4TH YEAR PROJECT REPORT

ON

JUICE CLARIFICATION

UNDER THE SUPERVISION OF-Dr. Gunjan Goel

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PART-2(I)

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CERTIFICATE

This is to certify that the work entitled “Clarification of juice using enzymatic treatment ” submitted by Komal kanungo at department of Biotechnnology and Bioinformatics ,Jaypee University of Information Technology,Solan,under my supervision towards the partial fulfillment of degree of Bachelor of Technology in Biotechnology from Jaypee University of Biotechnology,Waknaghat.

It is also certified that no part of this work has been done by anyone and submitted for the award of any degree or diploma.

Dr. Gunjan Goel

INTRODUCTION

The liquid water inside fruit cavities on top of base is juice.it means to liquid and liquid water.asthetically pleasureful to many it means robust situation to demand there where it is desiredand pleasure of heavenmake it essence. Fully appatable it means to protect against germs in loby as it makes more number of guards.The juice is medically also important as it is second most easily digested food after water.also it stops metabolic insults as it lets all metabolic processes run simultaneously and do notLet any poison being made. And to let metabolic processes run in same manner .for example the secondary metabolite tannins are being made by result of many metabolic processes.Juices are of many types depending on type of fruit for example apple has many varities like delicious, golden delicious,mcintosh,roman beauty,granny smith.Now juices also depend on time period of maturation of fruit ,some take 100 days and some take 200 days.The TSS value of juice is defined as total amount of soluble solids ,minerals and nutrients.The TSS value is counted in degree brix The TSS of juice should be 4 to 5 degree brix.it is basically the sweet content of juice.it is measured by the haze present in the juice.The taste of juice given by the haze is due to metabolites it produces . one of the metabolites is tannins which for example give puckry feeling to the taste of berries and they look sweetishly sour.For example the right attire makes the person same way right carnel makes better juice. So do not let the fruit dry and spoil so let the juice being prepared and then taste it.

