzyme activity determination

CONCLUSIONS

The enormous quantity of organic wastes that are generated every year to support the hunger needs of growing population creates piles of heap of waste. The wastes from agricultural areas can be used as a nutrient source for the growth of micro-organisms. The use of Apple pomace to cultivate *Aspergillus awamori* provides a cheap alternative source of media and also cleans up the environment.

Apple pomace, a development made in broad entireties by the agro sustenance industry, is a fair trademark medium for microorganism advancement in solid state fermentation. The ease of apple pomace makes it conceivably a decent crude material for the creation of high included esteem items. The principle cost in the generation of mechanical compounds is the medium of maturation, apple pomace in this test has been assessed for the creation of a scope of hydrolytic catalysts including xylanase, exo-PG and α-amylase.

In this investigation, the capability of improving pectinase creation by Aspergillus awamori utilizing apple pomace as a wellspring of carbon was assessed. To begin with, apple pomace was examined as the wellspring of pectinase creation because of financial and ecological advantages. Second, additionally investigations were.carried out at various conditions to discover the streamlined conditions for the most extreme creation of the enzyme.

Keeping the concentration of substrate constant as 4gm and the volume of media as 8ml (the substrate to medium concentration constant 1:2) the maximum production of enzyme was with 1ml inoculum. With regard to the stability against temperature, Pectinase activity remained highest at 30 ºC and decreased for higher temperatures. The activity of the enzyme decreased at higher incubation temperatures. The probable reason for thermal denaturation of enzyme. At higher temperatures, there could be disruption in non-covalent linkages. The maximum growth of fungus was found on the 5^{th} day, indicating higher amount of pectinase production.

Thus, the present study was useful in providing a cheaper source of carbon for pectinase production. The response was good from the cheap pectin sources like pomace and fruit leftovers. This helped in the designing a production media at a low cost.

REFERENCES

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1 Ashfaq Khan, S. S. (2012). Production and optimization of pectinase enzyme using *Aspergillus niger* strains in solid state fermentation. Research in Biotechnology, 3(3) , 19-25.

2 Beatriz Gullon, E. F. (2007). Evaluation of Apple Pomace as a Raw Material for Alternative Applications in Food Industries. Food Technology and Biotechnology 45(4) , 426–433.

3 Cordoba Sancho J. & Delgado Fernando A. (2012). Apples: Nutrition, Consumption and Health: 1-37.

4 Danielle Biscaro Pedrolli, A. C. (2009). Pectin and Pectinases: Production, Characterization and Industrial Application of Microbial Pectinolytic Enzymes. The Open Biotechnology Journal, 2009, 9-18

5 Joshi, V., & Atri, D. (2005). Solid state fermentation of apple pomace for the production of value added products. Natural Product Radience, 5(4) , 289- 296.

6 Martin, N., de Souza, S. R., da Silva, R., & Gomes, E. (2004). Pectinase Production by Fungal Strains in Solid-State Fermentation Using Agro-Industrial Bioproduct. Brazilian Archives of Biology and Technology , 813-819

7 Oliyad Jeilu Oumer and Dawit Abate (2017). Characterization of Pectinase from *Bacillus subtilis* Strain Btk 27 and Its Potential Application in Removal of Mucilage from Coffee Beans. Enzyme Research, 2017, 1-7.

8 Optimization of the process parameters for the utilization of orange peel to produce polygalacturonase by solid-state fermentation from an *Aspergillus sojae* strain. (2012). Turkish Journal of Biology 36, 394-404.

9 Pasha, K.M., Anuradha, P., & Subbrao, D. (2013), Screening of pectinolytic fungal strain *Aspergillus foetidus* MTCC 10367 for the production of multienzymes of industrial importance. International Journal of Pharma & Biosciences, 4(2), 1205-1209

10 Sethi B.K., Nanda P.K., Sahoo S. (2015), Enhanced production of pectinase by *Aspergillus terreus* NCFT 4269.10 using banana peels as substrate. 3 Biotech 6:36

11 Reis, S., Rai, D. K., & Ghannam, N. A. (2012). Apple pomace as a potential ingredient for the development of new functional foods. International Conference on Food Safety, Quality and Nutrition (ICFSQN) , 61-66.