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Natural, Culinary Fruit Peels as a Potential substrate for Pectinolytic Enzyme

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Abstract:
Pectinases or Pectinolytic enzymes are the one which have broadest applications in the food processing, alcoholic beverages and textiles industries. These enzymes are chiefly produced from the plants and microorganisms. The fruit peels are regarded as waste by most of the industries. And the disposal of them becomes the serious problem, as it leads to the environmental pollution. On the other hand, it is of low-cost and it contains pectin, a natural substrate that contains selective chemical compound which is suitable for the production of pectinase enzyme. This review mainly concerned about the selection of substrate as peels and the production of pectinolytic enzymes using different fruit peels, comparison of fermentation method that is suitable for enzyme production using peels as substrates, different enzyme assay methods, computer software controller for fermentation used and also applications of pectinase.

Keywords: Fruit peels, Pomace, Pectinase, Substrate, Solid State Fermentation (SSF), Submerged Fermentation (SMF).

Introduction

Enzymes are bio-active compounds or catalyst that regulate many chemical reactions in living tissues and cells (1). Pectinases are posh cluster of enzymes which degrade different pectic substances present in plant tissues. These enzymes have useful applications in paper, fruit and textile industries. Almost 75% of the estimated sale value among industrial enzymes in 1995 has been contributed by pectinases (2). On the other hand, these enzymes also have biological importance in identification of plant diseases and protoplast fusion technology. Microorganisms have more number of advantages and can be used for enzymes production at a higher level. Pectinases have great biotechnological potential and can be used in many industrial processes (3,4). Hence, applications of pectinases in various fields are widening, it is necessary to understand the properties and nature of these enzymes for efficient and effective usage (5). Pectinase enzymes are yield from a vast variety of microbial sources such as Fungi, Bacteria, Yeast and Actinomycetes of them the maximum producer is Fungi. In this review paper, we discuss about the selection of fruit peels as substrate for production of pectinase and works carried out using different fruit peels, mode of fermentation which is compatible for this substrate, enzyme assay methods and applications.

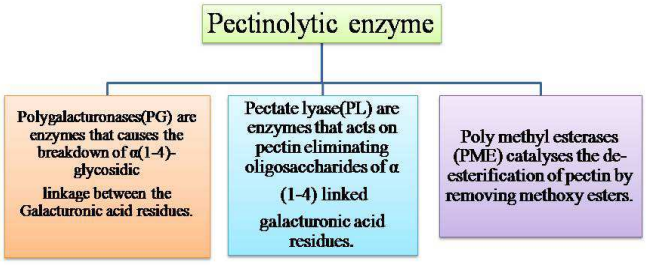

Fig 1: Classification of Pectinolytic enzyme

Substrate

Pectic substance

Pectic substance, a generic name used for the compounds in which the pectinolytic enzymes are acted upon it. These compounds are of negatively charged, large molecular weight, acidic in nature, glycosidic macromolecules (polysaccharides) in complex manner which are present in the plants. The pectin is present as the large constituent of middle lamella between the cells in the form of calcium pectate and magnesium pectate.

Classification of pectic substrate

Pectic substances are classified according to the American Chemical Society into four major types as follows:

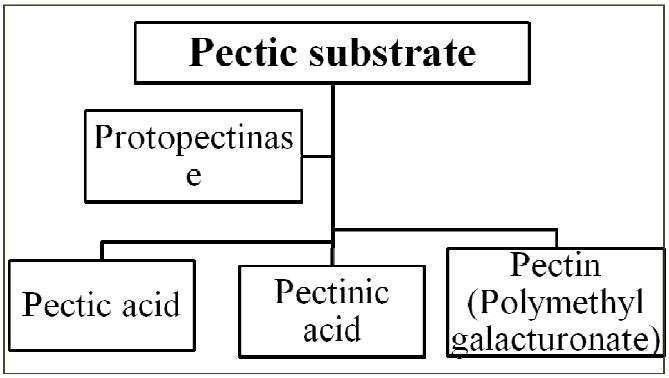

Fig 2: Classification of Pectic Substrate

Protopectin: It is the pectic substance which is insoluble in water and present in intact tissue. On restricted hydrolysis, protopectin yields pectin or pectic acids.

Pectic acid: It is the soluble polymer of galacturonase which contains insignificant amount of methoxyl groups. The normal or acid salts of pectic acid are known as pectates.

Pectinic acids: It is the polygalacturonan chain that contains > 0 and < 75% methylated galacturonate units. The normal or acid salts of pectinic acid are called as pectinates.

