EFFICACY OF PURIFIED PECTINASE OBTAINED FROM *A. NIGER* IN EXTRACTION AND CLARIFICATION OF JUICE FROM GRAPES AND POMEGRANATE FRUITS

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Abstract
Pectinases are one of the important and approaching enzymes of the commercial sector, especially, in the fruit juice industry as a pre-requisite for obtaining well clarified and stable juice with higher yields. Microbial pectinases has been employed vigorously for the juice clarification since recent times. The present study was carried out to investigate the application and competitiveness of commercial and purified pectinase obtained from A. niger in fruit juice (grapes and pomegranate) clarification of different enzyme concentrations like 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, varying incubation time (30, 60, 90, 120 and 180 min) at constant temperature of 40ºC to optimize the enzymatic treatment for the yield and clarity of the juices. The optimum conditions suggested for enzymatic (crude, purified and commercial pectinase) treatment for clarification and yield of fruit juices were 3.5 mg/20g pulp of enzyme concentration and 180 min incubation time at a constant temperature of 40ºC. A maximum yield of 62% and 65.5% whereas clarity of 3.4 and 3.7 % were obtained from grape juice and a significantly high yield of 68% and 72.5% whereas, clarity of 2.7 and 3.6 were acheived from pomegranate juice when compared to the unclarified grape and pomegranate juices (50 and 57.5% of yeild and clarity of 1.0 and 1.5 respectively). Therfore it was found that purified pectinase obtained from pectinolytic fungus, A. niger enhanced juice yield and clarity of grape and pomegranate juices when compared to untreated juices.

Keywords: Pectinase, *A. niger*, extraction, clarification, fruit juice

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1. INTRODUCTION

Pectinases are main enzymes for the biotechnological sector, which have been presenting a constant increase in their market share and presently hold a leading position among the commercially-produced industrial enzymes. Pectinolytic enzymes are also referred to as pectinases, include a group of enzymes that are responsible for the degradation of pectic substances and have important applications in the food industry (Sandri *et al.*, 2018).
These enzymes are useful for fruit juice extraction and wine clarification; tea, cocoa, and coffee concentration and fermentation; vegetable oil extraction; preparation of jam and jellies; and pickling (Upadhyaya *et al.*,2020). Furthermore, these enzymes are used in paper and pulp industries, bleaching of paper, bio-scouring of cotton, retting and degumming of plant fibers, oil extraction, wastewater treatment, poultry feed additives, protoplast fusion technology and bioenergy production (Kubra*et al.*, 2018).
Pectinases are high molecular weight, negatively charged, acidic glycosidic macromolecules which breakdown complex polysaccharides in plant tissues into simpler molecules with remarkable specificity, catalytic power and substrate specificity (Approvi and Vuppu, 2012). Pectinases are produced by several fungi including *Aspergillus sp., Botrytis cinerea, Fusariummoniliforme, Rhizoctoniasolani, Rhizopusstolonifer, Trichoderma sp., Neurosporacrassa, Penicillium* and *Fusarium* (Joshi *et al.*, 2006). Pectinases have attracted attention globally as biological catalysts in many industrial processes. These enzymes are used in processing agricultural and agro-industrial waste (Patil and Dayanand, 2006) for the production and clarification of fruit juices to improve the cloud stability of fruit and

vegetable juices and nectars, for depectinization in order to produce high density fruit juice concentrates and for haze removal from wines. The great industrial application of pectinases is in fruit juice extraction and clarification. Pectinscontribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases are used to clarify fruit juices. Treatment of fruit pulps with pectinase also exhibited an increase in fruit juice volume from banana, grapes and apples (Kaur*et al.,* 2004). Furthermore with the addition of pectinases, the viscosity of the fruit juice drops, the pressability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained with higher yields.Keepingalltheaboveadvantagesofpectina sesin consideration, the aim of the present study was designed to assess the efficacy of the purified pectinase in clarification of fruit juices.

2. MATERIALS AND METHODS

Collection of fruit samples: Completely ripe fresh grape and pomegranate fruits without any optical blemishes were purchased from local market of Warangal, Telangana state. The fruits were washed and rinsed with running water and were ground using a lab mixer for 2-3 min to obtain a homogenous fruit pulp. The grape juice was extracted from the whole pulp and the pomegranate juice from the seeds (Figure 1).

