PRODUCTION OF PECTINASE BY SOLID STATE FERMEN TATION OF APPLE POMASE

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

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF

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DECLARATION BY THE SCHOLAR

I confirm that the work which is done in this thesis is my own . This work is to be reported in B.tech thesis having title " **Production of the pectinase by apple pomaceusing solid state fermentation**".to be submitted at **Jaypee University of information Technology, Waknaghat ,H.P..**The work presented in this project is mine (Arshiya Chauhan) amd of my project partner (Nishtha Thakur) who worked for 6 months from July 2018 to December 2018 This work was done under the guidance of **Dr.Anil kant Thakur.**The work is not submitted in any other institution for any degree or diploma.

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SUPERVISOR'S CERTIFICATE

This is to certify that the work incorporated in this project titled" " **Production of the pectinase by apple pomaceusing solid state fermentation"** is a work of Arshiya Chauhan (151510) Nishtha Thakur (151820) was also the part of the project for six months i:e July 2018 to December 2018 under my guidance at **Jaypee University of information Technology, Waknaghat,H.P.**

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We all need someone who inspires us to do better than we know now, I must express my gratitude to the people who helped me & guided me during project. It is my pleasure to say that I find myself writing down these lines to express a sincere thanks to various people to help me along the way in completing my training successfully. I would like to thank all of them for their enthusiasm and support.

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Thank you!

Arshiya Chauhan (151510)

INDEX OF SYMBOLS AND ACRONYMS

SSF	Solid State fermentation
Smf	Submerged fermentation
U/gds	Units per gram of dry substrate
min	Minute
hrs	Hours
℃	Degree celcius
U/ml	Units per millie liter
gm	Gram
NA	Nutrient agar
PDA	Potato dextrose agar
WB	Wheat bran
DNS	Di-nitro salicyclic acid
RPM	Round per minute
O.D	Optical density
Nm	Nano meter
d/w	Distilled water
L	Litre
M	Molar
Conc.	Concentration

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ABSTRACT

Apple pomace is waste which is not used in the fruits and juice manufacturing industries..In a Pomace is also used for manufacturing of some products because it is a part of apple.It has a wide commercial application in food , pharmaceuticals, paper and textile and pulp producing industries. Solid state fermentation is utilized for the better productivity of the enzyme.SSF increase the level of protein in raw material which makes it the good cattle cake .Use of the apple pomacefor good manufacturing of enzymes would be more beneficial to HIMACHAL PRADESH where is no use of raw material and it would be simple to assemble small scale manufacturing unit.This study attracts the manufacturing of pectinase exploits SSF by *Aspergillus niger* Analysis were done to progress the different fermentation parameters alike basic moisture level, fermentation time ,pH, and diffrent substrartes mixture mixing with apple pomase. This project surveys the work which is done to use the asset, which can be helpful for setting small ventures.

Keywords: Pectinase, pomase, fermemtation, manufacturing, substrates.

CHAPTER -1

(INTRODUCTION)

Apple is the most favourite fruit among millions of people. India is the 9th largest producers of the apples.Apples are considered as the 4th largest crop in India. About 73% fresh apples are consumed as it is and left 27% is used for producing some products like apple cider, apple juice,wine, apple purees, jams and dried apple products. Himachal Pradesh, J&K and Utaranchal are the largest producers of the apples. In industries 74% of apples are used for juice manufacturing whereas 26% is the pomase. During winter season large amount of apples get wasted by falling down or due to improper packaging are then used for feeding animals.

The waste in the large scale industries are categorized into two parts one is apple pomase and second is the apples which are damaged and disposed into the sorting unit. The rejected apple are also dumped in the apple pomase dumping field. Industries just dump this unutilized segment into the fields. As the water content present in the apple pomase is high it got easily fermented and create disposal problems. It creating some serious environmental pollution. The sugar level is high and this leads to creating soil problems. Using it as a feed for animals reduces this problem of causing pollution, but only few amount of apple pomase is utilized as feed source for animals because it easily and very fastly get spoiled.

The apple pomase genrally consists of seeds,stems and apple peels. The water content which is present in the apple pomace is depend upon the processing technique and the ripening of the apple. It generally contains the starch, carbohydrates, dietary fibers, volatile compound, pectin , reducing sugars and a source of nutrients, antioxidant property.

Pectinase is an enzyme which is found in many fruits like banana apples. It break down the polysaccharide which is present in the cell wall of plant known as pectin.

Keeping all these things the project work is carried out on the manufacturing of the pectinaseenzyme by apple pomase .

There are few fermentation processes which utilizes the liquid medium for the growth of the microorganisms. The medium which is used in this process is in the solid form. The growth of the microorganism required solid medium with few or very little amount of moisture content. SSF is used for the production of some fermented food products, enzyme, organic acids.

Advantages of SSF-

- Simple solids are used as the media in SSF.
- Low energy is required and requirement of investment is very less .
- Less need for sterilization as the microbial contamination is less.
- Industrial and agricultural can be utilized.

Disadvantages of the SSF-

- Utilization of only those microorganism which grow under low moisture content are used
- The monitoring of the SSF is difficult(oxygen level, moisture content)

Objectives of this study

- Quantification of the pectin of the apple pomace which is procured from the HPMC (Horticultural Produce Marketing & Processing Corporation) Shimla Himachal Pradesh.
- To escalat diffrent fermentation conditions which are required for the growth of *Aspergillus niger*
- To check the best propotion of agro- wastes with apple pomase to get the higher yield of pectinase.

CHAPTER 2

REVIEW OF LITERATURE

India is in top 10 countries which are the largest producers of the apple. Apple is the 4th most grown crop in India. Apples are used for making juice, some value added products, jams, apple cider and dried apple products. After the manufacturing of such food items the surplus substance is called as Apple pomase. In the world for the development of fuels,feed ,ehanol, vinegar ony 30-35% of apple pomase is used. In India it is no at all used it all goes waste. It

create a huge economic loss with environmental pollution (Rachana Shalini,Gupta D.K et al.,2009) thus now a days to solve this issue people are developing different techniques for utilizing the apple pomase and developing some products out of it. Using apple pomase as raw material various techniques for producing pectin, ethanol, citric acid, enzymes are developed. The figure below shows the utilization of apples in industries.