TERMINOLOGY

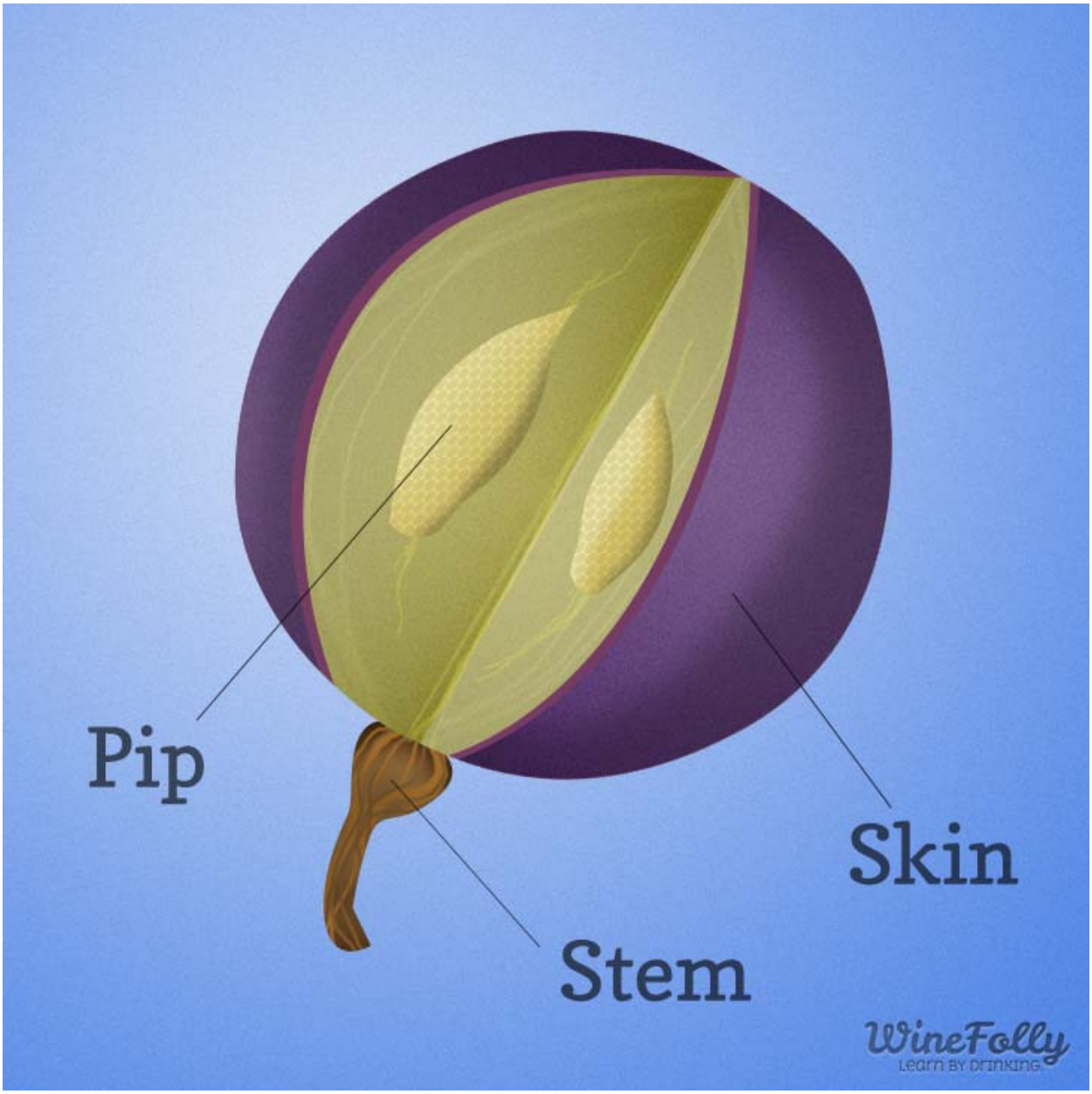
According to united kingdom juice is a quantity already present in the fruit. FDA makes it named as nectar.According to US the juice with some of the corn syrup juice is called as juice cocktail orjuice drink.Usually juice does not contain any added sugar ,but it may carry natural occurring sugar and that is labeled as carbohydrate.A juice carry many nutrients like orange juice carry vitamin C ,folic acid, potassium.

Benefits of fruit juices

The fruit juice help in weight loss.they have anantioxidant property which can help in curing many diseases like heart attack, cancer etc.3)they are easily digestible which can lead to spontaneous energy.they boon with immunity.The treated juice is often called as fortified juice for example minute maide ‘s heart check and Tropicana original.

