CHARACTERIZATION OF PECTINASE ENZYME PRODUCED BY ASPERGILLUS NIGER USING PINEAPPLE PEELS AS SOLE SOURCE OF CARBON

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Abstract-The agricultural wastes generated frompineapple (Ananascosmosus)represents about 35% of the entire fruit. These wastes can be converted to most useful products such as pectin. Pectin was extracted from pineapple peels with a percentage yield of 8.33% at pH 2.2 and temperature of 70° C.The Aspergillusniger was isolatedfromsoilcontainingdecomposingpineapplepeels and was induced to produce extracellular pectinaseinsubmergedfermentationusingpectinextracts frompineapplepeels. The extracted enzyme was purified and then characterized for its enzyme activity.

Keyword-Pineapple peels, Pectinase, enzyme activity and Aspergillusniger.

Introduction

Pineapple (*Ananascosmosus*) belongs to Bromeliaceae family. This is a tropical plant and its edible fruit is a multiple fruit consisting of coalesced berries. Pineapple waste can be bio-transformed in to by-productssuch as pectin, dietary fibers and pectinases. Pectin is one of the major components of the primary cellular walls in the middle lamella of plant tissues. Pectin was first isolated and described in 1825 by Henri Braconnot (Bracconot and Keppler, 1825. Pectinasescanbeproducedbybothsubmergedandsolidstatefermentation(SSF). Submergedfermentationiscultivationofmicroorganismsinliquidbroth.Itrequireshighvolumesofwater,continuousagitationandgenerateslotofeffluents. SSF incorporatesmicrobial growthandproductformationonorwithinparticlesofasolidsubstrate(Mudgett,1986)underaerobicconditions. Pectinasesareagroupofenzymes,whichcausedegradationofpectinthat,arechainmoleculeswitharhamnogalacturonanbackbone;associatedwithotherpolymersandcarbohydrates.Thesepectinaseshavewideapplicationsinfruitjuiceindustryandwineindustry.Infruitjuiceindustry,itisusedforclarification;reductioninviscosityiscausedwhichultimatelyleadstoformation ofclearjuice.

Abbreviations

UDP-D-Uridinediphosphate
PDA-PotatoDextroseAgar
SmF-Submerged fermentation
SSF-Solidstate fermentation

History and Description of Pineapple

Pineapple (Ananascosmosus) is the common name for a tropical plant and its edible fruit, which is actually a multiple fruit consisting of coalesced berries. It was given the name pine apple due to its resemblance to a pine cone. The pine apple is the most economically important plant in the Bromeliaceae family. The word "pineapple" in English was first recorded in 1398, when it was originally used to describe the reproductive organs of conifer trees. The term pine cone for the productive organ of conifer trees was first recorded in 1694. When European explorers discovered this tropical fruit, they called them pineapples (Wikipedia, 2011). The popularity of the pineapple is due to its sweet-sour taste. The core of the pineapple is continuous with the stem supporting the fruit and with the crown, a feature unique among cultivated fruits. The stems and leaves of the pineapple plant are sources of fiber, which can be processed in to paper and cloth. The cloth made from pineapple fiber is known as 'pinacloth'and was in use as early as 1571. Parts of the pineapple plant (Fig.1) are used as silage and hay for cattle feed such as the processed wastes in the form of pomace or centrifuged solids from juice production (Wikipedia, 2011).

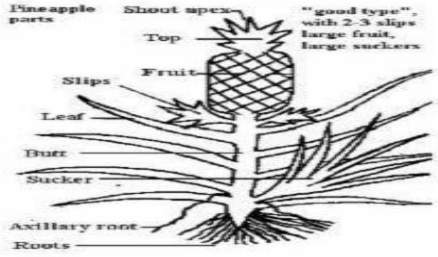

Figure.1: Parts of a pineapple fruit (Elfick, 2007).

Plant Cell Wall

Plant cell walls consist of plant middle lamella, primary cell wall and secondary cell wall as can be seen in Fig.2. The primary walls of enlarging plant cells are composed ofapproximately 30% cellulose, 30% hemicellulose and 35% pectin with about 1-5% structural protein (glycoprotein) on a dry weight basis (Cosgrove, 1997).