Pectin (Polymethyl galacturonate): It is the polymeric substance in which, at least, 75% of the carboxyl groups of the galacturonate units are esterified with methanol. It is the one which benefits rigidity on cell wall when it is bound to cellulose in the cell wall.

Substrate selection

Substrates should provide all needed nutrients to the microorganisms for its growth. Other factors like moisture level, particle size also taken into consideration for the selection of substrate. Various substrates like Banana waste, sugarcane bagasse, wheat bran, rice bran, saw dust, apple pomace, lemon peel, Orange peels, etc., are generally employed for the pectinase production (6). The agricultural residues contains cellulose, starch, lignin, xylan and pectin as their major component which is utilized as a energy and carbon sources by several microbes for manufacturing a vary range of enzymes in numerous environmental conditions (7). Substrates which are utilized for the production of pectinolytic enzyme must be a solid substrate and give good encourage to the growing cells. For the bioconversion and use of the various residues of agro-industries such as orange peel, lemon peel, it contains valuable amount of pectin that acts as support and inducer, so it can be utilised as the substrate for the making of pectinase enzymes by microbes (8). The agro-industrial residues, primarily to apple bagasse, lemon peel, citrus peel, obtain after a thermal pretreatment followed by washing with water for many times to achieve less concentration of soluble acids and sugars before dry (9).

Table 1: Composition of pectin in different fruits and vegetables Source: (10)

Fruit/Vegetable	Tissue	Pectic substance (%)
Apple	Fresh	0.5-1.6
Banana	Fresh	0.7-1.2
Peaches	Fresh	0.1-0.2
Strawberries	Fresh	0.6-0.7
Cherries	Fresh	0.2-0.5
Peas	Fresh	0.9-1.4
Carrots	Dry matter	6.9-18.6
Orange pulp	Dry matter	12.4-28.0
Potatoes	Dry matter	1.8-3.3
Tomatoes	Dry matter	2.4-4.6
Sugarbeet pulp	Dry matter	10.0-30.0

Grape pomace

Grape pomace is the important wastes which are discard from wine industries, it can be used as a natural substrate for different groups of microbes to grow for the production of enzymes. Although the cost of this substrate is low and its chemical compounds are having great deal of major nutrients that is necessary for the growth of microorganisms it can be used for the yield of pectinase. In SSF, pectinase enzymes are produced by *Aspergillus awamori* on grape pomace shows that the exo-polygalacturonase activity is high when it is compared with related values in the literature and endo-polygalacturonase shows repression due to catabolite when the medium has large reducing sugars and its activity increases with decrease in the concentration of reducing sugars in the medium (11). The mixed substrate of grape pomace and orange peels (dry weight 1:1) are utilized for the yield of enzymes by *Aspergillus awamori* in packed bed reactor, it shows that exo-polygalacturonase is produced at optimum air flow rate of 90ml/min is observed and there is no increase in the enzyme productivity above this optimum level (12). The grape must is also used as substrate for the production of cold-active polygalacturonase by *sacchromyces sp*. and it is about (18 U/ml) (13).

Orange peels

Citrus peels, main solid by-product of the fruit processing industries which constitute about 50% of the fresh fruit weight becomes a serious problem in disposal of peels from industries. As an alternative to the disposal of peels, it can be utilised as a substrate for pectinase enzyme (12).The Orange peels contains great quantity of pectin, it can be a desired substrate for the growth of pectinase producing microbes. The pectin lyase is produced and characterized by *Aspergillus niger* on orange peels. The optimum pectin lyase activity were analysed by pectin lyase activity assay. It can be purified by 60% of ammonium sulfate and shows higher activity at 30°C and 8 pH (14). The optimum conditions for the pectinase production by *Aspergillus niger* on orange peel as substrate are temperature is 50°C, pH 5, incubation time 96hrs, moisture ratio 1:2(v/w),Inoculum size 2.5ml, carbon source sucrose and surfactant Triton- X 100. The partially purified enzyme shows maximum activity at 50°C and pH 5 (15). Under favourable conditions, *A.niger* was the highly potent strain in polygalacturonase and pectate lyase production on Orange peels under SSF (16). The cold-active polygalacturonase have been produced by *sacchromyces sp.* on raw orange peel as substrate is about (21U/ml) (13).

