



Production of ethanol and clarification of apple juice by pectinase enzyme produced from *Aspergillus terreus* NCFT 4269.10

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Abstract

Aspergillus terreus NCFT 4269.10 was evaluated by liquid static surface fermentation (LSSF), shaking fermentation (ShF) and solid state fermentation (SSF) for the production of pectinase. Among various substrates tested, banana peels supported maximum production of pectinase i.e. 1000 ± 141.42 U/ml. The biomass of *A. terreus* was maximum with wheat bran (0.55 ± 0.07 g/50ml). Pectinase produced by *A. terreus* displayed higher specific activity when wheat bran was used as the sole source of carbon and energy. After successful fermentation, crude enzyme was purified to electrophoretic homogeneity with a specific activity of 1846.50 U/mg from an initial specific activity of 1282.05 U/mg. The cell free-dialyzed-enzyme containing 107100 U was purified to 1.44 fold with an overall enzyme yield of 35.70%. Immobilization study revealed that the production of pectinase was increased up to third cycle and decreased thereafter when further pectinase production was carried out by immobilized spores of *A. terreus*.

Keywords: *Aspergillus terreus*; Banana Peels; Fermentation; Wheat Bran.

1. Introduction

Pectinases are the complex hydrolytic enzymes that include pectin esterase (EC. 3.1. 1.11) which catalyzes the hydrolysis of methylated carboxylic ester groups in pectin into pectic acid and methanol. Pectinases are one of the highflying enzymes of contemporary biotechnology industries, especially, in juice preparation and food industries (Kashyap et al. 2001), paper and pulp industries (Beg et al. 2001; Viikari et al. 2001). It has been reported that microbial pectinases account for 25% of the global food enzyme sales (Sethi et al. 2016). Among all the microbial pectinases, fungal sources are the most preferred one (Singh et al. 1999).

Pectinolytic enzymes can be derived from different sources (Namasivayam et al. 2011). However, pectinase producing microorganisms have due advantage over other sources because they can be subjected to genetic and environmental manipulations to increase yield (Vibha and Neelam 2010). Submerged fermentation (SmF) and solid state fermentation (SSF) have been successfully used in pectinase production by fungi (Pedrolli et al. 2008). Submerged fermentation is a well-developed system used in industrial scale to produce a large variety of microbial metabolites. SmF is technically easier than SSF and has been strongly developed from 1940s onwards. Microbial pectinase can be stated as the most important enzyme for the juice industry. Although, pectinase production is an inherent property of most of the organisms, only those microbes that produce a substantial amount of extracellular pectinase are of industrial importance and have been exploited commercially. Among these, strains of *Aspergillus* species dominate the industrial sector.

Pectinases are widely used in the food industries in the production of juices, fruit drinks and wines (Semenova et al. 2006). Pecti-

nases are widely used in extraction, clarification and removal of pectin in fruit juices, in maceration of vegetables to produce pastes and purées and in winemaking, are often produced by fungi, especially by *Aspergillus niger*. The crushing of pectin-rich fruits results in high viscosity juice which stays linked to the fruit pulp in a gelatinous structure, hindering the juice extraction process by pressing. Pectinase addition in the extraction process improves the fruit juice yield through an easier process, decreases the juice viscosity and degrades the gel structure, thus improves the juice concentration capacity (Kashyap et al. 2001). In case of fruit juice, extraction by enzymatic maceration can increase yields by more than 90% compared to conventional mechanical juicing, besides improving the organoleptic (color, flavor) and nutritional (vitamins) properties and technological efficiency (ease of filtering) (Rombouts and Pilnik 1980). In several processes, pectinolytic enzymes are applied in association with other cell wall degrading enzymes such as cellulases and hemicellulases (Bhat 2000). The mixture of pectinases and cellulases has been reported to improve more than 100% juice extraction yields (Kashyap et al. 2001). Soares et al. (2001) reported an improvement between three and four times in juice yields from papaya, banana and pear using enzymatic extraction instead of the conventional pressing process. The enzymatic treatment can help in decrease of 62% of the apple juice viscosity. When the depectinized apple juice is ultrafiltered, the permeate flux is much higher than undepectinized juice. The increase in the permeation rate is a result of both reduction in apple juice viscosity and total pectin content. Pectin is a fiber shaped colloid that causes severe fouling of ultrafiltration membranes (Alvarez et al. 1998). The commercially available pectinase preparations used in food processing are traditionally associations of polygalacturonases, pectin lyases and pectin methyl esterases.

