*International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)*
*An Open Access, Online International Journal Available at http://www.cibtech.org/jfav.htm*
*2017 Vol.7 (3) September-December, pp.55-60/Suryam and Charya*
***Research Article***

# FRUIT JUICE EXTRACTION AND CLARIFICATION BY PECTINASES OF *ASPERGILLUS FLAVUS* AND *A.NIGER*

***Aruri Suryam and M.A. Singara Charya**
*Department of Microbiology, Kakatiya University, Warangal Urban – 506 009, Telangana State, INDIA*
**Author for Correspondence*

## ABSTRACT
Thirty fungal species were isolated from infected fruits and tested for pectinase production, among *Aspergillus flavus* and *A.niger* showed their potentiality in the pectinase production. These two fungal strains were taken for the extraction and clarification of the fruit juice. Maximum juice was extracted from orange (82ml) by *Aspergillus flavus* followed by *A. niger* (80ml). Fruit juice was increased upto 90 ml after heat treatment at $40^0$C for 20 minutes. The enzyme extraction from these two fungal strains is very effective and replaces the application of costly commercial enzymes in fruit juice and related technologies.

***Keywords:*** *Pectinases, Fruit Rot Fungi, Fruit Juice, Extraction, Clarification*

## INTRODUCTION
Pectin found in primary cell wall and middle lamella of fruits and vegetables (Favela-Torres *et al.,* 2006). Pectinases are group of enzymes that attack pectin and depolymerise it by hydrolysis and trans elimination as well as by de-esterification reaction, which hydrolyzes the ester bond between carboxyl and methyl group of pectin (Satyanarayana and Panda, 2003). Pectic enzymes are classified into two main groups, namely de-esterifying enzymes (pectin esterases) and chain splitting enzymes (de polymerases). Pectin esterase, de-esterifies pectin, producing methanol and pectic acid. The de polymerases split the glycosidic bonds of their preferred substrate either by hydrolyses (hydrolases) or by β–elimination (lyases) (Ward and Mooyoung, 1989). Pectinases contain both cleavage activities, random (endo) or terminal (exo) (Kashyap *et al.,* 2001).

Fruit juices are generally extracted by pressing, but this is by no means, easy with soft fruits, like strawberries, partial destruction of pectin, through enzymatic treatment of the pulp, facilitates pressing and ensures high yields of juice and anthocyanin pigments. Commercial sources of fungal pectinases have been used in fruits processing for clarification, to increase the fruit juice. Clarification and complete depectimzation are pre requisites for juices, that are to be concentrated. It can be done at raised temperature (45-$60^0$C).

Saying both enzyme time and avoiding the problem of infection and unwanted fermentation (Palanivelu, 2006). Enzymatic treatment of pulp can be carried to effect almost complete liquefaction of fruits and vegetables (Pilnik and Voragen, 1993). Pectinase activities released 80% of the polysaccharide in apple, similar effect has been found for grape fruit segment membrane (Dasilva *et al.,* 2005). The immobilization of a commercial preparations of pectin lyase derived from *Aspergillus niger*, was studied in view of its possible application in fruit treatment (Spagna *et al.,* 1995).

Pectin lyase from *Penicillium cenescens*, successfully applied to production and clarification of juice (Synitsyna *et al.,* 2007). A non pathogenic fungus is a good source and has considerable economic importance, since it is employed to produce extra cellular pectinases used in food industry (Suryakanth 2008).

Fruit juices world market is about US $5 billions / year, in which Brazil is responsible for 33%, there is a creasent demand for fruit juices with the original characteristics of the fresh fruits and free from chemical additives. This results in the search of new technologies that are able to improve the nutritional and microbiological quality of the fruit juices (Lucia *et al.,* 2002). Traditional juices such as orange, grape, tomato and blends are well established in developed countries and tropical juices and their products are attracting new attention (Liew *et al.,* 2007).

Among pectin Degrading Enzymes producing Micro Organisms are yeast bacteria and fungi. However, fungi are the best producer. Enzyme activities present in crude extract is important in enhancing their industrial application. *Aspergillus sojae* is potential producer (Marco *et al.,* 2015).