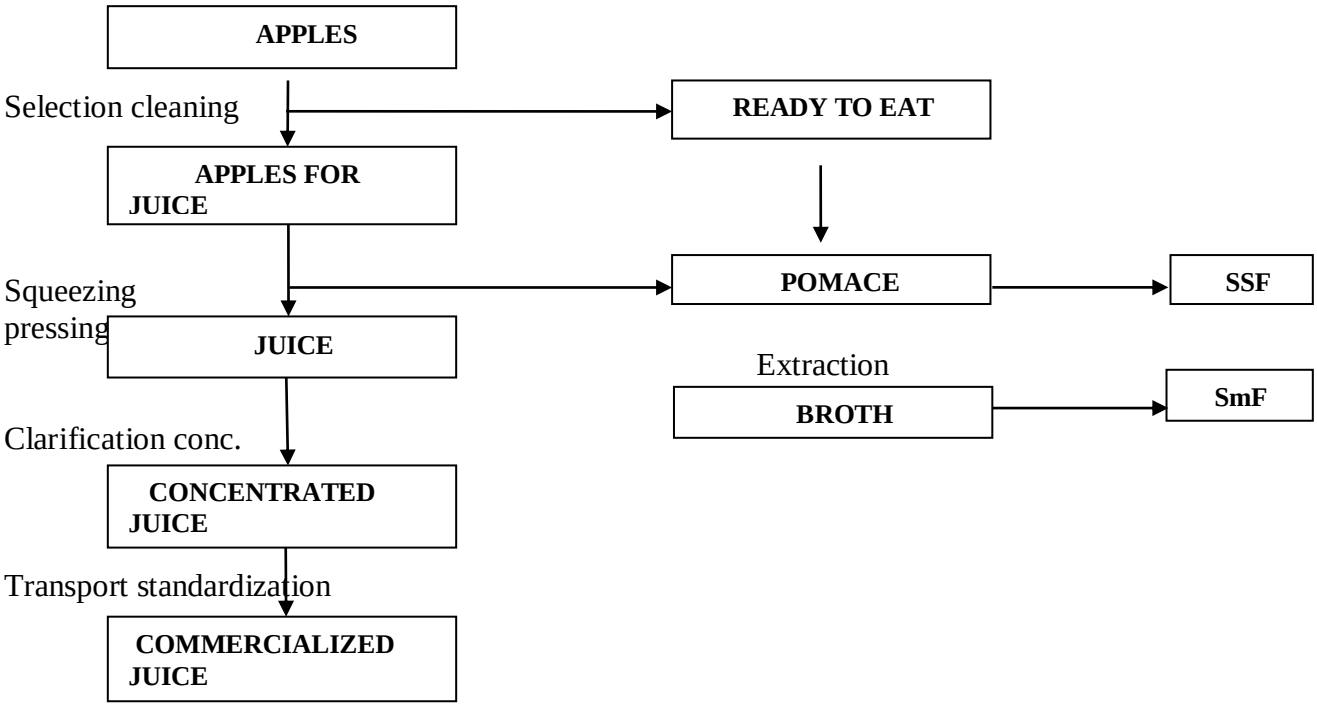

Fig 2.1 Figure showing the processing of the apples in various juice factories.

2.1 Apple pomace composition

Apple pomase is wet in nature so it contains the large amount of the moisture content. It also contains proteins (3.99%), crude fibre (16.16%), acidity(2.39%), pectin(16.95%), sugars(17.35%), Ash(1.65%).The percentage varies from research to research and depending on apples.

Enzymes are very costly in todays world .As in case of pectinase it costs about 1300/kilograms

Table 2.1- Apple pomace compositon (Hang and Woodams et al., 1987)

Composition	Percentage (Wet weight Basis)
Moisture content	75.6%
Protiens	5.1%
Lipid	4.2%
Fibers	4.3-10.5%
Ashe	2.8%
Carbohydrates	9.5-22%
Reducing Sugars	5.7%
Pectin	1.5-2.5%

2.2 Products produced using Apple pomase

2.2.1 Edible products

For the human consumption various products are produced such as jams , jellies, sauce.

1. **Jellys and jams**

   Apple pomace is used for preparing jams of good quality. Apple pomase is to be diluted for the prepararion of the pulp which is required for making of jam. The pulp:sugar is to be optimum. Jam, having the shelf life of only 6 months.
2. **Soft drinks**
3. **Cookies and bread**
4. **Pomase sauce**

2.2.2 Fermented products

Different products like cider, vinegar, vermounth likewise been made from apple pomase

1. **Cider**
2. **Beer**
3. **Vinegar**

2.2.3 Flavour compounds

By extracting with CO_2 and then rupturing it at various temperatures and acquiring the flavourless proportion and a excessive flavoured proportion.Flavour produced by this method

has wide spectrum as compared to the flavour compounds can also produced using distillation process(R.R.Sharma et al., 2003).

2.2.4 Citric acid

Apple pomase is used as substrate for the production of the citric acid by using five fungus strains. *Aspergillus niger,Aspergillus niger* NRRL567 produced the greatest amount of citric acid in the presence of the 4% methanol (Y. D. Hang,E. E. Woodams et al.,1984). The production of the citric acid depend on the apple pomase, content of methonal present in the apple pomase and time and temperature required for the fermentation.

2.2.5 Other industrial products-

1. **Pectin**

   Pectin is used as an main ingredient in the preparation of jellies. Dried apple pomace or apple pomace powder is utilized for the extraction of the pectin from the apple pomace.In this project pectin quantification was also performed by using rapid method described in 3.6. The amount of pectin quantified in this project was 56.85% .The percentage varies due to the variety of apple used in the research.

2. **Ethanol**

   S.cerevisiae is majorly used as the fermenting microbe for the manufacturing of the bioethanol from apple pomase using SSF.The final output of the ethanol depends upon the amount of apple pomace utilized during the process.It is the most valuable product which is produced by apple pomace (R.R.Sharma et al,2003).

3. **Industrial pigments**

   In various countries different strains of microoraganism are used for producing different pigments.*Rhodotorula* strain is used for producing carotenoid pigment, whereas *Monuscus sp.*are utilized for producing Monascorubramin,Rubropunctatin pigments.

Table 2.2 Naturally derived pigments from micro-organism

Micro-organism	Pigments	Color/appearance
Staphylococcus aureus	Zeaxanthin	Golden yellow
Serratia marcescens	Prodigiosin	Red
Phaffia rohodozyma	Astaxanthin	Red
Blakesela trispora	Lycopene β-carotene	Red Yellow-Orange
Flavobacterium spp.	Zeaxanthin	Yellow
Pseudomonas aeruginosa	Pyocyanin Blue	Green
Dunaliella salina	β-carotene	Cream
Monascus sp.	Monascorubramin, Rubropunctatin	Yellow,Orange,Red

4. Animal feed

For making the animal feed fresh apple pomase can be utilized .Fresh apple pomase have high nutritional value and is rich source of fibres, carbohydrates, proteins. As the water content present is in high content the chances of spoilage is high so it is neccasary to use it fresh as a feed .

2.3 Production of the pectinase by using apple pomase

Pectinases were one of the most used and commercially applied in the juice and wine industries(Kashyap, D.R., Vohra, P.K., Chopra, S. & Tewari et al.,2001). These enzye account for 30% of world wide enzyme market and can be produced by using fungi, bacteria, plants, yeasts etc (Uenojo et al.,2003). These help in the degradation of pectin which are used in the juice industries for thickening of juice (Kashyap, D.R., Vohra, P.K., Chopra, S. & Tewari et al.,2001). Pectinase can be produced using solid state fermentation by apple pomase .

2.3.1 Production of pectinase enzyme by using bacteria in SSF –

The bacterial species which are used for producing the pectinase enzyme are *Bacillus subtilis,Bacillus licheniform,Bcillus cereus, Bacillus coagulans and Pseudomonas* species (Babu et al.,1995) .