What are tannins

Tannin is a loose term for a whole collection of non-volatile phenolic substances found in apples, grapes and many other fruits, and which provide 'body' to fermented beverages. There are a dozen or more of these in apples, such as chlorogenic acid, phloridzin, epicatechin and the procyanidins. Only the procyanidins are 'true tannins' in the sense of being able to tan protein (e.g.animal hides) or to produce an astringent sensation in the mouth. However, in this essay I shall break with the habits of a professional lifetime and use the term 'tannin' in its loosest sense to cover all apple phenolics except where otherwise indicated! By and large the content of 'true tannin' in most apples is about half the level of 'total phenolics'. But the level of 'true tannin' parallels the level of 'total phenolics' in most apples pretty closely. Bittersweet apples have the most tannins or phenolics, and dessert apples the least.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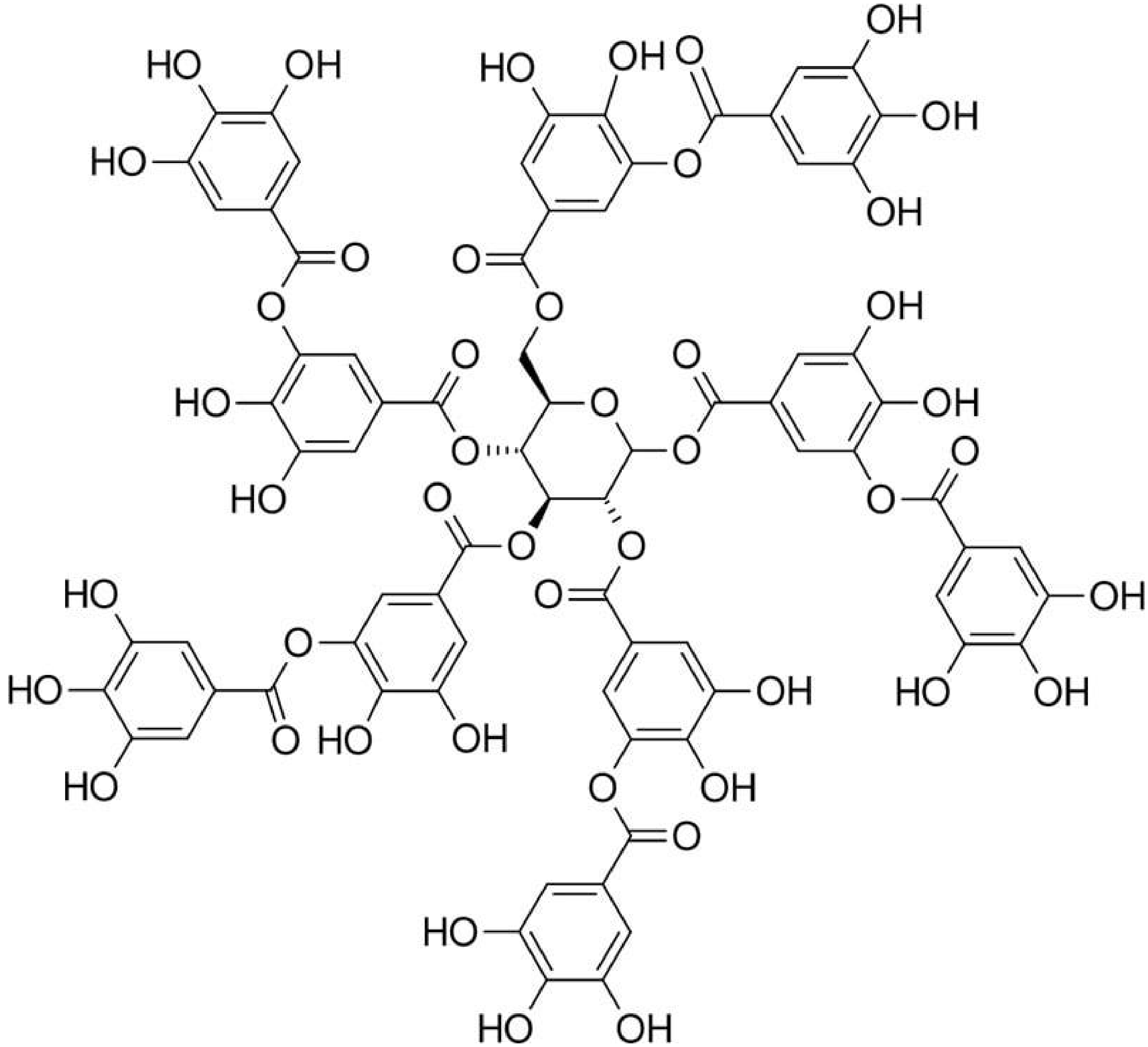


APPLICATION OF TANNINS IN FRUIT JUICES

PROBLEM: TO ACT ON tannins which are responsible for juice haze,colour, taste and aroma.

Definition :The plant secondary metabolite like tannins are responsible for plant haze,colour ,aroma and taste and these are to be removed for better colour and other induced properties for better product.The plant secondary metabolites like tannins are of two types, one is hydrolysable tannin and another is anthocyanin.The anthocyanin part is responsible for its colour,and aroma,taste.

TANNINS IN FRUIT JUICES AND FRUITS

A tannin is an astringent, bitter polyphenolic compound that binds to and precipitate protein and various other organic compound including amino acid and alkaloid.The word is derived from german word meaning fir tree or oak tree that’s why every fir tree has tannins in its bark.