Table 2: Composition of Orange peel Source: (16)

Component %	(w/w dry basis)
Crude fat	3.9± 0.1
Water soluble materials	41.1±1.2
Pectin	14.4±0.3
Protein	7.9±0.1
Cellulose	16.2±0.5
Hemicellulose	13.8±0.3
Ash	1.7±0.1
Lignin	1.0±0.02

Lemon pomace

Lemon peel pomace (LPP) consists of large amount of pectin content which can acts as the catalyst to induce the reaction and as a support for the growth of microbes. So, it can be used as a substrate for the yield of pectinolytic enzyme by the microbes (8). LPP constitutes about 19.8% (dry mass) of lemon peels and it becomes the important solid by-product outcome from lemon processing industries (9). In SSF, the maximum pectinase is produced by *Aspergillus niger* on lemon pomace (17).

Mango peels

The Mango peel contains a pectin content of 18.2 (w/w) and it can be used as a substrate for pectinase enzyme. With Solid state fermentation, *Aspergillus foetidus* shows extreme production of polygalacturonase and pectin lyase had optimum activities at pH 5 and 5.5, temperatures 35◦C and 30◦C respectively. The enzymes are utilized for mango juice processing for clarification and it shows maximum clarification of about (92.5±0.26%) at a temperature of about 40◦C and 150 minutes of incubation on mango pulp (18). The fungal isolate *Aspergillus sclerotioniger* produced polygalacturonase in the highest value on the 6th day of fermentation ranged from 0.448 – 4.7745 U/ml using mango peel as substrate on submerged fermentation (19).

Banana peels

The isolate of *Aspergillus niger* was able to produce polygalacturonase and pectin lyase enzymes utilizing the decayed banana peels as sole carbon source. Solid State Fermentation(SSF) produces the higher yield of pectinolytic activity compared to that of Submerged Fermentation(SMF). The Pre-treatment of banana peels like blanching the substrate with cold sodium chloride, treating the peels with wood ashes and also allowing the unripe banana to ripening stages increases the production of polygalacturonase and pectin lyase enzyme (20). On the 6th day of submerged fermentation, *Aspergillus parasiticus* produced 0.1183- 2.4683 U/ml of polygalacturonase using banana peels as substrate (19).

Apple pomace

Apple is grown worldwide including India and around 30% is used for the production of different products like juice, wine, canned slices, cider, etc., Apple pomace is a leftover residue after juice extraction containing peel, seeds and remaining solid parts representing about 25-35% of the quantity of processed apples. It consists of large amount of pectin compared to that of other nutrients. So it can be utilized as the natural substrate for the pectinolytic enzyme production. Pectin methylesterase (PME) can be produced by *Aspergillus niger* from apple pomace in SSF at a pH of 4.0, incubation temperature of 25◦C and incubation time of 96hrs, Ammonium sulphate is used as nitrogen source at the rate of 2% and Sodium chloride at the rate of 0.5% is used as additives for the highest yield of the enzyme. And the yield is 2.3 times greater compared to that of the PME production in Submerged fermentation (SMF) on same apple pomace (21).

Table 3: Composition of Apple pomace Source: (21)

Component	(w/w) dry weight basis
Total nitrogen	6.8 (g/kg)
Total carbon	127.9 (g/kg)
Cellulose	7.2 – 43.6 (% w/w)
Hemi- cellulose	4.26- 24.40 (% w/w)
Lignin	15.3- 23.5 (%w/w)
Pectin	3.5- 14.32 (% w/w)
Total carbohydrates	48.0- 83.8 (%w/w)
Fiber	4.7- 51.10 (%w/w)
Protein	2.9- 5.7 (% w/w)
Lipids (ether extract)	1.20- 3.9 (%w/w)
Reducing sugars	10.8 – 15.0 (%w/w)

Mosambi peels *(Citrus limetta)*

Citrus limetta peels (On dry weight basis), reported to contain 30.28% calcium pectate which represents a main substrate for pectinases. As *Citrus limetta* peels are pectin rich and easily available, it was selected as an important constituent in medium formulation for the extracellular pectinase production. In submerged fermentation, *citrus limetta* peels are used as a susbstrate for the pectinase production by marine *Bacillus subtilis*. The pectinase enzyme is produced maximum in the late exponential phase of the growth of the culture after 28 hours in medium containing *citrus limetta* peel powder (3%). The pH (5.0 at 40°C) of the medium containing peels shows the maximum pectinase activity (22).

