MediPharm

International Journal of MediPharm Research

ISSN:2395-423X www.medipharmsai.com
Vol.04, No.02, pp 12-20, 2018

Microbial pectinases: Wonderful enzymes in fruit juice clarification

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Abstract : Microbial pectinases has been employed vigorously for the juice clarification since recent times. The juice clarification procedures carried out in industries mainly involve bio-catalyzed depectinization and fining by incorporation of pectinases to achieve pectin degradation. Pectins are fibre-like structure which makes the clarification process more complicated. Pectinases are enzymes which degrade these pectins and cause the flocculation of pectin protein complexes. The resulting juice will be less viscosity and minimal amount of pectin that is suitable for subsequent filteration process. Microbial pectinases are more advantageous over other ultrafilteration and other chemical process that it can be produced by valorization of fruit peels which makes the process cost- effective, eco-friendly and also it provides remarkable results. This study mainly concerned about the utilization of pectinase enzyme in various fruit juice clarification and improvement in the characteristics of fruit juices, techniques and statistical methods for the optimization of the clarification process of various juices had been highlighted. Moreover, among pectinolytic microbes actinomycetes have ample scope in juice clarification process in near future.

Keywords: Pectin, juice, pectinase, clarification

Introduction

Pectin is a methoxylated galacturonic acid polymer which provides integrity and rigidity to the plant tissues and present as a complex macromolecular structure in between the cell walls[1].The pectin degradation can be attained by the synergistic and combined action of various pectinolytic enzymes. The pectinases which acts on the homogalacturonan smooth region are classified into three groups namely: 1- Pectin lyase (EC 4.2.2.10) which cleave the bonds present in pectin by beta- elimination; 2- Polygalacturonase (EC 3.2.1.15) which hydrolyses the glycosidic linkages present in pectin; 3- Pectin methylestrase (EC 3.1.1.6) which helps to remove methyl groups in pectin[2]. Enzymes were becomes big bio business because of their usage as commercial biocatalysts in various industrial sectors. About 45% of juice industries utilize food processing enzymes including pectinases[3].The juice clarification procedures carried out in industries mainly involve bio-catalyzed depectinization and fining by incorporation of pectinases to achieve pectin degradation. Fruit juices mainly contain colloids which ultimately causes fouling problem during the process of filteration. The investigation of these colloids after pressing reveals that they are polysaccharides namely pectins and starch. Pectins are fibre-like structure which makes the clarification process more complicated. Pectinases are enzymes which degrade these pectins and cause the flocculation of pectin protein complexes. The resulting juice will be of less viscosity and minimal amount of pectin that is suitable for subsequent filteration process. The enzymatic hydrolysis of pectic substances in juice mainly depends on various physicochemical factors like enzyme concentration, contact time, temperature of incubation and pH[4]. Sometimes, the loss of clouds may also lead to decline in consumer acceptability. These cloud particles are responsible for the characteristic colour, flavor of the juice. This cloud constitutes a complex mixture of pectin, protein, hemicelluloses, lipids and other minor components. During the processing of industrially processed fruit-based beverages which includes wines and fruit juices are clarified as there is a necessity to remove unwanted haze, turbidity as well as sediments present in the products. Visual insight of haze and turbidity in fruit juices are mainly due to the outcome of light scattering induced by suspended pectin particles[5, 6]. This review mainly discusses the utilization of microbial pectinase and techniques followed in different types of fruit juice clarification.

Orange juice

The cloud particles present in the citrus fruits range in size from 400 to 5000nm and it varies on the type and processing of fruit. In case of orange juice 2 µm cloud particles are normally stable[7]. Pectinmethylestrase de-esterifies methyl groups on galacturonic acid present in the backbone of pectin, producing charged regions that complex ca2+ and forms ca2+ pectate gels which helps in juice precipitation and clarification. In earlier reports, it has been shown that a degree of esterification of pectin backbone which is of <36% is imperative for the loss of cloud in orange juice[8]. The exo-polygalacturonase from *Aspergillus awamori* is utilized along with xylanase and CMCase for the Orange juice clarification. The enzyme extracts reduced the turbidity value upto 24.25 NTU. Moreover, after the enzyme extracts treatment the clarity of juice has been increased to 95% [9].

The Fungal crude pectinase enzyme from *Rhizopus oryzae* was employed for orange juice clarification. The results revealed that there was decrease in their turbidity from 184 NTU to 36 NTU and 51% reduction in viscosity when compared to untreated juice was observed[10].