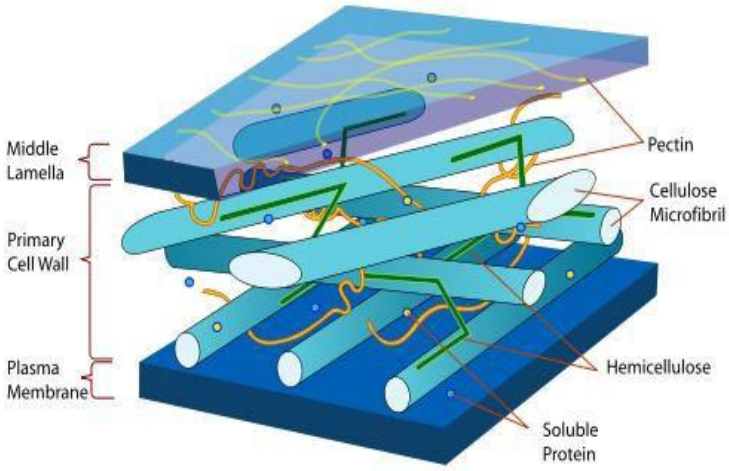

Figure.2: Structure of the Plant Cell Wall (Carpita and Gibeaut, 1993).

The Middle Lamella of the Fruit Cell

The middle lamella is the first layer formed during cell division, and can also be seen as the space between the cell walls, and as the connecting region between adjacent cells, binding cells together. The highest concentrations of pectin are found in the middle lamella of cell walls, with a gradual decrease as one passes through the primary wall toward the plasma membrane (Kertesz, 1951).

Pectic Substances

Pecticsubstance is the generic name used for the compounds that are acted up on by the pectinolytic enzymes. They are high molecular weight, negatively charged, acidic, complex glycosidicmacromolecules (polysaccharides) that are present in the plant kingdom. They are present as the major components of middle lamella between the cells in the form of calcium pectateand magnesium pectate(Rastogi, 1998). The synthesis ofpecticsubstances occurs in the Golgi apparatus from UDP-D-galcturonic acid during early stages of growth in young enlarging cell walls (Sakai *et al*., 1993).Compared with young, actively growing tissues, lignified tissues have a low content of pectic substances. The content of the pecticsubstances is very low in higher plants usually less than 1%. They are mainly found in fruits and vegetables, constitute a large part of some algal biomass (up to 30%) and occur in low concentration inagricultural residues (Table:1). Pectic substances account for 0.5–4.0% of the fresh weight of plant material (Kashyap*et al*., 2001; Sakai *et al*., 1993). Contrary to the proteins, lipids and nucleic acids, which are polysaccharides, pecticsubstances do not have defined molecular masses.

Pectin

Through various studies, it has been brought in notice that the structure of pectin is difficult to determine because pectin subunit composition can change during isolation from plants, storage and processing of plant material (Novosd'skaya, 2002). Pectin was first isolated and described in 1825 by Henri Braconnot(Braconnotand Keppler., 1825). At present, pectin is thought to consist mainly of D-galacturonic acid (Gal A) units (Sriamornsak, 2002), joined in chains by means of α(1-4) glycosidic linkage (Fig.3).

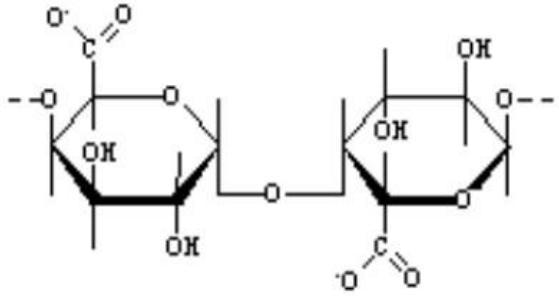

Figure.3: Structure of Galacturonic Acid (Pilnikand Voragen, 1993)

GeneralProperties ofPectins

Pectinissolubleinpurewaterasmonovalentcation(alkalimetal)saltsofpectinicandpecticacids;areusuallysolubleinwaterunlikedi-andtrivalentcationsaltsthatareweaklysolubleorinsoluble(Sriamornsak,1998).DilutepectinsolutionsareNewtonianbutatamoderateconcentration,theyexhibitthenon-Newtonian,pseudoplasticbehaviour and characteristics.