Sour orange peels *(Citrus aurantium L)*

Table 4: Chemical composition of sour orange peel Source: (23)

Parameters	Concentration
Moisture (%)	12.5±0.5
Ash (%)	3.5±0.1
Fat (%)	1.7±0.2
Crude fiber (%)	17.2±0.2
Total sugar (%)	14±1.3
Reducing sugar (%)	10±1.0
Non reducing sugar (%)	3.8±0.4
Pectin (%)	7.0±0.3
Lignin (%)	5.4±0.3
Vitamin C (mg/100g)	84±2.5

The Sour Orange peels are additionally used as substrate for the yield of pectinases. The disposal of peels became the serious problem in polluting the environment. Although the Citrus peels contain the known amount of pectin which can be used as a substrate for pectinase enzyme. The Crude pectinase is produced from the Sour Orange peels (*Citrus aurantium* L) by *Aspergillus niger* in Submerged Fermentation (SmF). The optimized conditions for producing crude pectinolytic enzyme shows that the substrate concentration (4%), inoculums size (9%), temperature (30°C), duration/time (120Hrs), pH (5) were evaluated (23).

Watermelon peels

The fungal isolate *Aspergillus parasiticus* produced highest level of polygalacturonase ranged from 0.212- 2.5153 U/ml using watermelon peels as substrate after 3days of cultivation (19).

Plaintain peel *(Musa paradisiaca)*

On the 6th day of fermentation, the isolate *Aspergillus flavus* produced the highest level of polygalacturonase enzyme ranged from 0.1154 – 2.4683 U/ml on the media containing plaintain peels (19).

Mode of Fermentation

The Solid State Fermentation (SSF) is a method that primarily involves the growth of microorganisms on a wet solid supports in the absence (or close to absence) of free water. New interest in this technique derives from merely possible fact that it is considered to be an actual approach for the processes that includes the bioremediation as well as removal of toxic substance from agricultural wastes, biopulping and biotranformation of crops etc., (24,25,26). The SSF uses a different variety of natural solid supports that supply the nutrients for growth that includes apple, citrus fruits, corn, potato and banana waste (27). The Agro-industrial residues are normally considered to be the excellent substrate for the production of enzyme through SSF technique. The SSF serve as an anchorage for the growing microbial cultures and also it prevents bacterial contamination due to low moisture and content of the fermenting medium, the pectinase synthesis is also less affected by the catabolic repression when

compared with SmF. Over all, SSF is much better than SmF for the production of pectinolytic enzymes using agricultural wastes (28,29,30).

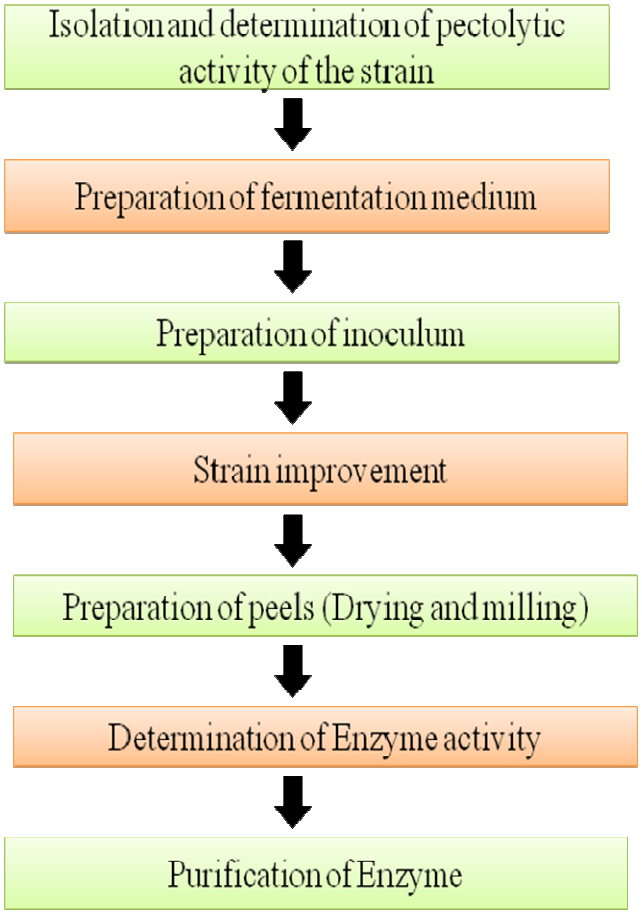

Fig 3: Overall steps involved in the Production of Pectinase using Fruit peels as substrate

Computer software for monitoring of fermentation process for pectinase

Fermentation processes have constant change, it can be nonlinear and non-stationary in nature, hence it leads to a lot of difficulties in controlling the fermentation process upto a certain set point. The computer software has already design to make the process much economical by reducing the man-power, time with increase in efficiency (31).