It is present in following fruits:

Grapes

Berries

Apple

Chocolate contain 6%tannins.

Limitations of tannins In context of juices

as they are present in black river water and some spoiled plants which can be harmful to drink.alkalinity can remove it from soil.it has a molecular weight 20,000 Da and is found in anthocyanin.some flavors of juices are spoiled by it .it is derived from bark of fir tree which is sometimes covered by lichen or algae.it is a bitter polyphenol which makes it unsuitable for health as cyclic groups are cancerous as they can release free radical in drink.

Affects on characteristics of juice

colour:the colour pigment is anthocyanin because it is responsible for dark colour in juice .it happens when tannin is present..this is present of hydrolysable tannin make it Dissolved as In show no colour.

taste:it has bitter taste as it is a polyphenolic compound so it makes juice bitter.

aroma:it gives a bitter taste to juice and aroma is punjent due to its presence.

haze:haze in fruit bark or some pieces of fruit itself which make it condensed juice due to which it has no antioxidant property as juice is not easily appatable.

Condensed tannins (proanthocyanidins, polyflavonoid tannins, catechol-type tannins, pyrocatecollic type tannins, non-hydrolyzable tannins or flavolans) are polymers formed by the condensation of flavans. They do not contain sugar residues.[1]They are called proanthocyanidins as they yield anthocyanidins when depolymerized under oxidative conditions. Different types of condensed tannins exist, such as the procyanidins, propelargonidins, prodelphinidins, profisetinidins, proguibourtinidins or prorobinetidins, formed from flavonoids structures corresponding tothe related anthocyanins. One particular type of condensed tannin, found in

grape, are procyanidins, which are polymers of 2 to 50 (or more) flavan-3-ol units joined

by carbon-carbon bonds. These are not susceptible to being cleaved by hydrolysis.While many hydrolyzable tannins and most condensed tannins are water soluble, several tannins are also highly octanol soluble.[2][3] Some large condensed tannins areinsoluble. Differences in solubilities are likely to affect their biological functions.

BENEFITS OF TANNINS

AS it is bitter in taste and aroma so it make predator away from plants carrying tannin mostly the fir trees etc.

Medical use

The tannin derived from Myocuodruon uvundeuva bark in its stem is neuroprotective in function carrying property of reversing 6-hydroxydopamine induced toxicity .the plant has shown promise as a potential therapeutic agent.Also plant carrying tannin have anti-inflamatory and anti-ulcer activity in rodents showing strong antioxidant property with possible

Therapeutic application.

Also natural polyphenol like tannins are derived from or are plant secondary metabolite which are capable of producing antioxidant properties which are responsible to remove metabolic insult.condensed tannins from lithocarpus glaber leaves have a potent free radical scavenging activity Proanythocyanidin oligomers, extracted from grape alopecia seeds, have been used for the experimental treatment of alopecia species. When applied topically to mice, they promote hair growth in vitro,and induce anagen in vivo. Procyanidin C2is the subtype of extract most effective.Experiments showed that both procyanidinC2 and Pycnogenol (French maritime pine bark extract) increase TNF-A secretion in a concentration- and time-dependent manner. These results demonstrate that procyanidins act as modulators of the immune response

in macrophages. Howeverfor health effects, one needs to look at randomized controlled trials to assess whether the claimed benefits are supported by evidence. A systematic review of randomized controlled trials that investigated the efficacy and safety of Pycnogenol for the treatment for chronic disorders found that currentevidence is not sufficient to support claims regarding its benefit in any chronic condition.[13]

FUNGAL CULTURE

Fungus help In cellulose degradation and cellulose production.because cellulose are final product of plants and for their degradation through various metabolic processes cellulose is produced.