FermentationConditions

Pectinasesareconstitutiveorinducibleenzymesthatcanbeproducedeitherbysubmerged(AquilarandHuitron,1999)orsolidstatefermentation(Acuna-arguelles*etal*.,1995).Variousfactorsaffectingtheproductionofpectinaseareconcentrationofnutrients,pH,temperature,moisturecontent,influenceofextractionparametersonrecoveryofpectinasesandtheeffectsplayedbytheinducers.Bothcarbonandnitrogensourcesshowoveralleffectontheproductivityofpectinases(Catarina*etal*.,2003;AlmeidaandHuber,2011).
Pectin,glucoseandsucrosewhenaddedtothemediainhigherconcentrationhavearepressioneffecton thestudiedenzymeactivity(Maria*etal*.,2000)ofthevariousnitrogenousmatters thatcanbeused.Optimumsourcesare$(NH_4)_2SO_4$,yeastextract,soyabeanpulppowder,soyapeptone. TemperatureandpHarealsoimportantparameters, wherepHis regulatedusingamixtureofsourcesofnitrogenwhen*Aspergillusniger*isbeingused,pHturnstobeacidic.Moisturecontentinthesubstratealsoplaysasignificantrole(Martin*etal*.,2004).Thepreviousstudies showthatitwasgenerallymaintainedaround50-55%fortheproductionofpectinasesbymicrobialmeans(Leda*etal*., 2000).

Twotypesoffermentationscanbecarriedoutforpectinaseproduction,theyaresolidstatefermentationandsubmergedfermentation.Thegrowthoforganismsisveryhighwithlargequantities ofenzymebeingproducedinsolid-statefermentation(RamanujamandSaritha,2008).Howeverintheproductionofextracellularpectinases,submergedfermentationispreferableastheextracellularpectinasesareeasierandcheapertouseingreatquantities.Submerged orsolidstate mediumsareusedforproducing ofthepectinoliticenzymesbyfungi(Bali,2003).

Types of Fermentation

i) SolidStateFermentation(SSF)
ii)SubmergedFermentation(SmF)

Solidstatefermentationisdefinedasthecultivationofmicroorganismsonmoistsolidsupports,eitheroninertcarriersoroninsolublesubstratesthatcanbeusedascarbonandenergysource.Thisprocessoccursintheabsenceornearabsenceoffreewaterinthespacebetweensubstrateparticles.Inthissystem,waterispresentin thesolidsubstratewhosecapacityforliquidretentionvaries withthetypeof material(Lonsane*etal*., 1985;Pandey*etal*.,2001).

Submergedliquidfermentationisthecultivationofmicroorganismsinliquidnutrientbroth.Industrialenzymescanbeproducedusingthisprocess.Thisinvolvesgrowingcarefullyselectedmicroorganismsinclosedvesselscontainingarichbrothofnutrientandahighconcentrationofoxygen(Grigelmo-MigeulandMartin-Belloso,1998).

ThereareseveraldisadvantagesofSSFwhichhavediscouragedtheuseofthistechniqueforindustrialproductionandthereforehavemadeSmFmoreapplicableintheproductionofenzymes.Theseinclude:thebuildupofgradientsoftemperature,pH,moisture,substrateconcentrationorCO_2duringcultivationwhichareddifficulttocontrolunderlimitedwateravailability(Holker*etal*.,2004).

Aimand Objectives of the study

- Tocharacterizethepurifiedpectinase enzyme.
- Todeterminetheeffect ofchangein pHonpectinaseactivity.
- Todeterminetheeffect ofchangein temperatureonpectinaseactivity.

MATERIALSANDMETHODS

Chemicals/Reagents

Allthechemicalsusedinthisresearchworkwereofanalyticalgrade.

Apparatus/Equipment

Autoclave, Centrifuge, Magneticstirrer, Microscope, Millingmachine, Oven, pHmeter, Waterbath, Weighingbalance.

PreparationofBuffers

Thestandardbuffersusedinthisstudywere pH4.0andpH7.0.Thesebufferswereusedtostandardizethe pHmeter.Theworkingbufferswere preparedbythis procedure.

Sodiumacetatebufferof0.05MandTris-HClbufferof0.05Mwerepreparedbydissolving4.10gsodiumacetatesaltand6.01gTrisbase,respectivelyin1000mlofdistilledwaterandstirredwithamagneticstirrertillahomogenoussolutionwasformed.Thesolutionsweretitratedagainstaceticacidand HCl,respectivelytilltherequiredpHwasobtained.