Mechanical crushing of pectin rich fruit yields a fruit juice with high viscocity, which remains bound to the pulp in the form of a jellified mass. It is difficult to extract this juice by pressing using other mechanical methods with the addition of pectinases fruit juice is easily obtained and with higher yields (Tapre and Jain, 2014).

In this way, the production abilities of pectinases of certain fruit rot fungi is a useful process for the extraction and clarification of fruit juice. This scientific approach showed its enhanced advantage in the collection, maceration, separation, clarification and liquefaction of variety of fruit juices. The survey isolation, identification and culturing of fruit rot fungi for their highest yields of pectinases has given a right direction for selection of fungal pectinases, in the fruit juice technology. The selected strains, which were thoroughly investigated and critically monitored are the safe candidates for the application in fruit juice technology.

## MATERIALS AND METHODS

***(a) Collection of Infected Fruits and Fungal Isolation Method:*** The infected fruits of tomato (*Lycopersicon esculentum*), mango (*Mangifera indica*), apple (*Malus pumila*), sapota (*Achras sapota*), orange (*Citrus sinensis*) and, grape (*Vitis vinifera*) were collected carefully in the separate poly ethylene bags from the fruit markets of Kumarpally, Hanamkonda, Kazipet, Warangal (Telangana) areas and carried to the laboratory.

The infected portions of fruits indicate post harvest fungal/bacterial diseases. The fruit was surface sterilized with 0.1%. Mercuric chloride for one minute and washed thoroughly and a small transitional portion of infected and healthy regions was separated and transferred onto the agar slants of Asthana & Hawker's Agar medium (A)(Glucose-5g, $KNO_3$-3.5g, $KH_2PO_4$-0.75g, $MgSO_4$-0.75g, Agar-Agar 20g) and incubated at room temperature for 3 days. After incubation period the emerged hyphal tips were picked up and transferred to Asthana and Hawker's Agar (A) slants in aseptic condition and incubated them at room temperature for one week to obtain pure cultures. About 50 fungal species were isolated and identified from different fruits and among these, the dominant cultures occurred very frequently were selected for the present study on (*in vivo* and *in vitro*) pectinase production. The important two fungal species used in the present study are viz. *Aspergillus flavus* and *A.niger*.

***(b) Extraction of Juice from Fruits:*** Ripened-and healthy apple, sapota, mango, grape, orange and tomato fruits were collected from the fruit markets and immediately carried to the laboratory. The fruits were washed and cleaned with muslin cloth and chopped into small pieces (5 mm x 5 mm x 5 mm). The 50g of fruit pieces/pulp was transferred in to four beakers each and was added with 2 ml of commercial pectinase enzyme, culture extract of *Aspergillus flavus*, *A.niger* and distilled water. The beakers were stirred thoroughly and incubated for 15-20 minutes at the room temperature. After incubation and extraction of the total juice from the pulp, the resultant clear fruit juice solution was filtered through glass funnel containing 3-4 folds of muslin cloth and the volume was quantified. Based on the obtained quantities with commercial enzyme, culture extract with pectinase enzyme was evaluated and compared. The fruit juice extracted with distilled water served as control. The fruit pulp was also extracted after heat treatment at $40^0$C for 20 minutes and the volume of fruit juice was compared with the normal treatments.

## RESULTS AND DISCUSSION

The two fungal strains i.e. *Aspergillus flavus* and *A.niger* was responsible for high pectinase production, were studied for their role in fruit juice extraction presented in table 1. From the table it was evident that, the fungal pectinases are viable and potential in extracting orange fruit juice, when compared to commercial pectinase enzyme. Substantial improvement in fruit juice production with heat treatment was witnessed. The fruit juice in orange was 82 ml and 80 ml by *A.flavus* and *A.niger* respectively, while it was 86 ml with commercial enzyme. Increased heat treatment and incubation time recorded increased fruit juice.