The addition of pectinase to the orange juice did not affect the particle size of juice. However, inclusion of pectin methyl esterase caused aggregation of the cloud particles suddenly and it also affected the aggregation kinetics. The change of cloud particles showed immediate aggregation and maximum particle size was observed at 2.5 (low pH) because of the decreased surface charge[11].

Mosambi juice

The commercial pectinase was taken for the treatment of mosambi juice clarification in response with Central Composite Design (CCD) and Response Surface Methodology (RSM). Decrease in viscosity and alcohol insoluble solid was observed in response to enzyme concentration and temperature. Clarification of juice increases with enzyme concentration and temperature[4].

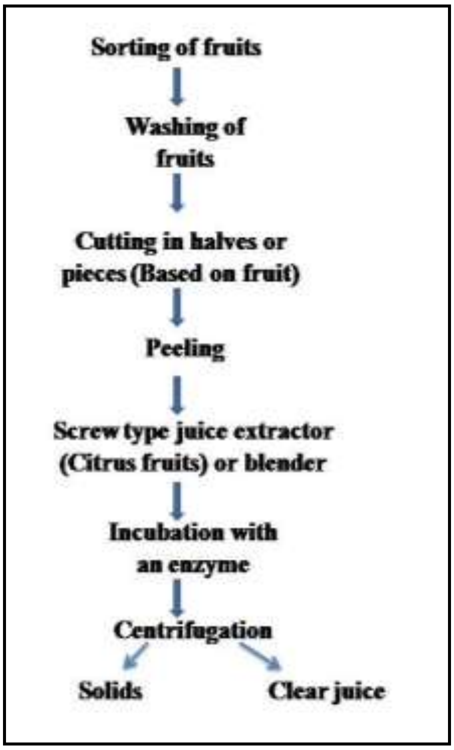

Fig. 1 Enzymatic process for the extraction of clear citrus fruit juices

Papaya juice

The utilization of crude pectin methylesterase from *Aspergillus tubigensis* have been carried out for the clarification of papaya juice reveals an increase in clarity from 3.1 to 19.5% with maximum activity till 50 °C and shows decrease in their pH from 4.3 to 3.0[12].

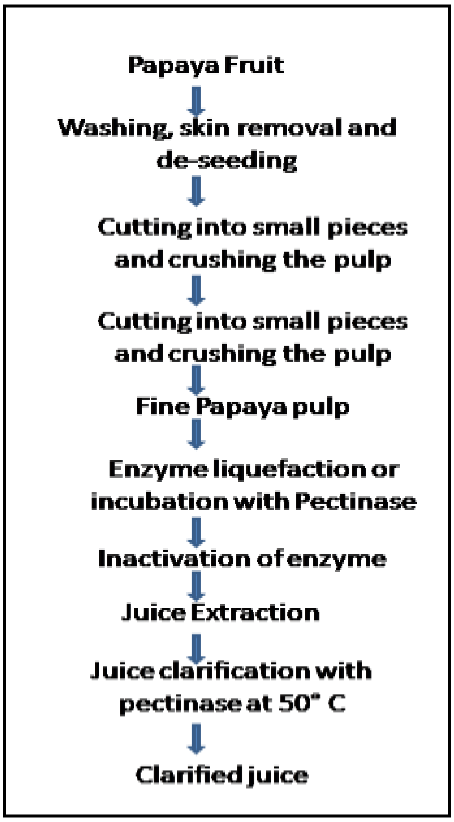

Fig. 2 Shows enzymatic extraction and clarification of papaya juice processing

Apple juice

The pectinase from *Aspergillus niger* was used for the clarification of apple and blueberry juices and it was compared with commercial pectinase. The juices were examined for the parameters such as turbidity, viscosity and degree of clarification which shows similar or superior results when compared to commercial enzyme[13]. The purified Polygalacturonase from *Aspergillus awamori* Nakazawa MTCC 6652 was utilized for the apple juice clarification. The clarified juice exhibited viscosity reduction of about 38%[14]. The pectinase from *Penicillium oxalium* F67 (PoPase) was immobilised with Magnetic cornstrach microspheres (MMCS) and it was also utilized for the apple juice clarification. The recovery of enzyme activity retained 60% of its activity after it has been reworked for eight times of juice clarification[15]. The commercial pectinase were also employed for the apple juice clarification. The enzymatic processing of juice showed significant increase in their yield ranged from 92.3% to 95.3% compared to control (81.8%). Moreover, higher turbidity and total polyphenol yield were also observed in the clarified juices[16]. The fungal pectinase in combination with gelatin was also utilized for apple juice clarification. There was an increase in clarification of juice of about 1.5- 2.0 times which contains both gelatin and enzyme compared to that contains enzyme alone in same incubation time. The effective clarification of juice was achieved with 15 IU/mL of enzyme with 0.01% of gelatin. Moreover, there is no haze development in the clarified juice during storage of about 2 months at 25°C[17].