The substrate which was used in the various studies for the production of the enzyme were wheat waste, ground nut waste, apple pomase. These are the substrate having less amount of the water or moisture content in it.

2.3.2 Fungus utilized for hight yield of the pectinase enzyme-

There are many species of fungus which are used for producing pectinase such as *Aspergillus niger, Aspergillus versicolor, Aspergillus favus, Rhizopus stolonifer, Penicillium jenseni, Trichoderma viridae, Mucor hiemalis*.The best producer of the pectinase was *Penicillium jenseni* and *A.niger* (Priya V &Shashi V et al.,2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Microorganism used

Aspergillus niger was utilized in this study for producing the pectinase using SSF by apple pomase.

3.2 Reviaval of culture and inoculums preparation

Aspergillus niger spores were taken from the deep freeze storage of dried medium. PDA plates were prepared for performing the revival and culturing of the Spores as shown in fig.3.6.

Inoculated the fresh culture of fungal spores. As shown in fig 3.6. After 6-10 days the Growth of the funus was checked. Then 10ml tween-80 was added & spores were removed by utilizing sterilized loop.

3.3 Fungal culture staining

Lactophenol cotton blue staining method was used for the staining of the fungal culture.

3.3.1 Methodology for staining of fungal culture

70% alcohol about a drop placed on a perfect magnifying slide . The wire was held over the Bunsen flame until the entire wire becomes red colour like hot .Wait until the wire properly cool down.The fungal culture was immersed by adding the drop of 70% alcohol.The needle and the inoculating loop were sterilized again.Before the alcohol dries out add one or at most two drops of the lacto phenol.cotton blue stain.Coverslip was lower down slowly onto the slide, avoiding the formation of theair bubbles. The slide was visualized under 40Xand 100x magnificationshown in fig 3.6. Coverslip was lower down slowly onto the slide, avoiding the formation of the air bubbles The slide was visualized under 40x and 100x magnification shown in fig 3.6.

3.4 Reagents required

3.4.1 Salt solution

Salt solution was prepared by the addition of KH_2PO_4 (2g/NaCl(1g/l),$MgSO_4$(1g/l) in distilled water & set pH to 5.

3.4.2 Tween-80

Adding 1 ml Tween-80 in the 50 ml distill water and then making up the volume to 1000ml.

3 4.3 1% Galacturonic acid

Adding 1mg galacturonic acid in 10ml distilled water , heated slowly and dissolved in galacturonic acid and make the vol. to 100 ml.

3.4.6 Phenol red indicator

Grind 0.1gm of dry powder in a mortar with 28.2ml of 0.01N NaOH . 250 ml distill water was added for diluting the mixture.

3.5 PDA media prepapropotionn

1 litre of PDA media was prepared by adding 39gm of PDA into the flask then added 1

litre distill water and then mixed it.kept it for boiling so that the PDA got dissolved properly .Autoclaved at 121℃ for 15 minutes. (shown in fig 3.1)

3.4 Rapid method used for the quantitative determination of pectinase-(Shelukhina et al., 1994)

0.5gm samples of dried apple pomace was weighed in vials.1-2ml ehanol was added and then dissolved in 10-20ml distill water.Per 10ml of solution 2ml of 1N NaOH was added

and thoroughly mixed.Kept at 20-25℃ for 20minutes for de-esterification.Then,suspension was acidified by adding 1N HCL(hydrochloric acid)and mixedthoroughly.50ml of 0.1N HCL was added .Kept at room temperature for about 5-7 minutes.Measured the volume of flask..Using whatman paper sample was filterate.From the filtrate ,20ml was pipeted into a 250ml flask.The residue remaining was mixed with the left filtrate.The filtrate in the flask and mixture were separately titrated with 0.1N NaOH using phenol red.. Level of HCL from the actual vol. was measured .Using the result of the titrationthat is mixed with the filtrate . Change in colour from yellow to red was observed as seen in fig 4.1.

Formula used for the quantification pectinase (in percentage(P%)) =

$$\frac{V_2 - V_1 \times 176 \times 0.1 \times K \times 100}{1000W}$$

3.7 Reducing sugar quantification in fermented apple pomase.

DNS of each sample was performed after the fermentation for checking the amount of sugars which are there in the pomase. The solution was prepared as explained in 3.10 dilute the sol. 100 times and after that different glucose standard were made (0,0.2,0.4,0.6,0.8,1.0mg/ml).Add 3ml of DNS sol. to each test tube and kept it in water bath for around 12minutes. Test tubes were removed from the water bath and wait until the temperature of the test tubes were cool down .Take O.D at 540nm .

3.8 Enzyme assay -

Enzme assay was calculated by adding 1.25ml of galacturonic acid,5ml of acetate buffer of pH5.Crude extract was diluted 10 times then added it in the flask. The solution was incubated at 50℃ for 12 minutes. Then DNS method was done.

Enzyme activity =

$$\frac{\mu Mol\ of\ product\ formed\ \times Total\ volume}{Incubation\ time(min) \times ml\ of\ crude\ enzyme\ extract}$$

3.9 Statistical analysis-

As the experiments were performed in triplicates data was analyzed using M.S Excel. to know the difference in all three samples.One way ANOVA was used .

3.10 Methods used to set SSF

10gm of dried apple pomace was added in the flask. 2mlSalt sol. was added. Then 60ml distilled water was added to make moisture level 60%. Added equal amount of water as the percentage mentioned.The flasks were autoclaved for 15 minutes at 121℃ . 2ml of spore suspension was inoculated into the flask.Then various culture conditions were made such as moisture level, pH, fermentation time, other agriculture waste ratio.100ml 0.1%tween-80 was added every flask. After fermentation process is completed the sol. was shaked for 60 minutes at 160rpm .Then centrifuged the solution at 18000rpm for 10 minutes.The pellets were discarded and the supernatant was kept. The sol. was divided into two equal divisions for performing reducing sugar quantification and checking the enzyme activity .The values got from 3.7 were substracted from 3.8 that was the actual sugar which was produced by the enzyme.