Pre-treatment of extracted fruit pulps: The extracted fruit pulps were pasteurized at 85ºC for 3 min to slow down the natural fruit enzymes and then cooled to 40ºC. The fruits are first cut into very small pieces and then, pre-treatments like steaming, cooling or heating earlier to enzymatic extraction were done to enhance juice recovery (Tapre *et al*., 2014).

Optimization of enzymatic treatment for the yield and clarity of fruit juice: To optimize the enzymatic treatment, each experiment with 20 g pulp was subjected to the treatment of enzyme obtained from *A. niger* (crude, partially purified and commercial pectinase) of different enzyme concentrations like 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 mg/20g of pulp, varying incubation time (30, 60, 90, 120 and 180 min) at constant temperature of 40ºC. Ultimately the enzyme in the sample was inactivated by heating the juice at 90ºC for 5min in a water bath.

Evaluation of juice yield: Extraction of juice using enzymes enhanced juice recovery from different fruits. But the enzymatic process should be optimized with respect to incubation, temperature, time and enzymatic concentration to increase yield and quantity of various fruit juices (Sharma *et al*., 2014).
The treated juices extracted from the pectinase treated pulp of grapes and pomegranate were centrifuged at 2000 rpm for 10 min and supernatant was collected and filtered through a muslin cloth spread on a glass funnel and the juice was collected as clear juice. Juice yield was estimated according to percentage of juice obtained from initial pulp. The juice yield was calculated using the following formula:

$$\text{Juice yield \%} = \frac{\text{Weight of clear juice}}{\text{Weight of sample}} \times 100$$

Evaluation of juice clarity: Fruit juices are cloudy, mostly in different degrees, due to the presence of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (Vaillant *et al.*, 2001). Pectinase degrades pectin consequently, resulting in viscosity reduction and cluster formation, which accelerates separation through centrifugation or filtration. Therefore, the juice presents higher clarity, as well as highly concentrated color and flavor.