In mango, the fruit juice was 44 ml with water extraction, while with *A.flavus* and *A.niger* the fruit juice was 52 ml and, 49 ml respectively, the commercial pectinase secreted 54 ml of fruit juice. The efficiency of fungal pectinase are almost equal to that of commercial enzyme. In sapota juice with water extraction, the production was 59 ml, while with *A.flavus* and *A.niger* it was 76 ml and, 74 ml respectively. The commercial pectinase enzyme secreted the fruit juice by 75 ml. Marginal increase was recorded in the fruit juice production by heat treatment at $40^0$C for 20 minutes. From the apple pulp in fruit juice extraction with water was only 48 ml, but substantially increased upto 64 ml by *A.flavus* and 63 ml by *A.niger*. The equal amount of fruit juice was produced by the application of commercial pectinase. Grapes were very much prone for fungal pectinase attack and 64 ml of fruit juice was extracted with water extraction 7l ml, by *A.flavus* and 70 ml by *A.niger* enzymes and, 74 ml of fruit juice was separated with commercial pectinase. With heat treatment the extraction was increased from 70-80 ml. In tomato fruit, juice extracted with water was 74 ml, while it increased marginally with *A,flavus* (80 ml) and *A.niger* (88 m1). The production of juice was 90 ml with commercial pectinase. Less amount of increase was noted in tomato juice extraction by treatment at $40^0$C for 20 minutes.

The *in vitro* and *in vivo* studies supported the statements of, the time required to obtain a clear juice is inversibly proportional to the concentration of enzyme used at 40 to $60^0$C temperature (Kilara, 1982). Fresh fruit juices are usually cloudy and have colloidal suspensions and in the case of orange and tomato, this cloudy effect is a desirable component in the juice and is acceptable (Isabella *et al.,* 1995). The use of crude enzyme resulted in good clarification of apple juice (Cristina *et al.,* 2005). When compared to the commercial products, the use of the crude preparation resulted in similar clarification in apple and blue berry juices (Ivana *et al.,* 2011). Pectolytic enzymes mixtures are having wide application to enhance pulp liquefaction and provide a higher yield of juice with high soluble solids content (Sharma *et al.,* 2014). Pactinases are ecofriendly in nature (Garge *et al.,* 2016). Enzymatic clarification of apple juice was stimulated by $Ca^{2+}$ ions (Szajer and Szajer 1982). The recommended temperature and incubation were $40^0$C for 120 minutes (Sin *et al.,* 2006) for maximum juice production, but according to our results the juice at $40^0$C for 20 minutes was maximum.

Improvement of cherry juice by the addition of pectinase and for large scale cherry juice processing, pre-centrifugation of juice, before clarification of juice, fining is recommended (Meyer *et al.,* 2001). Enzymatic hydrolysis with micro filtration can be attractive, alternative to sterilize tropical pulpy juices and the use of this technology can improve new trends for fruit juices market (Suryakanth, 2008) 28% ascorbic acid losses in orange juice, after membrane microfiltration process between 20-$35^0$C was reported (Venturini, 2003). Ascorbic acid losses in apple juice after enzymatic hydrolyses at $40^0$C for 60 minutes was also noticed (Youn, 2004).

*Aspergillus* genus especially *A.niger* as frequently responsible for post harvest decay of fresh fruits such as citrus, grapes, tomatoes (Ajayi *et al.,* 2014). Treatment of fruit pulps with pectinase also showed an increase in fruit juice volume from banana, grapes and apples (Kaur *et al.,* 2004).

Pre-treatment with cellulase and hemicellulase in order to reduce melon juice viscosity and obtained better fluxes in the subsequent concentrations and clarification by membrane process (Vaillant, 2005). The best particle size reduction was observed in lemon juice hydrolysed with 0.3% enzyme concentration and an incubation time of 40 minutes, the particle size ranging from 5 to 200 μm, with almost no particles above this size, different from other treatment (Carvalho *et al.,* 2006). To prevent the development of food borne pathogens in orange juice, it will be necessary to combine ultra sound with other processing methods with greater anti microbial potency, as well as to achieve a very low initial concentration of bacteria, yeast, moulds in use, such combinations will require further exploration of important synergistic effects, that are relevant for industrial use (Valero *et al.,* 2007). It is possible to reduce patuline level in apple juice, after pasteurization, enzymatic treatment, micro filteration and evaporation processes (Juliane *et al.,* 2009). Ultra sonification may be useful to extend the shelf life of orange juice (Gomez *et al.,* 2010).