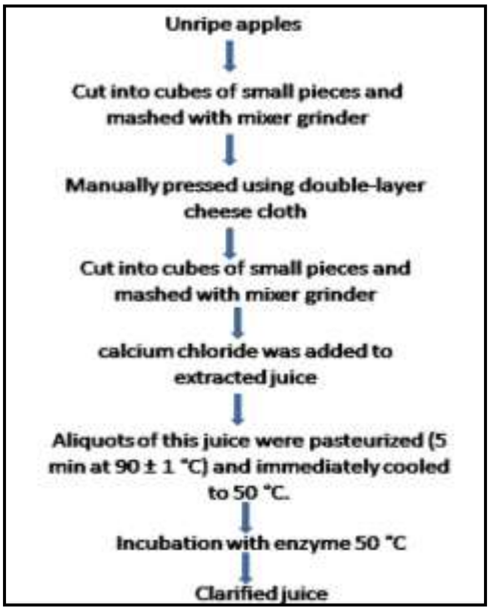

Fig. 3 shows the extraction and clarification of apple juice

Cherry juice

The pectinase from *Aspergillus sp.* was employed for the clarification of cherry juice which showed a weak effect on instant decline in turbidity, but had less chance of development in the turbidity during storage of juice[18].

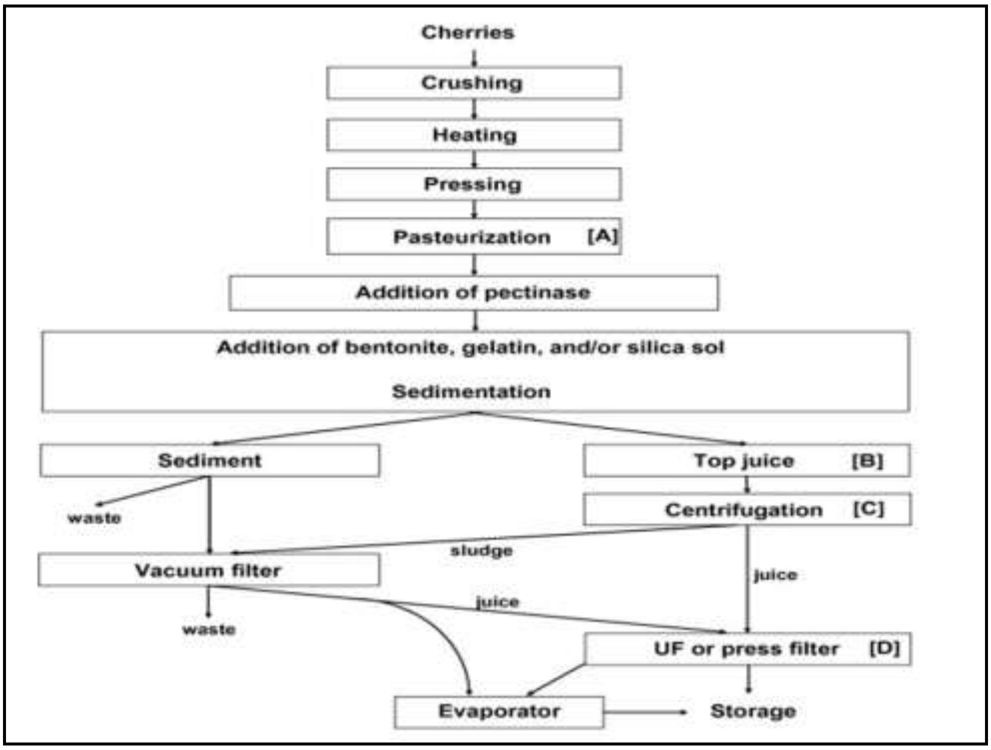

Fig. 4 shows flowchart for cherry juice processing [18]

Peach, pear, plum and apricot juices

The partially purified pectinase from *Aspergillus niger* was utilized for juice extraction and clarification of pear, peach, plum and apricot. The enzyme significantly increased the colour (TCU) and decrease in the pH, Brix acid ratio and relative viscosity. There was substantial improvement in colour and clarity scores in the extracted juice were also observed. The flavor of extracted juices was remaining unchanged by the addition of enzyme[19].