These preparations are usually derived from fungi, mainly from the genus *Aspergillus* (Gummadi and Panda 2003; Semenova et al. 2006). Among filamentous fungi, the genus *Aspergillus* is well recognized in different industries as it has the ability to produce many extracellular enzymes for nutrition and can use cheapest substrates to sustain and carry out its life processes. Filamentous fungi of genus *Aspergillus* have therefore received attention towards the development of expression secretion systems aiming at the economic production of high-valued industrial enzymes.

Agro industrial residues have been reported to be the suitable substrate for economic production of pectinase (Kiran Kumar et al. 2011). Fungal species have been studied extensively for the production of industrially proficient pectinase enzymes because of their ability to use complex and low cost substrates for growth and maintenance (Kumar and Duhan 2011). Most commonly used agro wastes used as substrates include sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soy hull, sago hampes, grapevine trimming dust, saw dust, corn cobs, banana waste etc. Among such substrates, wheat bran, however, occupies the key place and has most commonly been used in various processes.

Therefore, the present investigation aimed at the economic production of pectinase using both free and immobilized cells of *A. terreus* NCFT 4269.10. Further, applications of this enzyme have been evaluated for its industrial compatibility and suitable use.

2. Materials and methods

2.1. Collection of agro wastes and inoculum preparation

Banana peels (BP), saw dust (SD) and wheat bran (WB) were collected and dried at 60°C for 48 h or more until the moisture content was reduced. Cooled samples were later grounded in a blender and kept in sterile containers until required. The fungal strain, *Aspergillus terreus* NCFT 4269.10 (GenBank accession Number: KT222271) was maintained on PDA/Sabouraud dextrose agar slants at 4°C. Spore suspension (1 ml) having spore concentration of about 1×10^7 cells ml⁻¹ from 7 days old culture was used as inoculums in the experiments (Sethi et al. 2013).

2.2. Crude pectinase production by fermentation

In 150 ml capacity Erlenmeyer flasks, 50 ml of sterilized fermentation medium having individually BP, SD and WB as substrate was inoculated with 1.0×10^7 cells ml⁻¹ from 7 days old cultures of *A. terreus* and incubated at $30 \pm 2^\circ\text{C}$ both at liquid static and shaking condition. After 96 h, samples were processed for recovery of pectinase and its activity assay. The fermented broth (50ml) cultures were centrifuged at 10,000 rpm, at 4°C for 10 min to obtain the crude supernatant as crude pectinase for pectinase activity assay. Further, the clear crude supernatant was passed three times through Whatman No. 1 filter paper before pectinase production assay. The remaining crude enzyme was preserved at -20°C for further use.

2.3. Pectinase assay

Pectinase activity was performed as per the standard method of Khairnar et al. (2009). Test sample are prepared by mixing 4.9 ml of 0.5% pectin solution in 0.1ml of pectinase, 5.0ml of I₂/KI solution (50M/200mM), 1ml of 1M Na₂CO₃, 2 ml of H₂SO₄ (2M) and 1-2 drops of pectin indicator. Blank was prepared without adding enzyme in the reaction mixture. All reagents were mixed by gentle shaking. Titrated the Test and Blank with Na₂S₂O₃ (100mM) until it became light yellow. Then 1 drop of pectin indicator was added and continuously titrated with Na₂S₂O₃ until solution became colorless.

Protein concentrations of the crude pectinase were determined according to Lowry et al. (1951) method taking Bovine Serum Albumin (BSA) as the standard.

2.4. Measurement of dry cell biomass

The biomass content was determined by measuring the dry weight from a known amount of sample. The sample was centrifuged at 10,000 rpm, at 4°C for 10 min and the fungal biomass was rinsed with sterilized double distilled water and filtered out using Whatman No.1 filter paper. The biomass obtained was dried overnight inside Hot Air Oven at 100°C till constant weight was attained. Finally, the dry weight of the fungal mycelia was weighed and calculated (Sethi et al. 2013).

Weight of biomass = weight of dry filter paper with fungal biomass - weight of blank and dry filter paper

[Dry weight of biomass = dry weight of fungus]

2.5. Partial purification of pectinase

Partial purification of the crude Whatman paper filtered pectinase supernatant was performed by ammonium sulphate precipitation up to 80% saturation at 4°C of the crude pectinase enzyme. The precipitated protein was recovered by centrifugation at 10,000 rpm for 10 min at 4°C and partially purified by overnight dialysis at 4°C against 0.1 M phosphate buffer (pH 6.5) (Jana et al. 2013). Pectinase activity and total protein content of the partially purified enzyme was measured.