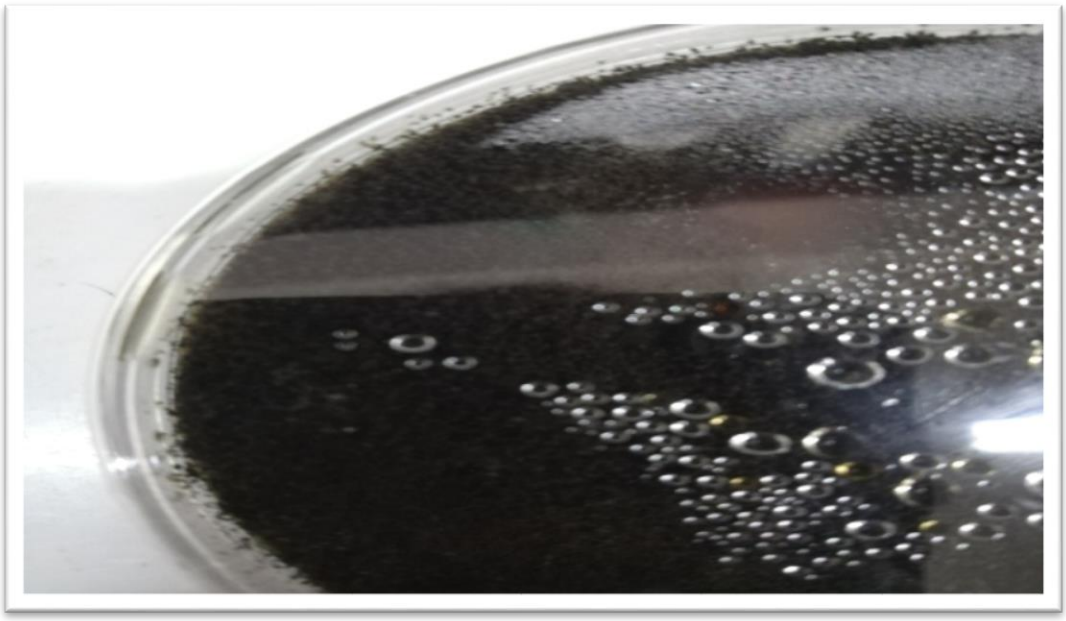

Fig 3.1-*Aspergillus niger* plate.

Fig. 3.2 Wet apple pomase.

Fig. 3.3 Sieved and dried potato peels

Fig. 3.4 Grinded and dried apple pomase.

3.11Experiment 1- Escalation of intial moisture level.

10gm of dried apple pomace was added to fifteen different flasks as the experiments was performed in triplicates. Three same percentages were labeled over three different flasks.2ml salt sol. and water was added in each flask. According to the percentage labeled (30%, 40%, 50%, 60%, 70%) in the flask then 2ml spore sol.was added in each flask and kept it for incubation.After the fermentation process was completed enzyme activity of each flask was calculated to check the growth of the fungus and the production of enzyme.

3.12 Experiment no. 2- Escalation of fermentation time.

10g of dried apple pomase was added to fifteen different flasks as the experiments was performed in triplicates .Three same fermentation time were labeled over three different flasks. 2ml of salt sol. and 60ml water was added in each flask(got result from experiment 4.3) 2ml of spore sol.was added in each flask and kept it for five different incubation time (44hrs 72hrs,96hrs,120hrs,144hrs). After the fermentation process was completed enzyme activity of each flask was calculated to check the growth of the fungus and the production of enzyme.

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3.13 Experiment 3- Escalation of pH .

10g of dried apple pomase was added to fifteen different flasks as the experiments was performed in triplicates .Three same pH were labeled over three different flasks. 2ml of salt sol. and 60ml water was added in each flask(got result from experiment 4.3) .Diffrent pH (pH3 ,pH4, pH5 ,pH6 ,pH7) of the solution was measured.Then 2ml of spore sol.was added in each flask and kept it for 96hrs incubation time (got result from experiment 4.4). After the fermentation process was completed enzyme activity of each flask was calculated to check the growth of the fungus and the production of enzyme.

3.15 Experiment 4- Escalation of the various propotion of the agro-waste(potatopeels) with apple pomase

Dried apple pomace and potato peels powder was added in the ratio of 1:1, 1:2, 1:3 into nine different flasks. The experiments was performed in triplicates.The three same ratios were labeled over three different flasks. 2ml of salt sol. and 60ml water was added in each flask(got result from experiment 4.3) .The pH of the sol. was set at pH5(got results from experiment 4.5)Then 2ml of spore sol.was added in each flask and kept it for 96hrs incubation time (got result from experiment 4.4). After the fermentation process was completed enzyme activity of each flask was calculated to check the growth of the fungus and the production of enzyme.

3.15Observation

3.15.1 Fungal culture

Aspergillus niger is known to be a filamentous fungi having the dark brown coloured spores which are conidia used for differentiating them from other species in the same genus.All these feature are clearly seen in fig 3.5

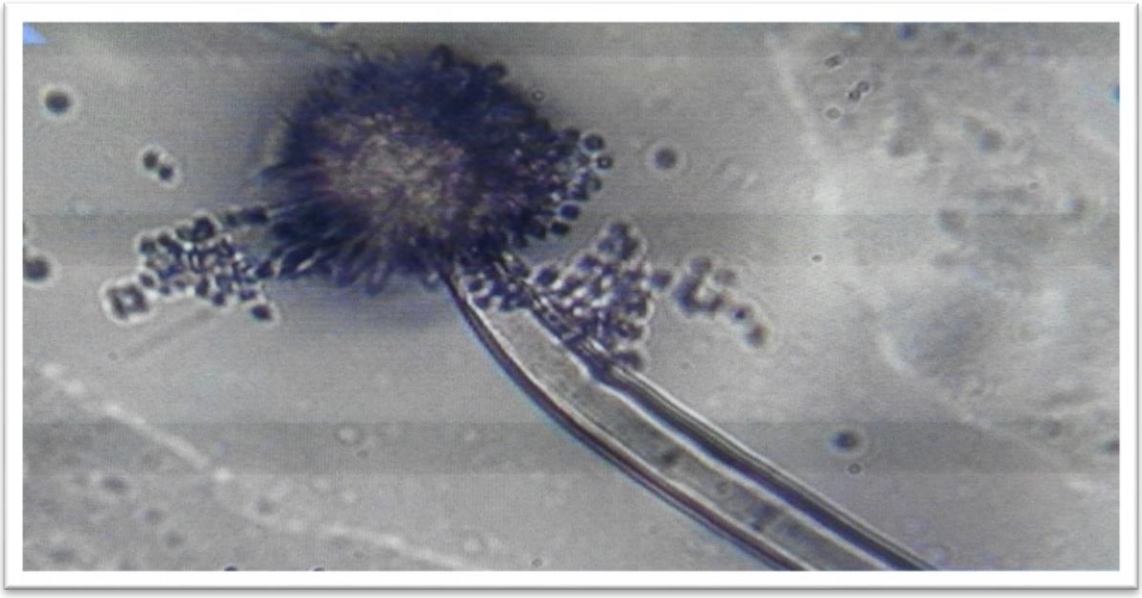

Fig. 3.5-Observation of fungus under 40x dramatization after lactophenol cotton blue staining method.

3.15.2 Spore count

Using haemocytometer spore count was calculated.The spores from the fungus plate were removed and collected in a centrifuged tube. The density of the spores was set to 1×10^7spores/ml then dilutions were performed to to set the density.

Concentration of spores =

$$\frac{Number\ of\ cells\ counted}{Chamber\ counted \times volume\ of\ chamber}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Average Pectin level found in apple pomase

Pectin quantification was performed in triplicates and the following values V1 and V2 were obtained from titration.By using formula mentioned above in 3.6 pectin percentage was calculated

Fig 4.1 Color change upon titration during the quantification of pectin level in apple pomace(yellow to red)

Table No.4.1 Showing the pectin percentage calculated.