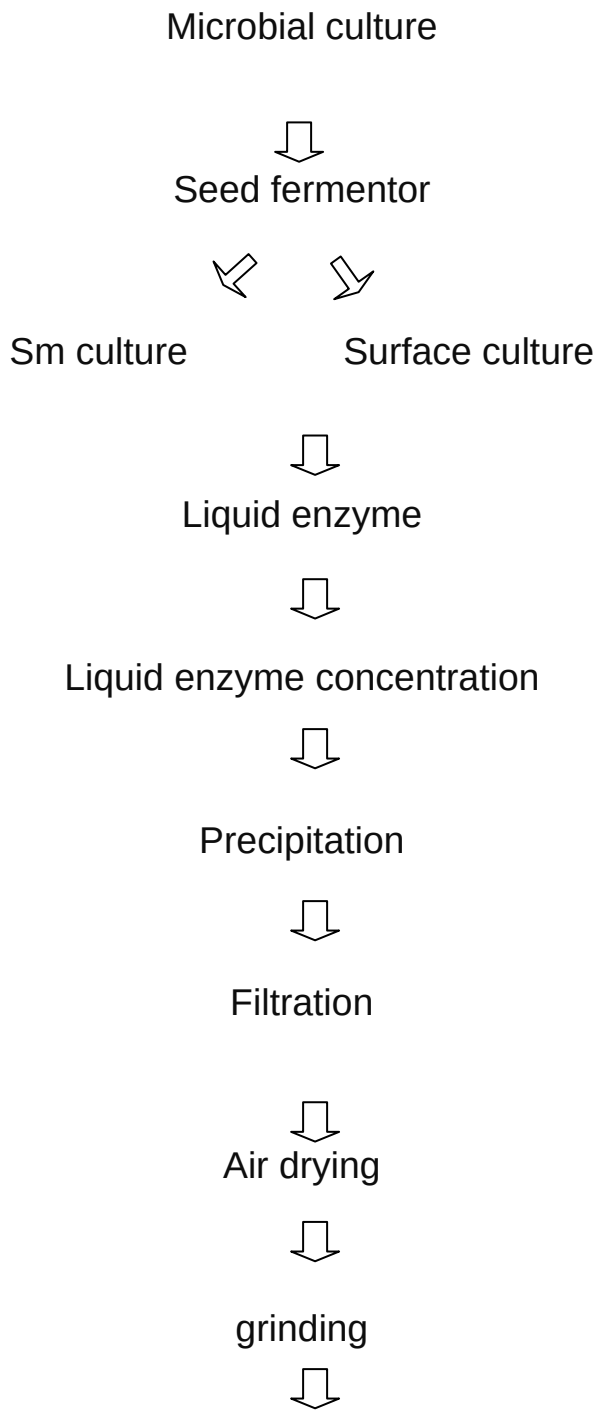
Cellulose->acetate,co2,formate,lactate,hydrogenFungi helps in degradation of cellulose by producing glucose.also they are characterized as thermotolerant as by producing hsp90,100.They are grown at 38degree Celsius.In fungus proteolytic system works by rumen enzyme work to degrade only glucose and not cellulose.The bacteria actinomycetes work same as fungus.

TANNNINS

Tannins are basically hydrolysable tannin are endoglucans (endo-1,4-b-D-glucan-4-glucan hydrolases) with E.C. no. 3.2.1.4 these attack ce;llulose chain and split the substrate cello dextrin to produceA product reducing sugar (as juice carry metal ion leaving lone pair so to reduce them we should have endoglucans which are cellulosic to produce glucose).The endoglucanases are of three types EG1.2.3.EG1 are characterized as to produce 43 to 55 KDa glycoprotein.

70% of EG1 carbohydrate was o-glycosidically linked which indicate less occupation of resulting N-glycosylation sites in EG1.It means that there is no glucose formation .EG1 and CBGHI are having substratesSpecificity.since enzymes is resistant to proteolytic and enzymatic deglycolysatiuon ,post secretional modification will result into high no. of proteins(55KDa).A no. of endoglucanases have been identified from T.reeseei cultures that could not be identified as EG1 or EG3.M.Bhikhabhai purified a 48KDa with PI 4.5 .the gene is responsible to isolate protein by glycosylation.B-GLUCOSIDASEAspergillus produces high molecular weight protein than does .trichoderma purified b-glusidases from Aspergillus niger hasmolecular weight 150KDa and consists of two polypeptide chains that canBe separated by SDS-PAGE.Aspergillus oryzae has been reported to form an enzyme with m.w.218KDa which also consists of two polypeptide chains.