2.6. Immobilization of *Aspergillus terreus* spores for production of pectinase

The alginate entrapment of cells/spores was performed according to the method of Sharma and Satyanarayana (2012). To the spore suspension, 3% of sterilized alginate slurry was mixed well in a ratio of 1:1 for 10 minutes to get a uniform mixture. The slurry was taken in a sterile syringe and added drop wise into chilled 0.2 M CaCl₂ solution from 5-cm height and kept for curing at 4°C for 1 h. Conidia immobilized in beads of calcium alginate were aseptically inoculated to sterilized fermentation broth (BP, SD and WB) and incubated at 30°C for 96h. Finally, the fermented media were filtered on Whatman No. 1 paper and beads were washed twice with sterile saline solution (0.8% NaCl) and kept in phosphate buffer (1M, pH 7.0). Then, the beads were again introduced into the fresh medium. After attaining maximum production of enzymes, the spent medium was replaced with fresh production medium (50 ml) and the process was repeated for five batches until the beads/blocks started disintegrating. The enzyme titers and cell leakage of each cycle were determined.

2.7. Ethanol production using agro-wastes

The fermentation broth media (500ml) for ethanol production were prepared using BP, SD and WB as the substrates. The flasks containing the sterilized fermentation media were aseptically inoculated with *Saccharomyces cerevisiae*. In another flask, *S. cerevisiae* co-cultivated with *A. terreus* and sealed tightly with wax so as to prevent the air flow. In co-cultivation experiment, the fermentation medium was first inoculated with *A. terreus* and incubated for 48h at 30°C. The spent fermentation medium was then inoculated with *S. cerevisiae* and incubated for another 10 days at $30 \pm 2^\circ\text{C}$ under anaerobic condition. The fermentation and production of ethanol were checked by the bubbles (CO₂) formed during the process and change of color of aqueous calcium hydroxide to white milky color. The fermented matter was allowed to settle for 1 day and then distillation was carried out for the recovery of ethanol.

Similarly, the sterilized fermentation media were aseptically treated with pectinase and cellulase individually and also in combinations (1:1) and incubated for 2h at 50°C. After that, the fermentation media were aseptically inoculated with *S. cerevisiae* and incubated for another 10 days at $30 \pm 2^\circ\text{C}$ under anaerobic condition. After the successful fermentation, the fermented broth was allowed to settle for 1 day and then distillation was carried out for the recovery of ethanol.

The fermented matter was transferred to alcohol distillation unit and sealed properly. Ethanol recovery was carried out at 68 ± 2 °C for 3 h and the distillate was collected in screw capped bottles, sealed with para film and finally kept in refrigerator at 4°C.

The ethanol content of the fermented broth was determined by measuring the specific gravity of the distillate according to the procedure described by Amerine and Ough (1984).

2.8. Enzymatic extraction and clarification of apple juice

Apples purchased from local market were washed, peeled and cut into small pieces. Ascorbic acid (0.5 g/kg) was added and the cut apples were mashed using a food processor for 30 sec. To the mash, 1ml/100 g of enzyme were added and extraction was carried out at 30 °C and 52 ± 2 °C for 30 min, 60 min, 90 min and 120 min respectively. The treated mash was heated to 90 °C for 5 min in a water bath to inactivate the enzymes. Juice from the mash was separated by centrifugation at 6000 rpm for 10 min and pomace was discarded.

Juice yield (%) = (Final juice weight/Initial mash weight) × 100

Clarity of juice was measured according to the method of Krop and Pilnik (1974). Percent transmittance was determined at 660 nm using a UV-Vis spectrophotometer.

The content of pectinacious materials present in the apple juices was measured in terms of alcohol-insoluble-solids (AIS). AIS were determined by boiling 5 g juice with 75 ml 80% ethanol, simmering for 30 min and filtering through Whatman No. 1 filter paper. The filtered residue was washed again with 80% ethanol. The residue was dried at 100°C for 2 h and expressed in percentage by weight (Hart and Fisher 1971). The density of juice was determined using a specific gravity bottle.