V1	V2	P%
18.3	33	56.9
16.2	30.6	55.75
17.1	32	57.9

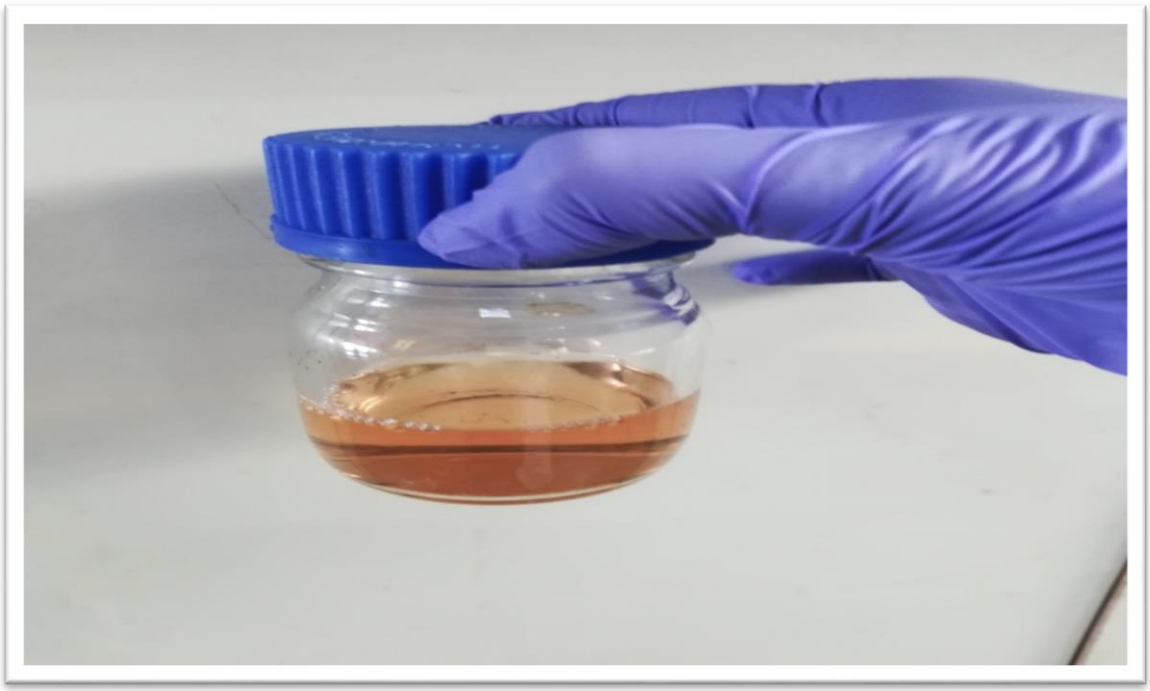

Fig 4.2 Change of color showing the presence of pectin in the apple pomase.

4.2 Escalation of moisture level

In this experiment the moisture level of the apple pomace was set. To check the moisture level required by fungus for growth. Five different moisture level were set (30%, 40%, 50%, 60%, 70%) (as shown in fig 4.3) out of which the initial moisture level of 60% giving the utmost enzyme activity after five days of the fermentation. At 30% and 40% of moisture level the enzyme activity was almost same. The highest enzyme activity was found at 60% which was 3666.667±478.42 U/gds. The enzyme activity declined at 70% moisture level. 60% is the best or optimum moisture level which is required by the fungus for the growth(as shown in fig4.4 and4.5).

Fig 4.3 Settling of initial moisture propotion (30% ,40% ,50% ,60% ,70%) for the highest yield of enzyme

Table No. 4.3 Mean enzyme activity of pectinase at various moisture level .

S.No.	Moisture level of Apple pomase	Enzyme activity in (U/gds)
1	30%	3666.667±478.42
2	40%	3961±256.12
3	50%	3455.333±345.36
4	60%	4448±96.03
5	70%	3384.667±198.25

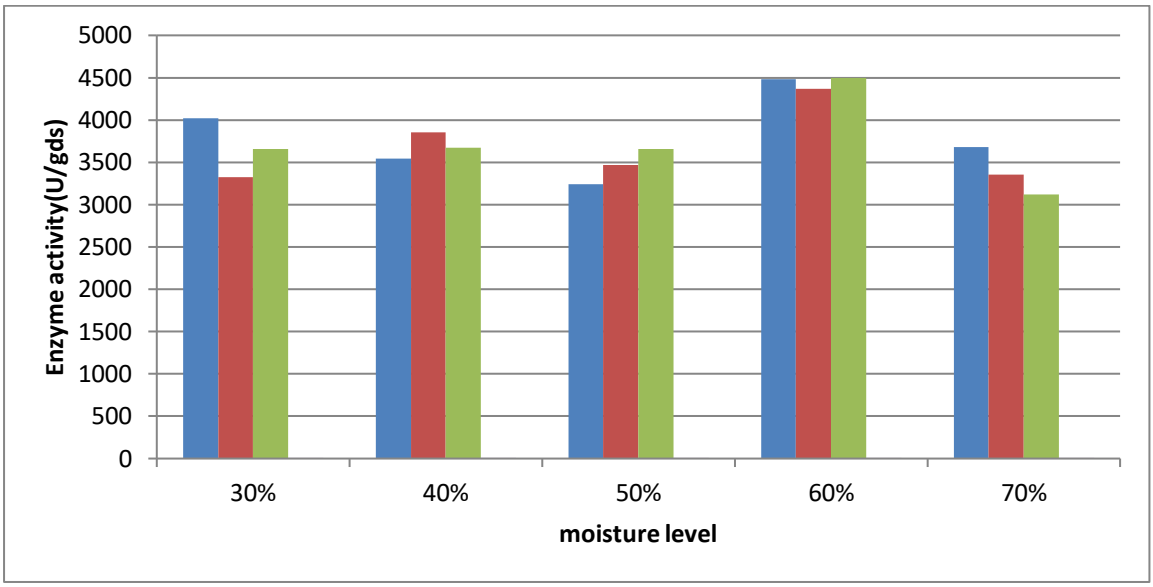

Fig4.4 Pectinase enzyme activity found at different moisture propotions.