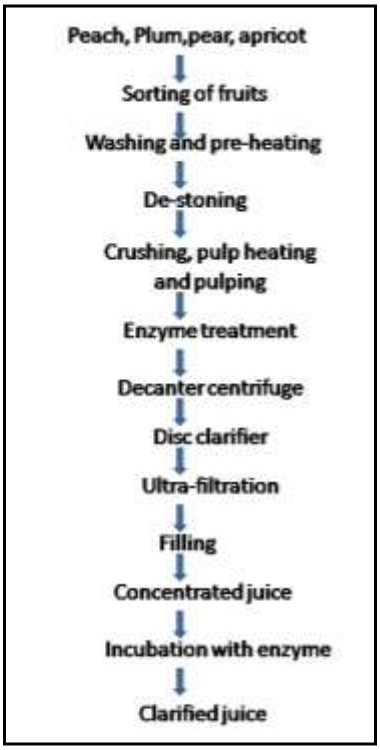

Fig. 5 Shows extraction and clarification processing of peach, plum and apricot

Carambola fruit juice

A typical analysis of carambola fruit juice reveals about 0.15% of pectin[20]. The commercial pectinase was used for the clarification of carambola fruit juice. The physical characteristics of juice which include clarity, turbidity, viscosity and color of the juice were evaluated. The results revealed that 0.10% of enzyme concentration at 30°C for 20 min showed significant changes to the clarified juice[21].

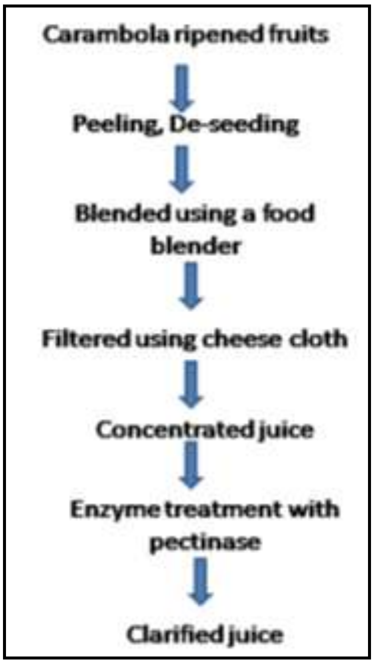

Fig. 6 shows extraction and clarification processing of carambola juice

White grape juice

The commercial pectinase from *Aspergillus niger* was employed for white grape juice clarification. It shows that it clarified the juice up to an extent of 98-99% and grape mash by 25-30%. After the clarification, total phenol and juice viscosity were reduced by 32% and 25% respectively[22].

Fig. 7 shows extraction and enzymatic clarification process of white grape juice

Butia palm juice

The crude enzyme extract from *Aspergillus niger* T0005007-2 (TE1) were utilized for butia palm fruit juice clarification and it was compared with commercial pectinases. The increase in time interval showed greater clarification, whereas temperature increase does not have much effect on the greater clarification[23].

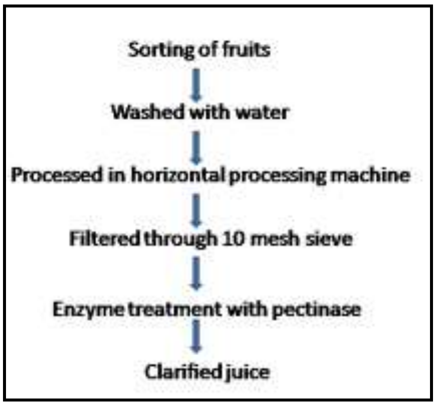

Fig.8 Shows extraction and enzymatic clarification process of butia palm juice

Banana juice

The commercial pectinase were tested for the increase in yield, viscosity reduction and clarification of banana juice. The clear juice yields of between 55 and 60% were obtained with 0.01% w/w of enzyme treatment by subsequent centrifugation at 2900 maximal centrifugal force for 20 min at 45°C. Untreated pulp yield was less than 5% under same conditions[24]. In other study, commercial pectinase at different concentrations were utilized for raw banana juice clarification. The enzymatic clarification process was optimized by response surface methodology (RSM). The factors namely enzyme concentration, temperature and time were taken into account to show the effect on turbidity, filterability, clarity and viscosity of the juice. It shows that an increase in enzyme concentration and time was associated with an increase in filterability and clarity, and decrease in turbidity and viscosity[25].