2.9. Fermentation kinetics study

Various fermentation kinetics parameters were also calculated (Sethi et al. 2015). They include Y_e/s (yield of enzyme per gram of substrate), Y_x/s (yield of biomass per gram of substrate), Y_e/x (yield of enzyme per gram of biomass), dx/dt [biomass accumulation in the culture medium ($g\ l^{-1}\ h^{-1}$)], dP/dt [enzyme accumulation in the culture medium ($U\ ml^{-1}\ h^{-1}$)], μ_{max} [highest specific growth rate observed during batch culture ($mg\ l^{-1}\ h^{-1}$)], x_{max} [maximum attainable biomass ($mg\ ml^{-1}$)], α [growth associated coefficient of enzyme production ($U\ g^{-1}$)], β [growth-independent coefficient of enzyme production ($U\ g^{-1}\ h^{-1}$)].

2.10. Statistical analysis

Statistical analysis was performed by SPSS, version 10 for windows (SPSS Inc; Chicago, IL, USA).

3. Results and discussion

Various agro-wastes like wheat bran (*Zea mays* L.), banana peels (*Musa paradisiaca* L.) and sawdust was screened individually for the selection of suitable substrate required for optimum production of pectinase. The nature and chemical composition of the substrate is also one of the most important parameter that greatly affects fermentation processes. Among various substrates used for the production of pectinase in LSSF, banana peels as the substrate supported maximum production of pectinase i.e. 1000 ± 141.42 U/ml when *A. terreus* was used for fermentation in BP fermentation medium. About half of the pectinase production (600 ± 141.42 U/ml) was obtained using saw dust as the sole carbon source (Table 1). Similarly, the total protein content of the crude extracts was different when the fermentation was carried out with different substrates. Maximum protein content was found in the crude enzyme extract when the fermentation was carried out in static condition using banana peels ($724.28 \pm 56.39 \mu g/ml$) (Fig. 1). After successful fermentation, the biomass was separated, processed and estimated. It is concluded that the maximum biomass of *A. terreus* was obtained with wheat bran ($0.55 \pm 0.07 g/50ml$) as compared to other substrates taken for this study. Banana peels were used as the substrate owing to its high pectin content which can be exploited very well as India has the first position in banana production which is about 16 million tons per year (Jacob et al. 2008). The use of other substrates (wheat bran, orange peel, sugar cane peel and banana peel) revealed the preference of banana peels by the isolates for pectinase production which is at par with the findings of Torimiro and Okonji (2013).

Table 1: Specific Activity of Pectinase Produced by *A. terreus*

Fungus	Substrates	Pectinase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
<i>A. terreus</i>	Sawdust	600 ± 141.42	0.46 ± 0.08	1295.05
	Banana peels	1000 ± 141.42	0.78 ± 0.05	1285.18
	Wheat bran	950 ± 212.13	0.26 ± 0.03	3612.16

Liquid shaking fermentation was carried out to find out the suitable shaking rate (rpm) and suitable fermentation type for the production of pectinase for its application in industrial sectors. It was concluded from the results that shaking fermentation was suitable for the production of enzyme using *A. terreus*. The shaking fermentation study revealed that maximum pectinase was produced using banana peels (1250 ± 212.13 U/ml) as the sole source of carbon at 100 rpm by *A. terreus* (Fig. 1). Similarly, maximum protein was also obtained from BP fermentation medium. Liquid shaking fermentation using orange peels by *A. terreus* at 120 rpm for 96 h at 30°C was suitable than static condition for pectinase biosynthesis (Sethi et al. 2015).