The experimental preparation extract enzyme (EE) showed results statistically similar or superior to those obtained with the commercial enzyme preparations strain of *Aspergillus niger* application in the production of enzymes to be used in the production of fruit juices (Ivana *et al.,* 2013).

***Research Article***

With use of agricultural wastes and agricultural waste generates gallons of wastes during preparation of different juices. It's dumping in nature causes pollution this problem can be solved by exploiting these agro wastes for pectinase production by using potential microgarnisms (Preeti *et al.*, 2015).

**Table 1: Fruit Juice Extraction with Pectinases of *Aspergillus Flavus* and *A. Niger* and Commercial Pectinase**

| Source | Volume Fruit Juice (in ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Un Heated | | | | | | Header at $40^0$C for Minutea | | | | | |
| | Orange | Mango | Sapota | Apples | Grapes | Tomatoes | Orange | Mango | Sapota | Apples | Grapes | Tomatoes |
| Distilled water | 72.0 | 44.0 | 59.0 | 48.0 | 64.0 | 74.0 | 76.0 | 50.0 | 62.0 | 52.0 | 70.0 | 84.0 |
| *Aspergillus flavus* | 82.0 | 52.0 | 76.0 | 64.0 | 71.0 | 80.0 | 86.0 | 70.0 | 78.0 | 70.0 | 78.0 | 88.0 |
| *A.niger* | 80.0 | 49.0 | 74.0 | 63.0 | 70.0 | 88.0 | 84.0 | 68.0 | 76.0 | 68.0 | 76.0 | 92.0 |
| Pectinase (PGase) | 86.0 | 54.0 | 75.0 | 64.0 | 74.0 | 90.0 | 90.0 | 74.0 | 80.0 | 68.0 | 80.0 | 96.0 |