Fig.9 Extraction and enzymatic clarification process of banana juice

Sapodilla juice

Sapodilla is globose fleshy berry contain one or more seeds. The fruit has thin dry scale skin and yellowish brown pulp with pleasent and extremely good taste[26]. The Commercial pectinase from *Aspergillus niger* were employed for the sapodilla juice clarification and optimized using response surface methodology. The Sapodilla juice was treated with different enzyme concentration, time and temperature. The factors namely turbidity, clarity, viscosity and colour were examined. It clearly shows that enzyme concentration plays a significant role in the clarification process and it influences all the other factors. The results revealed that 0.1% enzyme concentration at 40 °C for 120mins of incubation is optimized conditions for clarification process[27].

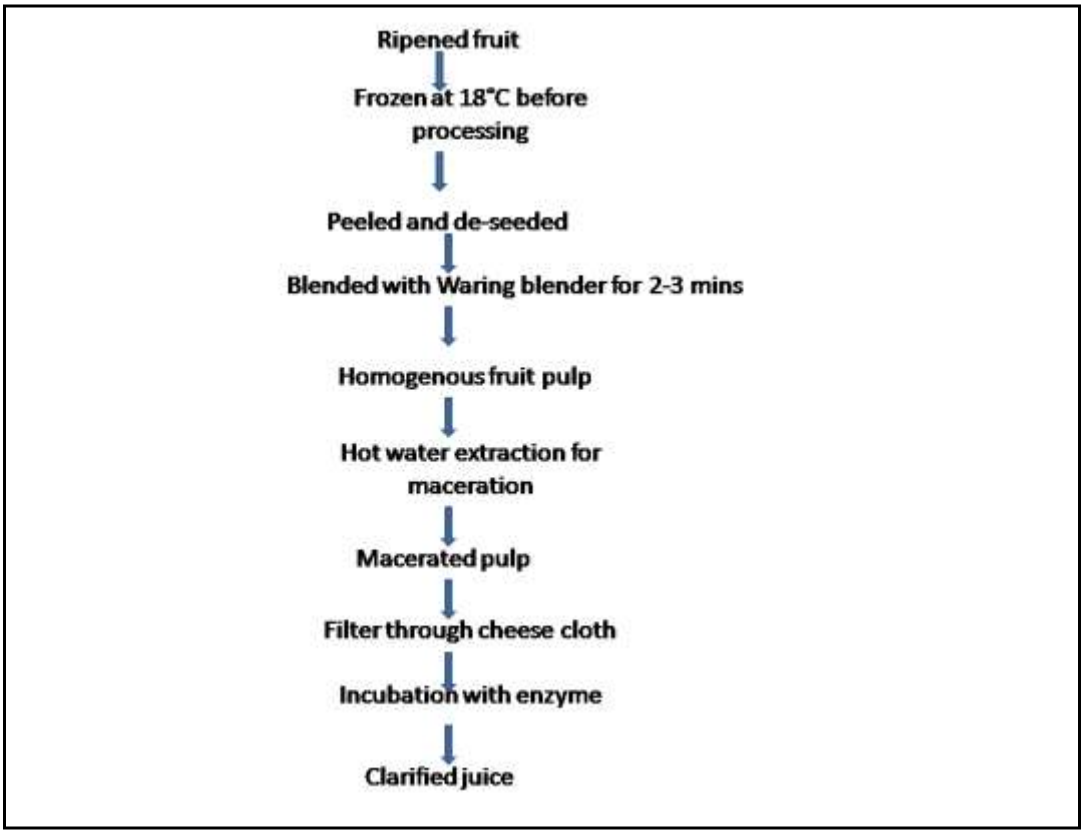

Fig10. Shows extraction and clarification of sapodilla juice

Conclusion

Pectinases plays an important role in fruit juice industrial sector. Utilization of enzyme helps in the improvement of clarity, filterability and decrease in their viscosity and turbidity. Moreover, it also helps in the color, flavor development which attracts more consumer acceptability. Recently, statistical designs like central composite design were applied for the optimization of juice clarification process. In some juices, the clarification process depends on the enzyme concentration and incubation time. Apart from ultra filtration and other process, microbial pectinase have more advantages that it can also be produced by valorization of fruit peels which makes the process cost- effective and eco-friendly. Thus, microbial pectinase will be most promising one for the juice clarification in fruit juice industries compared to other chemical engineering methods. Moreover, among microbes Actinomycetes will have ample scope in fruit juice clarification process in juice industrial sector in near future.

Acknowledgement

The authors want to express their gratitude to honorable Chancellor, Dr. G. Viswanathan, Dr. Sekar Viswanathan, Mr. Sankar Viswanathan, and Mr. G.V. Selvam for their constant encouragement and providing us good laboratory facilities of VIT University, Vellore, India to carry out this valuable work.

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