Phosphatebufferof0.05Mwaspreparedbydissolving7.10gdisodiumhydrogenphosphatesaltin1000mlstirredaswellwithamagneticstirrerandthentitrated againstthesolutionofitsconjugateacidwhichissodiumdi-hydrogenphosphatetilltherequiredpHwasobtained.

Studies onPurifiedPectinase Enzyme

EffectofpHChangeonPectinaseActivity

Theeffectof pHon enzymeactivitywasdeterminedusing0.05Msodiumacetatebuffer pH3.5-5.5,phosphatebuffer pH6.0-7.5and Tris-HClbuffer pH8.0-10.0atintervalsof0.5.0.1%pectinsolutionwaspreparedbydissolving0.1gpectinin100mlof0.05Mofthe respectivebuffers.Also0.5ml ofthepartiallypurifiedenzymeswas added to0.5ml ofeach ofthebuffers.Thenultimately,0.5mlofeachoftheenzyme-

buffersolutionwasmixedwith0.5mlpectinsolutionatthecorrespondingpHforpectinaseassaysasdescribedpreviously.

Effectof TemperatureChangeonPectinaseActivity

Theoptimumtemperaturewasdeterminedbyincubatingtheenzymewithpectinsolutionat25-70^{O}Cintervalof5^{O}Cfor1hourandatthepHwiththehighestactivity.Theactivitywasthendeterminedasdescribedin previously.

RESULTS

PineapplePectinExtraction

Pectinextractionyieldwasfoundtobe8.33%atpH2.2,temperatureof70^{O} Candextractiontimeof1 hour.

Photographof PineapplePectinExtract:-

Fig.4shows aphotographofthepineapplepectinafterextraction.

Figure.4: PhotographofPineapplePectin

Characterizationof Pectinase

EffectofpHChangeonPectinaseActivity

Fig.5 showsthatthehighestpectinaseactivitywasrecordedatpH5.5.Also,observedinFig.19was adeclinein activitywhenpHwasincreasedordecreasedbeyond5.5.

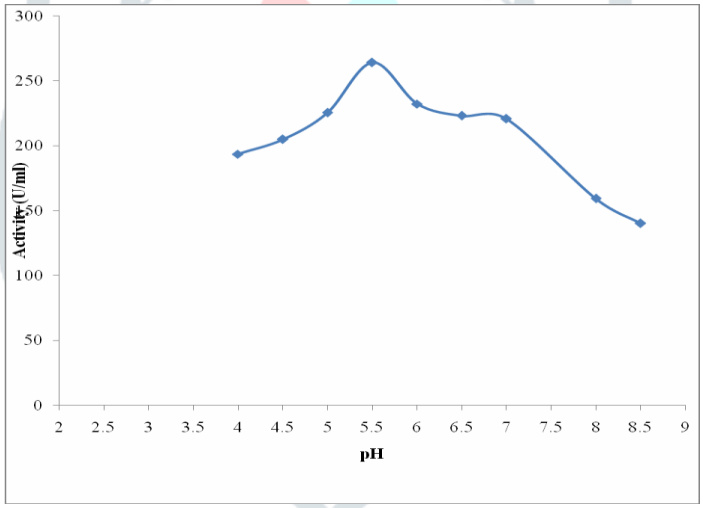

Figure5 : Showingtheeffect ofchangein pHonpectinaseactivity

EffectofChangein TemperatureonPectinaseActivity

Fig.6 showsgradualdeclineoftheenzymeactivityatatemperartureof40˚Cwithsubsequentriseattemperatureof45and50˚C.Thepeakoftheenzymeactivitieswasobservedatatemperatureof55˚Caccompaniedwithasharpdropoftheenzymeactivitywithcorrespondingrisein temperature.