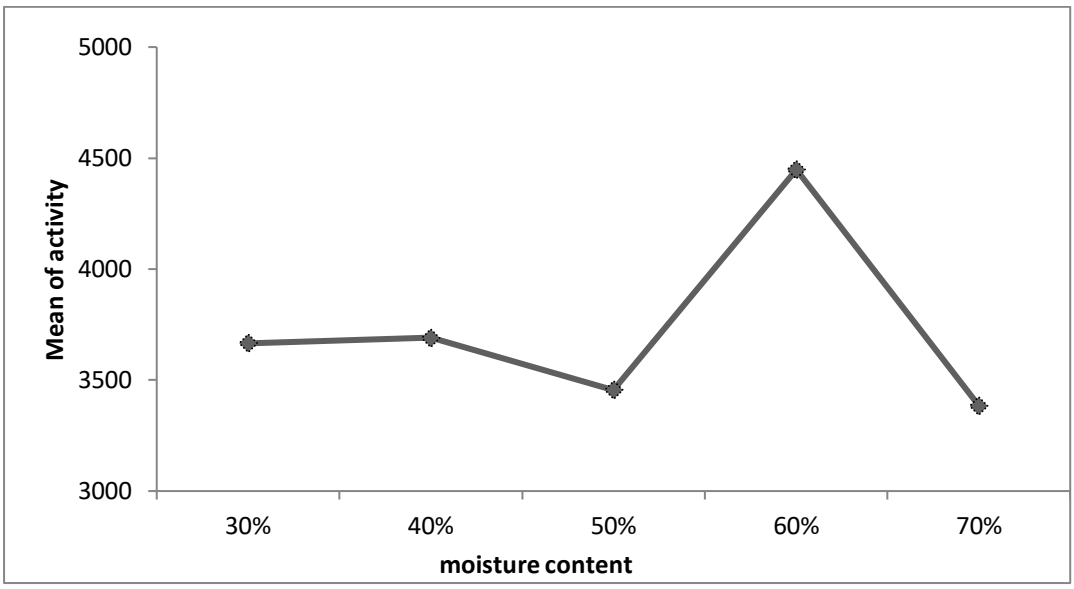

Fig4.5 Mean enzyme activity of pectinase depicting the best moisture propotion(60%).

4.4 Escalation of fermentation time

The fermentation time was set to check the best fermentation time required by the fungus to grow and to get the maximum enzyme production .The fermentation was set at five different

time (48hrs, 72hrs, 96hrs, 120hrs, 144hrs,)(as shown in fig 4.6)and the moisture level was set to 60% as it shows the maximum enzyme production (results from previous experiment 4.3).The best time for the manufacturing of enzyme was found out to be 96hrs as seen in fig 4.6 that the enzyme production was highest at 96hrs. The highest enzyme activity was 6513±65.32U/gds at 96hrs and the least enzyme activity was at 144hrs which was 846.667±17.02U/gds. The enzyme activity was increasing from day2 to day4. After day 4 the activity decreased as seen in fig 4.7. The difference between the highest and the lowest enzyme activity was 5666.333±48.3U/gds ,it is a huge diffrence. Results depicting that the *Aspergillus niger* takes four days for the best growth in SSF.

Fig 4.6 Settling of the different fermentation time(48hrs, 72hrs, 96hrs, 120hrs, 144hrs)

Table No. 4.4 Mean enzyme activity of pectinase at various fermentation time .

S.NO.	Fermentation time (in hours)	Enzyme activity in (U/gds)

1	**48hrs**	**1183±19**
2	**72hrs**	**4383.333±37.26**
3	**96hrs**	**6513±65.32**
4	**120hrs**	**3128.667±55.98**
5	**144hrs**	**846.667±17.02**

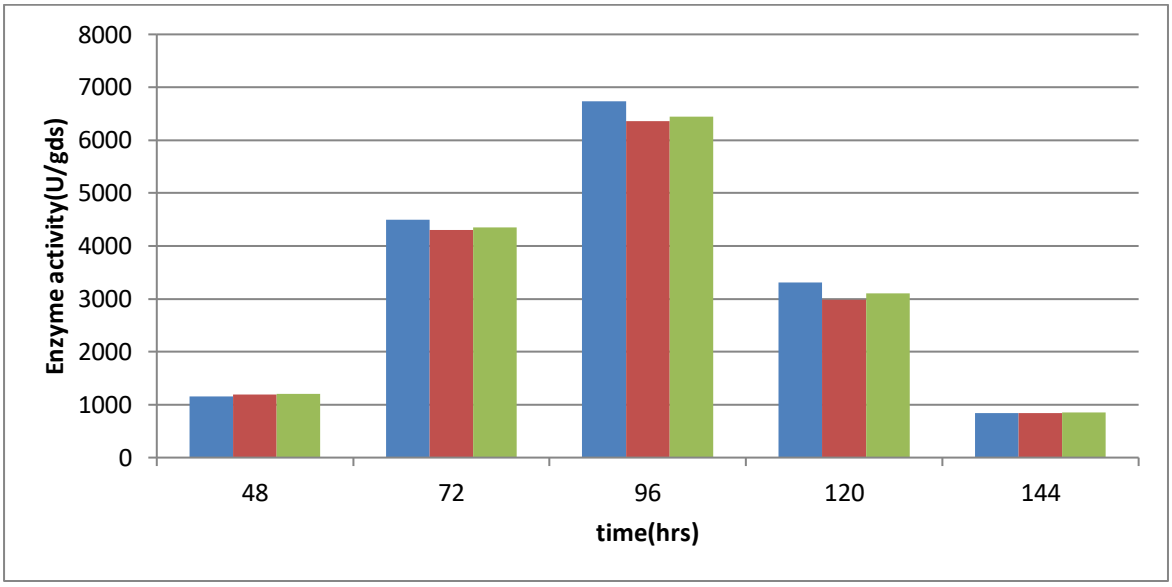

Fig 4.7 Pectinase Enzyme activity in which was done in triplicates found at different fermentation time.

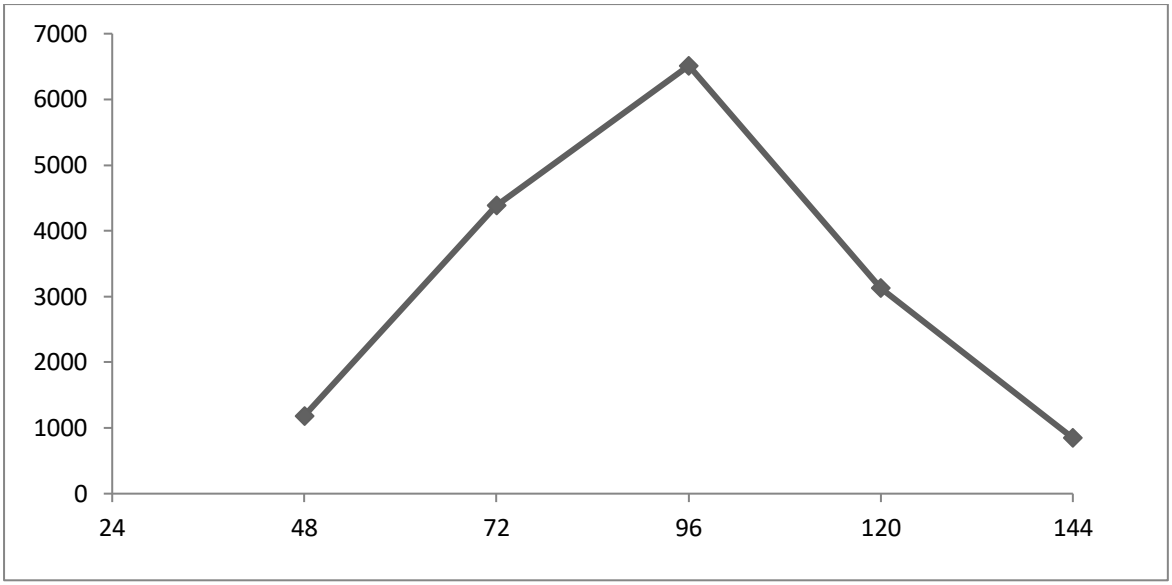

Fig 4.8 Mean enzyme activity of pectinase depicting the best fermentation time (96hrs) .