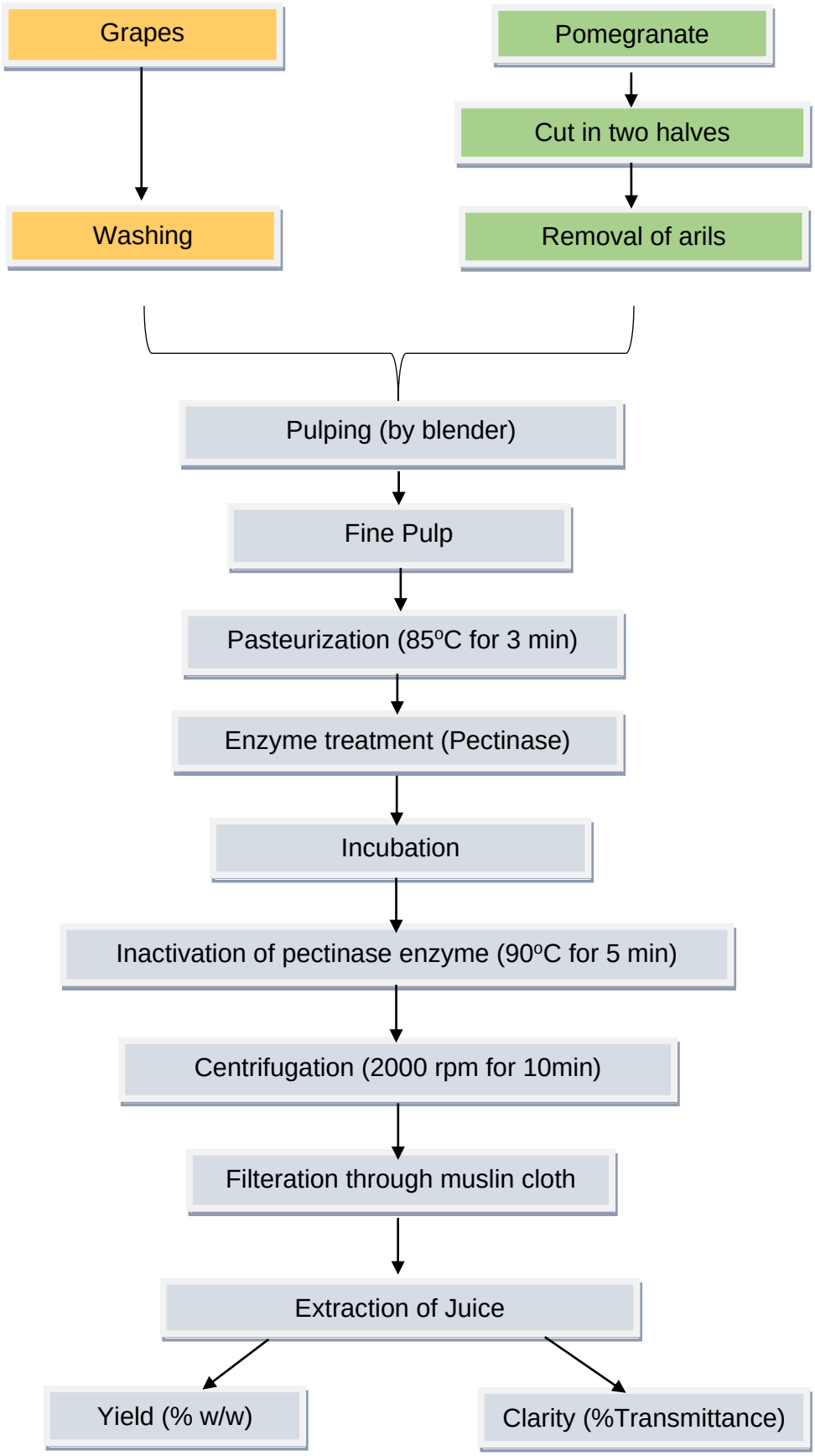

Fig.1 Flowchart showing extraction of grape juice and pomegranate juice

Clarity of the juice was determined by measuring % transmittance at a wavelength of 660 nm using UV-VIS spectrophotometer according to Tapre and Jain (2014). Distilled water was used as a blank. The percent transmittance was considered as a measure of juice clarity.

$$\% \text{ Clarification} = \frac{\text{O.D at 660 nm (treated juice - untreated juice)}}{\text{O.D at 660 nm untreated juice}} \text{x100}$$

Statistical analysis: In order to evaluate and determine the significant of the findings and also to compare the differences among the findings, the statistical analysis was used. One way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) using SPSS Statistics, version 17.0 was used to analyze the significant different of the mean of experimental data.

3. RESULTS AND DISCUSSION

Initially**,** experiments were performed to determine the optimum conditions like enzyme concentration and incubation time for maximum yield and clarity of fruit juices. For optimization of enzyme treatment, 20 g pulp of grapes and pomegranates were weighed, treated with different concentrations were incubated at a temperature of 40˚C at different incubation time.

Optimization of different parameters for the yield and clarity of enzyme treated fruit juices.

Effect of enzyme concentration and incubation time on grape and pomegranate juice yield:

Data from tables 1 and 2 it is evident that with increasing enzyme concentration and incubation time, an increased juice recovery was observed. The yield of grape juice was recorded high with increasing pectinase (crude, purified and commercial) concentrations and incubation time. The results shown mainly high yields of grape juice (68%, 71.5% and 73%) and pomegranate juice (60.5%, 66% and 71%), (crude, purified and commercial enzymes respectively) using 0.5 to 3.5 mg/20g pulp concentration for 30 to 180 min of incubation. On enzyme treatment, degradation of pectin leads to reduction in water holding capacity of pectin. Free water is released to the system and as a result the viscosity decreases and yield increases (Kashyap *et al.*, 2001 and Lee *et al*., 2006).

Table 1. Optimization of enzyme concentration and incubation time on grape juice yield