ENZYME EXTRACTION



WHY CLARIFICATION

Juice clarification is being done for so long. All over the world this is being implemented for various reasons .There are many reasons to clarify juices and are of medical importance and aesthetic importance as it directly provide whole nutrition without providing us the waste content. Clarification was done to have a pleasant taste and color and odors. Many methods and techniques are employed to clarify juices and lot of hard work is done to have a better product of economic value. Many non clarified juices are being clarified for the sake of better quality.

WHAT ARE JUICES?

Juices are the extracts of cells and tissues. They basically contain minerals and vitamins. Juices are liquid extracts of cells of fruits and vegetables. But juices are having non-clearance which is due to pectin and cell debris. Clarification is necessary also because when TSS of juice is raised very high it gets jellified. Clarity in fruit juices is necessary to preserve its taste, color. The naturally clarified juices are apple, pomegranate, pineapple, mango, carrot etc.The non clarified juices are guava, citrus fruits etc.

NEED FOR CLARIFICATION

One question arises that why there is a need for clarification. The non clarified juices basically contain pectins, tannins and cell debris. This may be the reason for immediate turbidity in the juice and later haze. Turbidity is the immediate effect whereas haze is the consequence during storage. The clarification is required to affect its turbidity and haze. Turbidity is due to the light scattering in suspended particles in fruit juices .The first appearance of naturally non clarified juices contain turbidity. Haze is due to interaction between haze active protein and polyphenols that form insoluble multi molecular structure.

APPLICATIONS

Juices are mainly clarified to remove bitterness and unwanted color. This bitterness is basically due to polyphenolic compounds present in juices. So to remove bitterness we have to clarify juices that carry bitterness. Juices

are clarified for preservation purpose also. They are preserved under cold storage as many microbes and bacteria can destroy them so to avoid their growth juices need to be clarified. The phenolic constituents of pomegranates give color, astringency and
bitterness to the juice . These compounds
are also responsible for the formation of cloudy appearance of fruit juices during concentration and storage

REVIEW

1) The activity of pectic enzymes was measured in culture filtrates of four selected fungal strains. Pectin lyase (PL) activity was produced by all fungi.

2) The effect of gelatin on fungal pectinolytic enzyme for apple juice clarification was experimented and it was analysed that juice was two times more clearer than normal.

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Occurance of tannins

Tannins refer to the diverse group of chemical compounds in wine that can affect the color, aging ability and texture of the wine. While tannins cannot be smelled or tasted, they can be perceived during wine tasting by the tactile drying sensation and sense of bitterness that they can leave in the mouth. This is due to the tendency of tannins to react with proteins such as the ones found in saliva In foods that are high in proteins (such as red meat) are often paired with tannic wines to minimize the astringency of tannins. However, many wine drinkers find the perception of tannins to be a positive trait—especially as it relates to mouthfeel. The management of tannins in the winemaking process is a key component in the resulting quality.[14]

Tannins are found in the skin, stems, and seeds of wine grapes but can also be introduced to the wine through the use of oak barrels and chips or with the addition of tannin powder. The natural tannins found in grapes proantrocyanidins due to their ability to release red anthocyanin pigments when they are heated in an acidic solution. Grape extracts are mainly rich in monomers and small oligomers (mean degree of polymerization <8). Grape seed extracts contain three monomers (catechin, epicatechin and epicatechin gallate) and procyanidin oligomers. Grape skin extracts contain four monomers (catechin, epicatechin, gallocatechin and epigallocatechin), as well as procyanidins and prodelphinidins oligomers.[15] The tannins are formed by enzymes during metabolic processes of the grapevine..