4.5Escalation of the pH

Different pH (3,4,5,6,7(shown in figure4.9)) were set to check the best pH required by the fungus for the growth and the manufacturing of the enzyme was best.The pH at which the enzyme production was found highest was pH5 with 60% moisture level and 96hrs of fermentaion time.Figure 4.10 and 4.11 showing that at pH3 and pH4 enzyme activity was low. Then there was an increase in the pH at pH5 and then it decreased again .The graph showing that at pH5 the enzyme activity was at highest peak 4597.66±176U/gds The difference between the highest and lowest enzyme activity was around 1280±77.24U/gds).

Fig4.9 Preparation of the setting the different pH for the manufacturing of enzyme

Table No.4.5 Mean enzyme activity of the pectinase at various pH obtained .

S.No.	pH	Enzyme activity(U/gds)
1	pH3	3211.33±267.67
2	pH4	3832.667±456.45
3	pH5	4597.66±176
4	pH6	4006.33±125.65
5	pH7	3317±98.76

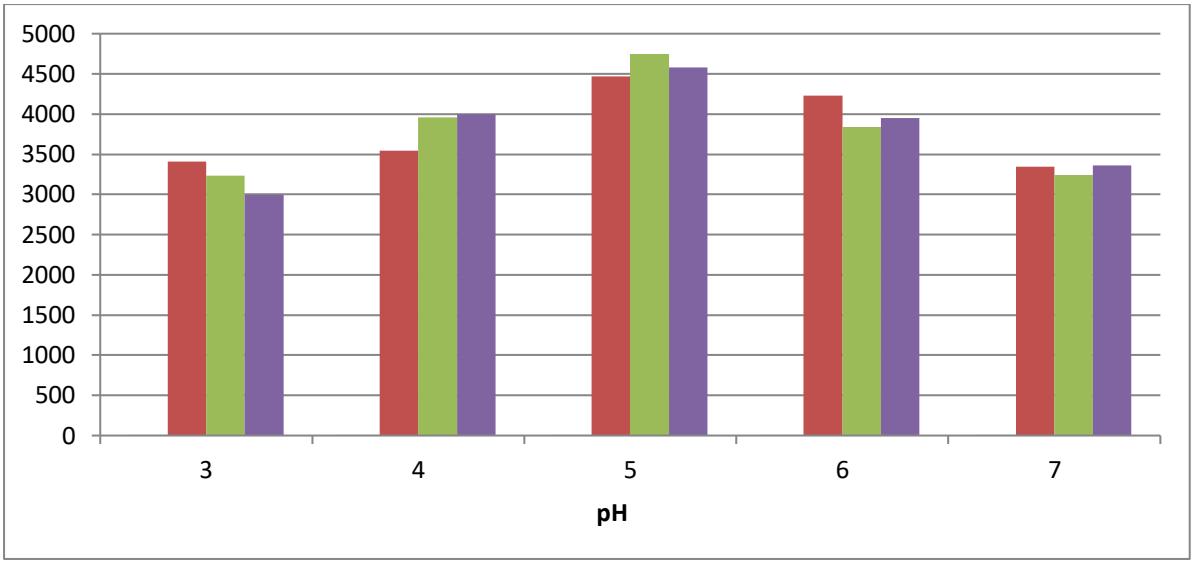

Fig 4.10 Enzyme activity of pectinase found after setting various pH after fermentation of apple pomase.

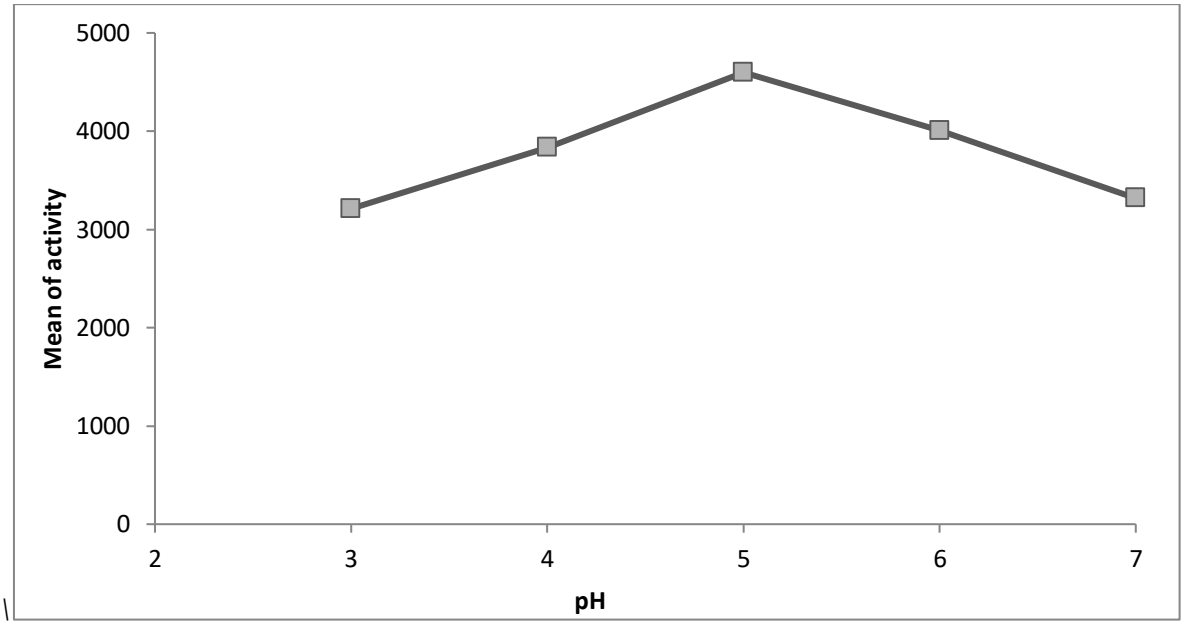

Fig 4.11 Mean enzyme activity of pectinase depicting the best pH (pH5) for the fermentation of apple pomase

4.6Escalation of different propotion of potato peels with apple pomase

Used peels of potato with the apple pomase as a mixed substrate for the growth of the fungus and the manufacturing of the enzyme.Using these both or combining these both together increased the yield of the enzyme.Table 4.7 showing the three different propotion which were used for the production. The propotion(1:2) of potato peels with applepomase depicting the best enzyme activity as comparing with another propotions.The enzyme activity was found to be 6812.66±2.67U/gds .The least enzyme activity was found at 1:3 propotion with 4966±176.98 enzyme activity.Fig4.11showing the graphical representation of differences in the enzyme activity. Fig 4.12 showing the mean enzyme activity at various propotions.