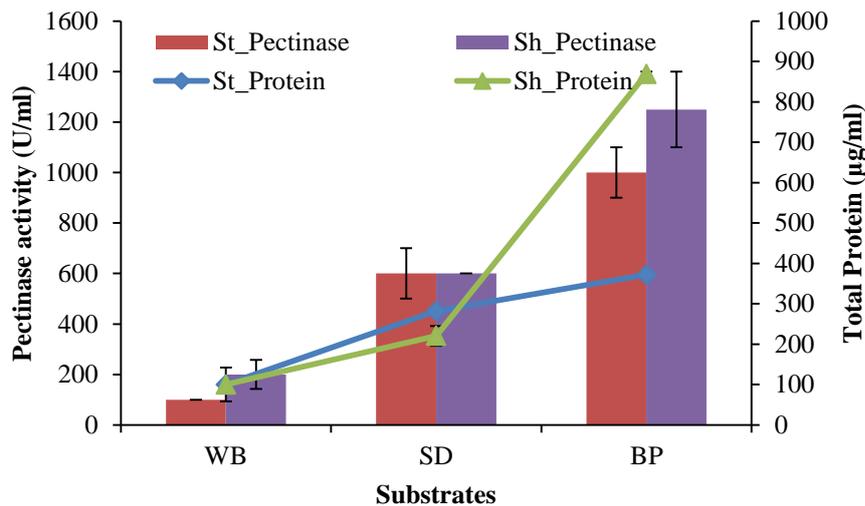


Fig. 1: Production of Pectinase, Total Protein and Sugar Released in Liquid Static Surface Fermentation and Liquid Shaking Fermentation using Banana Peels (BP), wheat Bran (WB) and Saw Dust (SD) as Substrates by *A. Terreus* (St= Static Condition; Sh= Shaking Condition)

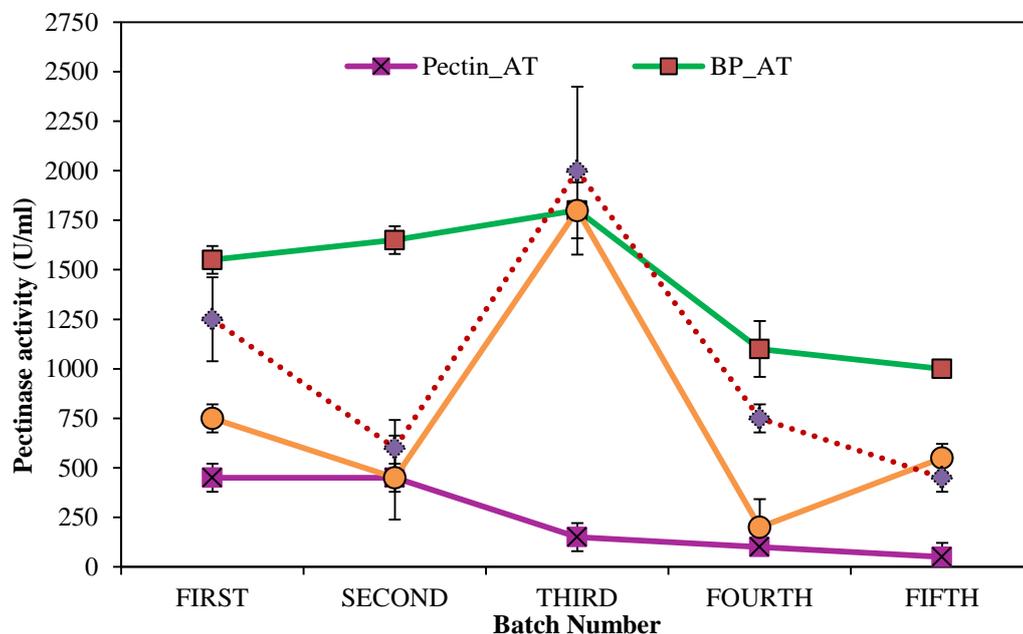


Fig. 2: Production of Pectinase Using Calcium Alginate Beads Entrapping the Spores of *A. terreus* Using Repeated Batch Cultures. (AT: *Aspergillus terreus*)

Out of these three agro waste (banana peels, wheat bran and saw dust) banana peel showed highest enzyme activity in both the fermentation process, hence, pectinase enzyme extracted from banana peel are used for further purification, ethanol production and apple juice clarification activity.

Due to the maximum purification of pectinase enzyme from lower range to higher range, the initial maximum enzyme activity (Maximum enzyme activity at static condition) was taken into consideration. The enzyme was purified to electrophoretic homogeneity with a specific activity of 1846.50 U/mg of protein from an initial specific activity of 1282.05 U/mg of protein. After dialysis, the cell free-dialyzed-enzyme containing 107100 U was purified to 1.44 fold and the overall enzyme yield was 35.70% (Table 2). The analysis of enzyme activity in the crude extract does not indicate either an isolated action or the presence of a multi-enzymatic system working in synergy on the substrate degradation. The characterization of purified enzymes is an important research line since it provides discrimination between the enzymatic complex components about substrate degradation mechanism, optimum activity conditions and enzyme synthesis regulation. Higher fold of purification of crude pectinase enzyme than the present investigation was also reported before (Celestino et al. 2006).