n the vineyards, there is also a growing distinction being made between "ripe" and "unripe" tannins present in the grape. This physiological ripeness, which is roughly determined by tasting the grapes off the vines, is being used along with sugar levels as a determination of when to harvest.The idea is that "riper" tannins will taste softer but still impart some of the texture components found favorable in wine. In winemaking, the amount of the time that the must spends in contact with the grape skins, stems and seeds will influence the amount of tannins that are present in the wine with wines subjected to longer maceration period having more tannin extract. Following harvest, stems are normally picked out and discarded prior to fermentation but some winemakers may intentionally leave in a few stems for varieties low in tannins (like Pinot noir)

in order to increase the tannic extract in the wine. If there is an excess in the amount of tannins in the wine, winemakers can use various fining agents like albumin,casein and gelatin that can bind to tannins molecule precipitate them out as sediments. As a wine ages, tannins will form long polymerized chains which come across to a taster as "softer" and less tannic. This process can be accelerated by exposing the wine to oxygen, which oxidize tannins to quinone-like compounds that are polymerization-prone.

consumption has shown that tannins, in the form of proanthocyanidins beneficial effect on vascular health. The study showed that tannins suppressed production of the peptide responsible for hardening arteries. To support their findings, the study also points out that wines from the regions of southwest France and Sardinia are particularly rich in proanthocyanidins, and that these regions also produce populations with longer life spans.[19]

Reactions of tannins with the phenolic compound anthocyanidins creates another class of tannins known as pigmented tannins which influences the color of red wine.

Immobilization

Culture broth

cenrifuge

Cell slurry

Pre-treatment

Cross-linking

Dewater

Particle shaping

Drying

Screening

TANNASE

In many countries including asia and Europe tannase is used to clear tannins from fruit juices.tannase is the enzyme that converts tannin to gallic acid.the gallic acid basically the ellagic acid and ellagy tannins to gallate and water basically ellagate.

Ellagins->2ellagate

Basically it works on esters so named as acylhydrolases.

Plate fermentation

Also called mSSF on plates .fermentation is the process which converts substrate to the product via an enzymatic reaction.tannase is the enzyme which is used to convert tannin to gallic acid or gallate.tannins are basically of two types gallic acid and anthocyanins.gallic acid is for conversion to gallate to have relief from bad color,aroma ,texture which is due to anthocyanin part of the tannins.

Requirements for growth of fungus

Ph-4.5.-5

Temperature-30degree Celsius

Time for growth-72-86hours

Harmful effects of fungus

CASE STUDY

When I worked with fungus it was quite a good experience.the fungus was ascomycetes.the high variety adds to benefit that it was a food technology project and I got the best of the results.ascomycetes in food is a boon to the era.

But with good something gets as bad also I could not handle it properlyso I got infection and it was toenail infection.it was trychoderma ,which is a common dermal infectious agent.according to the internet there were many remedies to it,but Doctors got the remedy as whole nail removal.

Some of the symptoms of the causing agent were:

1)toenail rupture

2)coloured nail

3)ruptured from side

4)dry and thick nail

OBJECTIVES OF PROJECT

1) Isolation of tannase:

Tannase to be isolated is given conditions to grow.

2) Screening: then isolated tannase is screened for verification.

3) Extraction of juice: juice is extracted or bought readily.

4) Clarification: clarification is done mechanically or enzymatic ally.

Mechanically: using sieve or other device.

Enzymatically: using various hydrolyses or proteases.

5) Juice processing: juice processing involves pasteurization, preservation and sealing.

I am trying to clarify juice enzymatic ally by isolating tannase.

The various steps we followed are:

1) ISOLATION ON PLATES
2) SCREENING FOR MORE PRODUCTION
3) ENRICHMENT
4) RESULTS
5) PRODUCTION OF TANNASE
6) JUICE CLARIFICATION

MATERIAL-:

100ml flasks, soil sample, nutrients, measuring cylinder, petri plates.

Enrichment basically involve supplying nutrients for the growth of desired enzyme .It basically involves providing C source and nutrients(czpak dox media) for the desired enzyme production. The desired is grown on three different temperatures i.e.18, 37 and 30 degree Celsius.