Table No.4.6 Mean enzyme activity of pectinase at various propotions of potato peelswith apple pomaceobtained under SSF of apple pomace

S.No.	Substrate propotion	Enzyme activity in(U/gds)
1	**PA:AP (1:1)**	5831±274.98
2	**PA:AP(1:2)**	6812.66±2.67
3	**PA:AP(1:3)**	4966±176.98

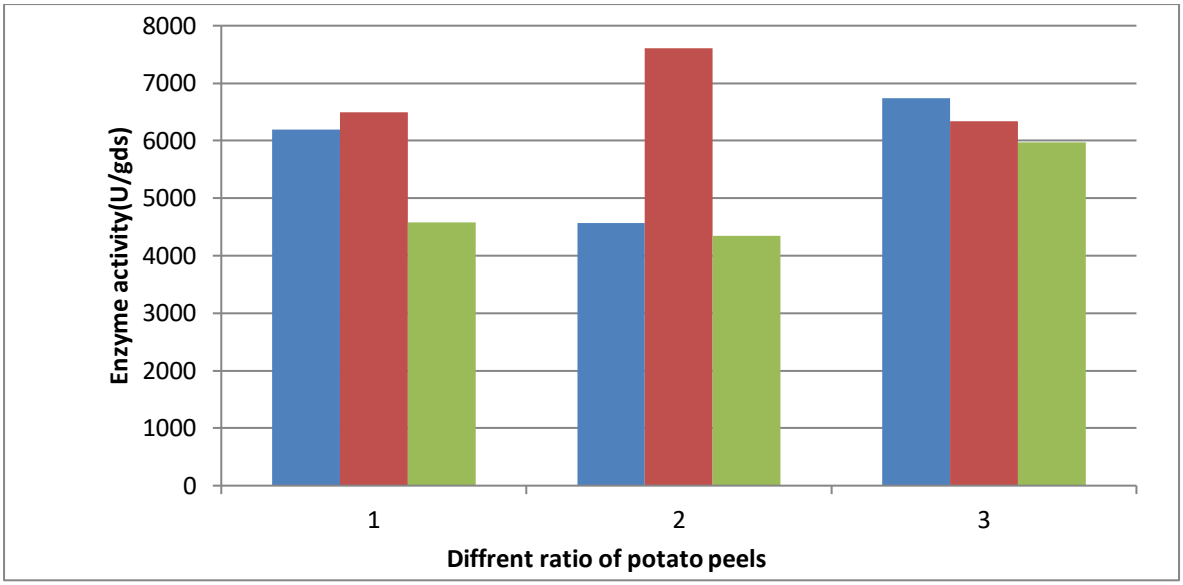

Fig 4.12 Enzyme activity of pectinase in which was done in triplicates found after different propotions of potato peels with apple pomase(1:1(potato peels and apple pomase)), (1:2(potato peels and apple pomase)), (1:3(potato peels and apple pomase)) .

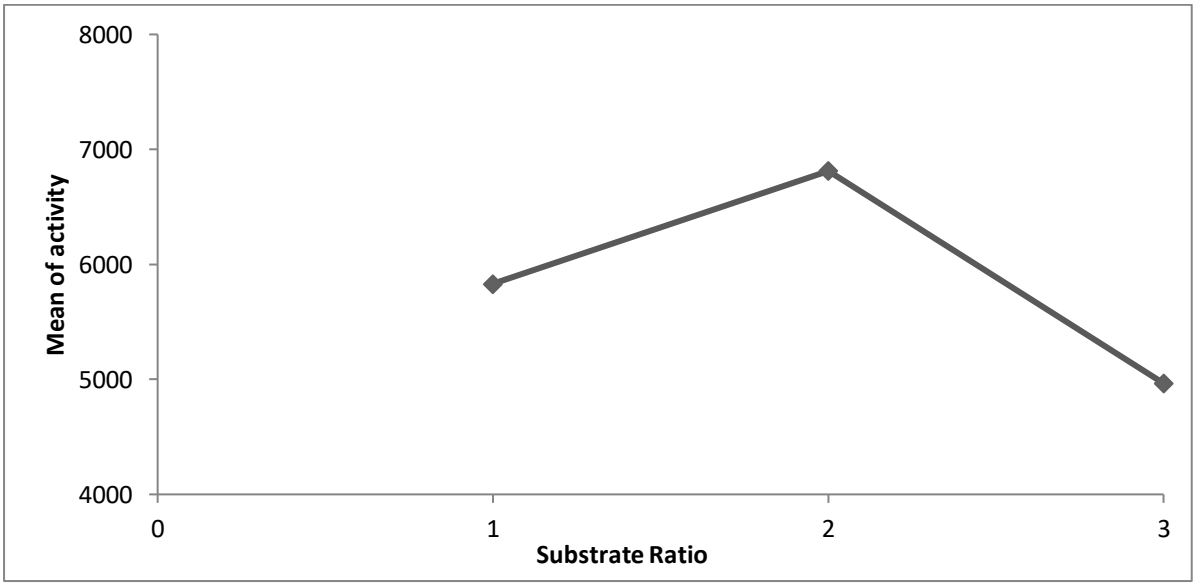

Fig 4.13 Mean enzyme activity of pectinase which was done in triplicates found after different propotions of potato peels with apple pomase(1:1(potato peels and apple pomase)), (1:2(potato peels and apple pomase)), (1:3(potato peels and apple pomase)) .

TableNo. 4.7 Effect of different parameters(Fermentation time , moisture level, pH, different propotion of potato peels)on enzyme activity of pectinase produced .

Moisture level	**Enzyme activity in (U/gds)**	**Fermenta -tion time**	**Enzyme activity in (U/gds)**	**pH**	**Enzyme Activity in (U/gds)**	**Different propotio n of potato peels**	**Enzyme activity in (U/gds)**
30%	3666.667± 478.42	48hrs	1183± 19	pH3	3211.33± 267.67	1:1	5831±274.9 8
40%	3961± 256.12	72hrs	4383.333 ± 37.26	pH4	3832.667± 456.45	1:2	6812.66± 2.67
50%	3455.333± 345.36	96hrs	6513± 65.32	pH5	4597.66± 176	1:3	4966±176.9 8
60%	4448± 96.03	120hrs	3128.667 ± 55.98	pH6	4006.33± 125.65		
70%	3384.667± 198.25	144hrs	846.667± 17.02	pH7	3317± 98.76		

CHAPTER -5

CONCLUSION

The study was done using apple pomace which is a waste product and was utilized for producing good yield of pectinase. The apple pomase was procured from HPMC Shimla,Himachal Pradesh.The study was done to check or escalate the different conditions used for pectinase manufacturing using SSF of apple pomace.Various paramaters like moisture level , fermentation time, pH ,supplementing another agro-industrial waste with apple pomace.

The combination found in this study was moisture level 60%, fermentation time 96hrs, pH5, 1:2 propotion of apple pomase with potato peels, gives the best yield.

This project was performed at small scale to check the average production of the enzyme using the agro-industrial waste by SSF. This study found the good amount of enzyme production in small scale. As Himachal Pradesh is one of the largest producers of apples. Farmers just dump the extra ripened apples and juice industries also dump the left part which is pomase. By using this study it may result in an earning source for the farmers and local people of Himachal Pradesh.

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