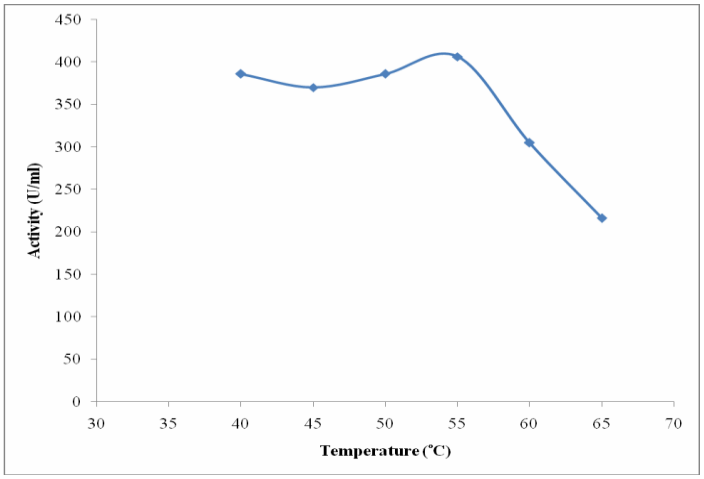

Figure6: EffectofChangein TemperatureonPectinaseActivity

Table 1 :Pectinasecharacterization

Properties	***Aspergillusniger***
pH	5.5
Temperature(°C)	55°C

Table1 shows thecharacterizationofthepectinaseobtainedfrom*Aspergillusniger*withthepHandtemperatureoptimabeing5.5and55˚C respectively.

Discussion

ThepartiallypurifiedenzymewascharacterizedbasedontheeffectofchangeinpH andtemperature.ThemaximalactivitywasobservedatpH5.5asseeninFig.5 forpectinasefrom*Aspergillusniger*usingpineapplepeelsassubstratewhichiscomparabletotheoptimumpHforpectinaseactivityfromthermo-tolerant*Aspergillussp*(Freitas*etal*.,2006).ChangesinpHcanchangetheshapeoftheactivesiteinanenzyme.Extremelyhighorlowp Hconcentrationsusually resultincompletelossofenzymeactivityduetodenaturation(Helms*etal*.,1998).Atextremelyhighandlowp Hvalues,thetertiarystructureoftheprotein(enzyme)maybedisruptedandtheproteindenatured;evenatmoderatepHvalues,wherethetertiarystructureisnotdisrupted,enzymeactivitymaydependonthealterationofionisationstatesof:certainaminoacidsidechainsresponsibleforsubstratebinding,certainsidechainsinvolvedin catalysis andcertaingroupsonthesubstrate(Lukong*etal*.,2007).

Temperaturehas acomplexeffectonenzymeactivity.The"optimumtemperature" ofanenzymeisthetemperatureinwhichtheenzymefunctionsmostefficient.Anincreaseintemperaturebelowtheoptimumresultsinanincreaseinthekineticenergyofenzymes.Thisleadstohigherefficiencyofenzyme-substratecomplexformation;thereforeahigherrateofreaction.Itdiffersineachenzyme,dependingonitsnatureandstructure.Atextremetemperaturesabovetheoptimum,theincreasedkineticenergydisruptsthebondsholdingtheactivesite;theenzymeisunstableandtheshapeofitsactivesitechanges.Thismeansthattheenzymeislessefficientandsuccessfulatenzyme-substratecomplexformation.Whentheenzymeissaidtobedenatured;ithaslostitsabilitytocatalyzereactions.Fig.6 showsthat55˚Cwasthetemperatureatwhichpectinasefrom*Aspergillusniger*exhibitedthehighestactivity,whichfallswithintherangeasexo-polygalacturonasefrom*Monascus*and*Aspergillussp*.(Freitas*etal*.,2006)whichexhibitedmaximumactivityat60 ºCand50ºCrespectively.Itwasalsoreportedthatpolygalacturonaseby*Aspergilluskawachii*(EsquivelandVoget,2004)and*P.frequentans*(Chellagatti*etal*., 2002) exhibitedmaximumactivityat60ºC.Atahighertemperature,theactivitydroppedandthiscouldbeasaresultofthedenaturationoftheenzyme.Itcouldbethatenzymesareproteinsandareheatsensitive,oncetemperatureshavereachedextremeheat,theenzymesdenature,therebyexposingtheproteinstructurewhichleadstoinactivation.Allenzymeshaveanoptimaltemperatureatwhichreactionratesgofastestwithoutdenaturingtheenzyme(CampbellandReece,2002).

Conclusion

From these investigations it is evidenced that the pineapple peels with 8.33% pectin content were successfully used to induce the production of pectinase under submerged fermentation process.Thus the Pectinase enzymes obtained using natural raw materials with biologically natural methods can be further characterized for its purity and activity at various physiological conditions. Theenzymesobtainedcanbeindustriallyusedintheproductionoffruitjuice,papermaking,retting ofplantfibres,etc.Ultimately,the rationalebehindthis researchwastheconversionofwastetowealthwhichcouldincreasetherevenuebaseofanyestablishmentorcountryobtainedandalsogearedtowards acleanerandsaferenvironment.

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