METHODOLOGY

- ISOLATION ON PLATES

1. POURING
    - Ignited the burner and Put plates around it.
    - Opened flask and put in front of burner.
    - Poured on plates.
    - Put half cover on it.
    - Covered plates for half an hour or 40 minutes.
    - Let them solidify.
    - Put paraffin wax around it.
    - Sealed plates and labeled them.
    - Put on incubation.

- ENRICHMENT

1. Mixed 2g of tannic acid in 25 ml water. Then pipette out 5ml of it in 4 nutrient flask.
2. Collected soil samples from three different regions and mixed them in flask with approx 25ml it and mixed in nutrient flask.

3. Incubated three flask at three different temperatures 18, 37,30 degree Celsius.

STREAKING

- ✓ Dipped needle in the spirit.
- ✓ Incineration on burner.
- ✓ Dip in flask and streak on plates.
- ✓ After streaking I put plates and flasks in respective incubators at 18, 30,37 degree Celsius.

PART-2

Step-1

Objective is to prepare media for polyphenol tannin for juice clarification.

Requirement-PDA,plates,cotton,spirit,autoclave,LAF,match stick,spirit lamp etc.

Theory-for growth of the enzyme we need substrate or media which can help in growth of the enzyme.for juices many polyphenols, free radicals are present in the preparation of better quality juice.For nutritious juice these polyphenol and free radical are to be removed. As free radicals are cytotoxic and polyphenol like tannin mainly gallic acid can help in making anti ,malarial drugTannins are helpful in converting hydrolysable tannin.

requirements like:

Temperature-121 degree Celsius

Ph-3.5

Pressure-15psi

Time-15minutes

streaking

Spot inoculation

EXTRACTION OF FUNGAL ENZYME FROM SOIL.

MATERIALS AND MATHODS

CZPAK DOX MEDIUM

$NaMoO_4.2H_2O$-0.008%

$(NH_4)_2SO_4$-0.87%

KH_2PO_4-0.44%

$MgSO_4.7H_2O$-0.088%

$CaCl_2.7H_2O$-0.009%

$MnCl_2.6H_2O$-0.002%

$FeSO_4.7H_2O$-0.012%

MEDIA as potato dextrose agar

potato-20g

Dextrose-2g

Agar powder-2g

Water-100ml

Key points:

HOLDING TEMPERATURE

This is the temperature at which enzyme starts working.we have given certain temperatures like 18, 30,37 degree Celsius as holding temperatures.

ACETONE PRECIPITATION

Enzyme mixed with acetone at 4degree Celsius in1:2v and after 4hours of incubation.

Centrifugation of enzyme and acetone mixture at 10,000 rpm for 10 minutes.

TANNASE ASSAY

Tannase activity determined by spectrophotometry (Iibuchi method) at 650nm.

One unit (1U) of enzyme activity is amount of enzyme required to hydrolyze 1micro mol of ester in 1minute.

REVIVAL SCHEME

The enzyme is revived three times for its purification.the bacteria revived from soil is aspergillus for C source dextrose medi a is used.in which I prepared 2.4g PD broth in 1g miller agar in 10ml water at ph 6.4 in one flask and autoclave it. In second flask PDB and water is mixed with agar-agar to make it PDA.

RESULTS

1.Clear zones seen which indicate the degradation of tannic acid.

2.the first growth was seen after 48 hours of inoculation.

DISCUSSIONS

After the solid state fermentation media was decomposed and tannase as product was recovered .This SSF lead to conversion of tannic acid to tannase.

WORK TO BE DONE

After results verified with screening -clarification and processing of juice is to be done.

INTERPRETATION

1)usually growth of fungal culture takes 4days. But I gave 2-3months to grow so that’s why it couldn’t be interpreted.

2)as per holding temperature 18,30,37degree Celsius.growth was seen at 37 degree Celsius and in reality it occurs at 30 degree Celsius.

3)for results we have to check revival time.

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