ASSAY METHODS

There are lots of assay methods used to determine the enzyme activity. Only some methods are briefly as follows:

i) Protopectinase activity

Protopectinase activity is assayed by measuring the amount of pectic substance liberated from protopectin by the carbozole-sulphuric acid method (32). The concentration of the pectin is measured as D-galacturonic acid from its standard curve. One unit of Protopectinase activity is defined as the enzyme that liberates pectic substance corresponding to 1µmol of D-galacturonic acid per millilitre of reaction mixture under assay conditions.

Protopectin + H_2O (Insoluble) $\xrightarrow{\text{PPase}}$ Pectin (Soluble)

ii) Reduction percentage

Pectinase activity was also measured as the reduction percentage in pectin solution viscosity. In this technique, 2ml of the culture fluid was added to 20ml of the test pectin solution and the mixture was incubated in a theromostatically controlled water bath at 30°C for 45 mins. 5ml of the mixture was then placed in each of two Ostwald viscosimeters and the dropping times at 30°C were noted. Control tests were run concurrently employing culture fluid which had been heated at 80°C for 10 mins. The Decrease in viscosity was expressed as a percentage according to the formula:

A-B/B×100

Where A= Viscosity of test pectin solution + heated medium

B= Viscosity of test pectin solution + unheated solution.

The same two Visocosimeters were used throughout the study (33).

iii) Dinitrosalicylic acid reagent (DNS) method

This method is additionally supported on the determination of reducing sugars yielded as a

result of enzymatic hydrolysis of pectin. Add (0.2 ml) 1% pectin solution, (2.0 ml) sodium citrate buffer (pH 5.0) and (1.0 ml) enzyme extract. The reaction mixture was incubated at 35°C±1°C for 25 minutes. After 25 minutes, 1.0 ml of reaction mixture was collected and added to 0.5 ml of 1M Na_2Co_3 solution containing testubes. To all test-tube, 3.0 ml of Dinitrossalicylic acid (DNS) reagent was added and the test tubes were shaken for the contents to be mixed. Then, the test tubes were heated to boiling on the hot water-bath for 10-15 mins (34).

APPLICATIONS OF MICROBIAL PECTINASES

Since last many years, pectinases are employed in many industrial processes includes fruit juice extraction, textile processing, degumming of bast fibres, paper industry , treatment of industrial waste water, coffee and tea fermentation etc., The below table shows the applications of these pectinolytic enzymes.

Fields	Process	References
Fruit juice	1.Clarification of fruit juice 2. Treatment of enzyme to banana, grapes, apple fruit pulp showed increase in juice volume.	(35) (36)
Pickle	Promotes excessive softening during the process of fermentation and storage.	(37)
Coffee and Tea	1. Enzyme accelerates the tea fermentation process and also destroys foam forming property. 2. Enzyme is used to eliminate mucilaginous coat from coffee beans.	(38) (10)
Textile	1. Degumming of plant bast fibres: Removal of bast fibres which contains gum by enzymatic degradation used as a pretreatment for textile making. This method also presents an eco-friendly and economic alternative to chemical degumming as they are toxic and polluting. 2. Used in combination with amylase, cellulose and hemi-cellulose to remove sizing agents in safe and eco-friendly nature. 3.Bio-scouring: Used in the removal of non-cellulosic impurities from cotton fibres with no side effect on cellulose degradation.	(39)
Wastewater treatment	Pre-treatment of waste water of vegetable food processing industries helps in removal of pectinaceous material and allows it suitable for the decomposition by activated sludge treatment.	(39)
Paper and pulp	In Paper making, pectinolytic enzyme depolymerise pectin content and during peroxide bleaching, it lowers the cationic demand of pectin solutions and filtrate.	(40,41)
Purification of plant virus	Sometimes, virus particle is present in the phloem, alkaline pectinase are used to liberate the virus from the tissues to give pure preparations of the virus.	(42)
Oil extraction	Citrus oils like lemon oil can be extracted with the help of pectinase as it destroys the emulsifying properties which affect collection of oils.	(43)
Improvement of chromaticity and stability of red wine	During wine production, pectinase is added to macerated fruits before the addition of yeast which improve colour and turbidity to liquid. And also, it gives greater chromatic characteristics and stability compared to controlwines.	(44)

CONCLUSION

The Disposal of fruit peels is becoming the threatened problem over fruit industries. So, fruit peels will be used as a potential substrate for the production of pectinase. Among them, citrus,apple and mosambi peels are most widely used because it contains large amount of pectin.

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SSF is more suitable for the production of enzyme from peels. And, the purification of this microbial enzyme in using the fruit peels as substrates is also can be achieved without difficulty. Hence, Fruit peels are the most promising potential substrate for the production of pectinolytic enzyme in future as it has lot of applications in various fields.

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Source of Support: NONE