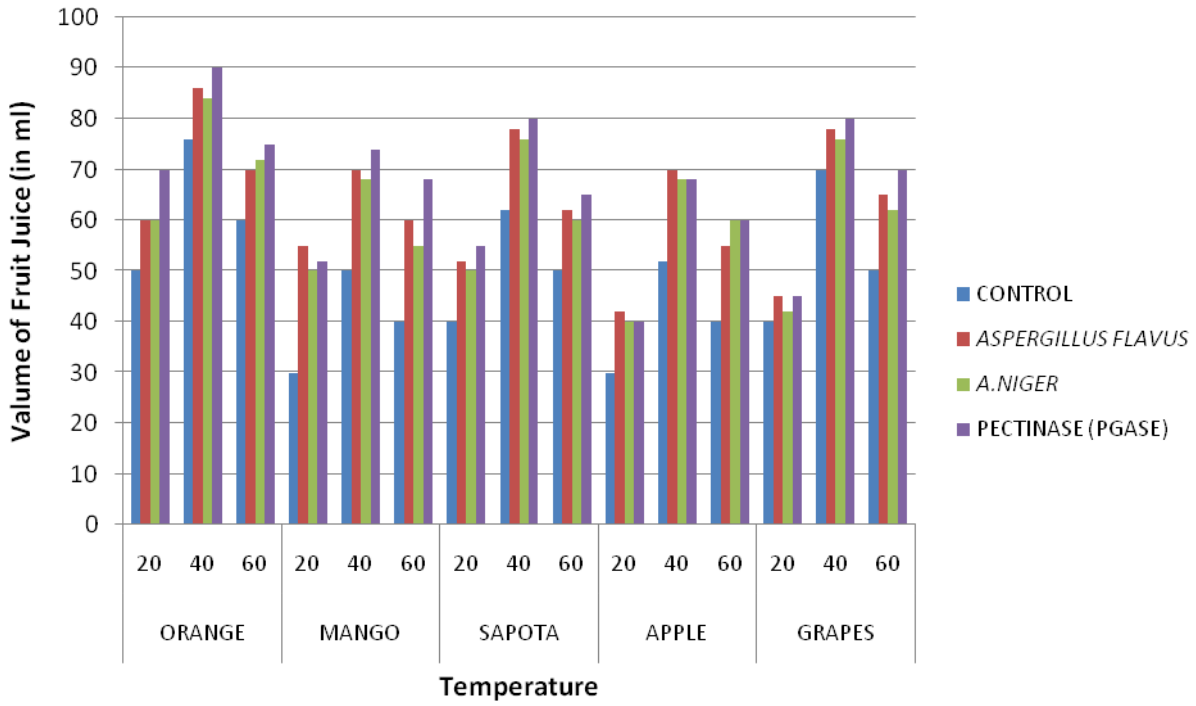


**Figure 1: Effect of Temperature on Pectinase Activity in Six Fruits**

***Conclusion***

It was clearly noticed that, the enzyme extraction from these two fungal strains is very effective and replaces the application of costly commercial enzymes in clarification and extraction. This scientific approach showed its enhanced advantage in the collection, maceration, separation, clarification and liquefaction of variety of fruit juices. As this fruit juice technology is attaining its momentum, this type of biotechnological methods, using cost effective, eco-friendly, non toxic approaches are utmost important.

These two selected strains i.e. *Aspergillus flavus* and *A.niger*, which were thoroughly investigated and critically monitored are the safe candidates for application in fruit juice technology.

**ACKNOWLEDGMENT**
The authors are thankful to Head Department of Micro Biology, Kakatiya University, Warangal Urban, Telangana, India, for providing lab facilitiesand one of the author Aruri Suryam is very thankful to University Grants Commission (U.G.C.), for award of Rajiv Gandhi National Fellowship (R.G.N.F.) during research.

**REFERENCES**
**Ajayi AA, Osunkoya FA, Peter ACF and Olaschinde GJ (2014).** Clarification of apple juice with laboratory produced pectinase obtained from the Deterioration of apple fruits by *Aspergillus niger. International Journal of Advanced Biotechnology and Research* **15** 134-140.
**Carvalho LMJ, Borchetta R and Silva EMM (2006).** Effects of enzymatic hydrolysis on particle size reduction in lemon juice, *Brazillian. Journal of Food Technology* **9** 277-282.
**Cristina FM, Mercedes PG and Adriano B (2005).** Enzymatic clarification of fruit juices by pectin lyase. *Journal of Food Biotechnology* **19** 173-181.
**Dasilva EG, De Fathima BM, Medina C, Piceoli RH and Schwan RF (2005).** Pectinolytic enzymes secreted by yeasts from tropical fruits. *Yeast Research* **9**(5) 859-865.
**Favela-Torres Volke ST and Viniegra GG (2006).** Production of hydrolytic depolymerising pectinases. *Food Technology and Biotechnology* **44**(2) 221-227.
**Garg G, Singh A, Kaur A, Singh R, Kaur J and Mohan R (2016).** Microbial pectinases, an eco-friendly tool of nature for industries. *Biotechnology* **6** 47.
**Gomez LVM, Orsolani L, Martinez YA and Tapia MS (2010).** Microbiological and sensory quality of sonicated calcium added orange juice. *Food Science and Technology* **43** 808-813.
**Harsh SP, Hiral P and Sugandha S (2014).** Enzymetic extraction and clarification of juice from various fruits: A review. *Trends in Post Harvest Technology* **2** 01-14.
**Isabella MB, Geraldo AM and Raimundo WF Physical (1995).** Chemical changes during extraction & clarification of guava juice. *Food Chemistry* **54** 383-386.
**Ivana GS, Cristiane MTL, Roselei CF and Mauricio MS (2013).** Use of pectinases produced by a new strain aspergillus niger for the enzymatic treatment of apple and blueberry juice. *Food Science and Technology* **51** 469-475.
**Ivana GS, Roselci CF, Debora MB and Mauricio MS (2011).** Clarification of fruit juices by fungal pectiuases. *Food Science and Technology* **44** 2217-2222.
**Juliane EW, Michele H, Horacio AD and Isa BN (2009).** Effect of processing stages of apple juice concentrate on patulin levels. *Food Control* **20** 48-52.
**Kashyap DR, Vohra PK, Chopra S and Tewari R (2001).** Applications of pectinases in the commercial sector: A review. *Bio Resource Technology* **77** 215-277.
**Kaur G, Kuar S and Satyanarayana T (2004).** Production, characterization and application of a thermopolite apinis. *Bio-Resource Technology* **94** 239-43.
**Kilara A (1982).** Enzymes and their role in the processed apple industry: A review. *Process Biochemstry* **23** 35-41.
**Liew AAG, Sulaiman NM, Aroua MK and Noor MJMM (2007).** Response surface optimization of conditions for clarification of Carambola fruit juice using a commercial enzyme. *Food Engineering* **81** 65-71.
**Lucia C, Irala DSS, Flavia DSG, Virgini MM and Lourdes MCC (2002).** Cold sterilization of pine apple juice by trangenial micro filteration. *Journal of Desulination* **148** 93-98.
**Marco AMG, Doreen H, Inigo OG, Francis B, Marco RP and Marcelo FL (2015).** A novel pectin degrading enzymes complex from Aspergillus sojac ATCC 20235 Mutants. *Journal of the Science of Food and Agriculture* **95** 1554-1561.