Table 2: Partial Purification of Isolated Crude Pectinase by *A. terreus*

Step	Volume (ml)	Activity (U/ml)	Total enzyme activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude	300	1000	300000	234	1282.05	1	100
Ammonium sulphate precipitation	44	4100	180400	103	1751.45	1.37	60.13
Dialysis	17	6300	107100	58	1846.50	1.44	35.70

The reusability of alginate immobilized fungi for pectinase production was evaluated. Beads entrapping *A. terreus* spores (Fig. 3) were successfully employed in 5 repeated cycles in the presence of pectin, banana peels, saw dust and wheat bran as the carbon sources. When pectin was used as the carbon source, the activity of pectinase increased slightly with the use of recycled beads up to second cycle and fell thereafter. The production of pectinase in-

creased up to third cycle and drastically decreased thereafter when banana peels were used as the substrate for the production of pectinase by the immobilized spores of *A. terreus*. Further, the disintegration of beads started at the late phase of fourth cycle in case of *A. terreus*. Similarly, when wheat bran was taken for the production of pectinase, the biosynthesis of pectinase by *A. terreus* was continued up to fifth cycle displaying highest production at third cycle. The disintegration of beads started from the late phase of third cycle. The production of pectinase was not uniform in case of saw dust supplemented fermentation medium for both immobilized spores of *A. terreus*. However, the production of pectinase was utmost at third cycle and drastically decreased thereafter in case of *A. terreus*.

Immobilization of microbial cells and enzymes has become one of the most valuable tools in the field of biotechnology (El-Katatny et al. 2003). Moreover, microbial entrapment gives prolonged metabolic activity when microbial cells are reused and protects the organism from inhibitory compounds or metabolites.

The partially purified enzyme was used for the production of ethanol. The pre-processed pectinaceous agro-wastes (banana peels) were treated with the partially purified enzyme for 2 h and the enzymatic hydrolyzed substrate was used for the production of ethanol by fermentation using *Saccharomyces cerevisiae* as the fermentative organism. Three different conditions were maintained for the ethanol production. It was concluded from the Table 3 that the enzyme treated substrate was able to produce highest amount of alcohol with high purity as compared to untreated and co-cultivated fermentation conditions (Table 3). The alcohol weight (%) of the distilled alcohol was also estimated. Alcohol weight (%) produced by enzyme treated substrate was found to be highest i.e. 91.5%, with compare to other experimental conditions (Table 3). Many literatures revealed that the supplementation of pectinolytic enzymes tends to increase the level of methanol in pectic substrates because of the activity of pectin methyl esterase.

Table 3: Production of Ethanol from Enzyme Treated and Untreated Banana Peels

Treatment of substrate ^a	Specific gravity at 20°C	Alcohol weight percentage	g/100ml	Volume percentage
Only <i>S. cerevisiae</i> cells	0.9917	22	21.8	27.5
<i>S. cerevisiae</i> + <i>A. terreus</i> spores	0.9917	22	21.8	27.5
Pectinase_AT + <i>S. cerevisiae</i> cells	0.9502	91.5	89.3	112.5

The fermentation medium for production of ethanol was prepared using only banana peel as the substrate. (Pectinase_AT: pectinase produced by *Aspergillus terreus* NCFT 4269.10)

Therefore, an attempt has been taken for the bioconversion of the pectic substrates to fermentative substrate by fungal enzymes for ethanol production using *S. cerevisiae*.

Pectins present in juices are responsible for the turbidity and consistency of the juice causing an increase in their viscosity which hinders its clarification, filtration and concentration (Alkorta et al. 1998). The degradation of pectic substances in mashed fruit purees is possible through the addition of pectinase resulting in an enhanced juice yield and its clarification as well as a decrease in viscosity. Treatment with pectinases also provides filtering of the product (Ribeiro et al. 2010). Hence enzymatic treatments of apple fruit juice are carried out at 30°C (temperature treatment). As pectinases are the chief component for the fruit juice manufacturing process, their activities under the suitable conditions were studied for apple juice preparation. Higher juice yield and clarity were obtained with only partially purified pectinases at 90 min of incubation in comparison to only cellulase and pectinase with cellulase treatment (Table 4). Similar results were obtained for the clarification of the juice when treated with only pectinase enzyme. The maximum clarity (80%) was obtained at 30 min of incubation when pectinase was used as enzyme source (Table 4).