**Meyer AS, Koser C and Adier NJ (2001).** Efficiency of enzymatic and other alternative clarification and fining treatments on turbidity and haze in cherry juice. *Agricultural and Food Chemistry* **49** 3644-3650.

**Palanivelu P (2006).** Poly Galacturonases, activesite analysis and mechanism of action. *Indian Biotechnology* **5** 148-162.

**Pilnik W and Voragen AGJ (1993).** *Pectic Enzymes in Fruit and Vegetables Juice Manufacture in Food and Science Technology Enzymes in Food Processing,* G.Reeds (edition) 3rd edition, (Academic Press, New York, USA) 363-399.

**Preeti S, Abishek T, Deeja K and Suresh S (2015).** Isolation, screening and optimization of novel pectinase producing fungal strain for fruit juice clarification and extraction. *World Journal of Pharmaceutical Research* **4** 2114-2126.

**Satynarayana NG and Panda T (2003).** Purification and biochemical properties of microbial pectinases: A review. *Process Biochemistry* **38** 987-996.

**Sin HN, Yusof S, Hamid SKN and Rehman AR (2006).** Optimization of enzymatic clarification of Sapodilla juice using response surface methodology. *Food Engineering* **73** 313-319.

**Spagna G, Pifferi PG and Gilioli E (1995).** Immobilization of a pectinlyase from *Aspergillus niger* for application in food technology. *Journal of Enzyme and Microbial Technology* **17** 729-738.

**Suryakanth KN (2008).** Comparative biochemical and structural characterization of fungal poly galacturonases. *Biologia* **63**(1) 1-19.

**Synitsyna OA, Fedorova E and Semenova MV (2007).** Isolation and characterization of extra cellular pectinlyase from Penicillium canscens. *Journal of Biochemistry* **72** 565-571.

**Szajer I and Szajer CZ (1982).** Clarification of apple juice by pectin lyase from Penicillium paxili. *Journal of Biotechnology Letters* **4** 553-556.

**Tapre AR and Jain RK (2014).** Pectinases: Enzymes for fruit processing industry. *International Food Research Journal* **21**(2) 441-453.

**Vaillant F (2005).** Concentration of melon juice using membrane process. *Innovative Food Science and Emerging Technologies* **6** 213-220.

**Valero M, Recrosio N, Saura D, Munoz N, Marti N and Lizama V (2007).** Effects of ultra sonic treatments in orange juice processing. *Journal of Food Engineering* **80** 509-516.

**Venturini FWG (2003).** Tangential micro filteration of orange juice in bench pilot. *Ciencia E Technologia De Alimentos* **23** 330-333.

**Ward OP and Mooyoung M (1989).** Enzymatic degradation of cell wall and related plant polysaccharides. *Critical Reviews in Biotechnology* **8** 237-274.

**Youn KS (2004).** Effective clarifying process of re constituted apple juice using membrane filteration with filter aid pre treatment. *Journal of Membrance Science* **228** 179-185.