In the case of apple juice, the most frequently used enzymes are those that can depolymerize the highly esterified apple pectin. Juice from apple can be achieved through two-step process consisting of a first treatment of the crushed apple mush with pectinases to get the premium juice followed by pomace liquefaction i.e. treating with a different proportion of pectinases and cellulases for the complete extraction of the juice (Will et al. 2000). Hence in the present study extraction and clarification of the apple juice was carried out by both cellulase and pectinase treatment (Table 4).

Table 4: Enzymatic Extraction of Apple Juice Using Partially Purified Pectinase

Treatment Duration	Enzyme application	weight of only pomace (g)	weight of only juice (g)	% of juice yield	Clarity (% Transmittance at 660 nm)
30 min	Control*	1.112	1.363	0	0.3
	Cellulase	0.769	1.536	62.06	31.3
	Pectinase	0.207	1.67	67.47	80.9
	cellulase + pectinase	0.922	1.575	63.64	21.4
60 min	Cellulase	0.015	1.668	67.39	38.9
	Pectinase	0.223	1.685	68.08	68
	cellulase + pectinase	2.25	1.575	63.63	29.6
90 min	Cellulase	0.198	1.67	67.47	27.6
	Pectinase	0.3	1.737	70.18	52.9
	cellulase + pectinase	0.911	1.679	67.83	19.4
	Cellulase	0.34	1.699	68.65	19.7
120 min	Pectinase	0.527	1.733	70.02	46.5
	cellulase + pectinase	0.93	1.726	69.73	17.7

* Control refers to the untreated juice. Percentage of juice recovery was calculated as follows. Percentage of juice recovery= {weight of only juice / (weight of pomace and juice)} × 100

The initial weight of the concentrated apple fruit juice was taken which was found to be 5.87 g. After treatment of the juice with the alcohol as per the procedure described above, the final weight was found to be 1.091 g. Hence, it was concluded from the result that about 81.43% of alcohol soluble and 18.57% alcohol insoluble solids were present in the apple fruit juice.

After fermentation, the enzyme and biomass were separated as described above. The yield factors were calculated and presented in the Table 5. It was concluded from the table that the highest specific growth rate of *A. terreus* observed during the fermentation was with wheat bran i.e. 114 mg l⁻¹h⁻¹ (Table 5). Similarly, the enzyme accumulation in the culture medium per hour (dP/dt) was highest when banana peels were used as the substrate for *A. terreus*. The growth associated coefficient of enzyme production (α) was maximum when pectinase was produced using banana peels by *A. terreus*. But, the growth-independent coefficient of enzyme production (β) was optimum when fermentation was carried out by *A. terreus* using wheat bran as the substrate. Similar type of result was also proposed by Iftikhar et al. (2010) for the biosynthesis of lipase from *Rhizopus oligosporus* var. *microsporus*.

Table 5: Fermentation Kinetics of the Pectinase Produced by *A. terreus*

Optimum conditions	$Y_{e/s}$ (U/g)	$Y_{x/}$ (g/g)	μ (h^{-1})	α (U/g)	β (U/g/h)	$\frac{dx}{dt}$ (g/L/h)	$\frac{dP}{dt}$ (U/ml/h)	X_{max} (mg/ml)	μ_{max} (mg/L/h)
SDAT	600	0.0	0.	31479.	327.	0.01	6.25	0.99	19.85
	0	19	99	53	91	9			
BPAT	100	0.0	2.	21404	229.	0.04	10.42	2.43	48.67
	00	46	43	1.09	59	8			
WBA	950	0.1	5.	86805.	487.	0.11	9.89	5.70	114.0
T	0	12	70	55	55	4			

4. Conclusion

Enzymes that hydrolyze pectin and plant based pectic substances have significance in numerous biotechnological applications and even more so in food industries. The employment of these enzymes for bioconversion / hydrolysis of pectic substrates i.e. banana peels and other materials containing large amount of pectin is fundamental to achieve value added products. For these reasons, an escalating demand exists in the biotechnological industries for production of industrial-friendly, stable, highly active and competent enzymes. Several operating strategies have been employed for the production of biotechnologically imperative enzymes by filamentous fungi i.e. mainly from *Aspergillus*, *Trichoderma* and *Penicillium*. The development of technologies that allow the use of organisms or enzymes in bioreactors in various industrial production systems promise to revolutionize fermentation technology. Despite the progress, more studies are still needed to scale-up these applications efficiently to industrial levels.

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