Enzyme concentration (mg/20g pulp)	Crude enzyme (%)					Purified pectinase (%)					Commercial pectinase (%)				
	Incubation time (min)					Incubation time (min)					Incubation time (min)				
	30	60	90	120	180	30	60	90	120	180	30	60	90	120	180
0.5	60	58.5	59.5	59.5	60.5	65.5	65.5	66	66.5	66.5	70	70.5	70.5	71	71.5
1.0	60	59	59	59	59	67	67.5	68	68.5	69	70.5	71	71.5	71	71.5
1.5	65	60	60	61	61.5	67	67.5	68	68	68.5	71	71.5	72	72.5	73
2.0	60	60.5	66	67	67	68	69	69.5	69.5	69.5	71	71	71.5	72	72.5
2.5	60	61.5	66.5	67.5	67.5	68.5	69	69.5	70	70.5	71.5	71.5	72	72	72
3.0	61	60.5	67.5	67.5	67	68.5	70	71	71.5	71.5	71.5	71.5	72	72.5	72.5
3.5	61.5	62.5	67.5	67	68	69	70	71	71.5	71.5	72	72.5	73	73	73

Table 2. Optimization of enzyme concentration and incubation time on pomegranate juice yield

Enzyme concentration (mg/20g pulp)	Crude pectinase (%)					Purified pectinase (%)					Commercial pectinase (%)				
	Incubation time (min)					Incubation time (min)					Incubation time (min)				
	30	60	90	120	180	30	60	90	120	180	30	60	90	120	180
0.5	57	57.5	58	58.5	59	62	62.5	63	63.5	64	66	66.5	67	67.5	68
1.0	57	58	58.5	58.5	59	62.5	62.5	63	63.5	64	67	67	67.5	68	69
1.5	57.5	58	58.5	59	59.5	63.5	64	64.5	64.5	65	67.5	67.5	68	68	69
2.0	58	58.5	58.5	59	59.5	63	63.5	64	64.5	65	68	68.5	69	69.5	70
2.5	58	58.5	59	59.5	59.5	63.5	63.5	64	64.5	65	68.5	69	69.5	70	71
3.0	58.5	58.5	59	60	60.5	64	64.5	65	65.5	66	68.5	69	69.5	70	71
3.5	59	59.5	59.5	60	60.5	64.5	65	65	65.5	66	69	69.5	70	71	71

Thongsombat *et al.* (2007) observed that a high yield of guava juice using 0.15% pectinase concentration incubated for 2.5 hrs. Ahmed *et al.* (2014) who reported that the maximum juice yield at 2.5 hrs in different concentrations (500, 1000 and 1500 mg.kg1) as 76, 78 and 80% in guava juice, 76, 78 and 79% in jack fruit juice and 77, 80 and 81% in pineapple juice. Similar results were observed a high yield of guava juice using 0.15% pectinase concentration incubated for 2.5 hrs (Thongsombat *et al.*, 2007).

Effect of enzyme concentration and incubation time on grape and pomegranate juice clarity:

It may be concluded from the tables 3 and 4 that with increasing enzyme concentration and incubation time, the treated juices showed an increase in the clarity. The maximum juice clarity of 3.7, 13.0 and 18.6 % and 2.0, 3.0 and 3.6 % were obtained at an incubation time of 180 min and pectinase (crude, purified and commercial) enzyme concentration of 3.5 mg/20 g pulp in grapes and pomegranate juices respectively. Increase in enzyme concentration may increase the rate of clarification by exposing part of the positively charged protein beneath, and reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and after some time settled out (Sin *et al.*, 2006). Similar view was suggested by Yannam *et al.* (2012) who reported maximum clarification of about (92.5±0.26%) at a temperature of about 40°C and 150 min of incubation by pectinase from *Aspergillus foetidus*. The results are similar with the findings of Vijayanand *et al.* (2010) who observed that the litchi pulp added with 500 ppm of pectinase resulted in maximum transmittance of 80% at 660 nm. Robin *et al.* (2013) observed that the minimum clarity *of alu Bukhara* was 6.3%, when the pulp was treated with 0.05ml/50g, crude enzyme concentration for 210min time at 35°C temperature whereas, the maximum clarity was observed at crude enzyme concentration; 0.10 ml/50g, time; 375 min and temperature;45ºC. Akesowan and Choonhahirun (2013) demonstrated the optimized enzyme treatment by adding 869.36 ppm pectinase in guava mash incubated for 71.27 min which is on par with our study. Singh *et al.*, (2012) obtained maximum juice clarity of 28.9% at an incubation temperature of 61.82°C, incubation time 375 min and pectinase concentration of 4.0 mg/25g when compared to the untreated bael fruit juice sample (17.4%) which was similar to our result.

Table 3. Optimization of enzyme concentration and incubation time on grape juice clarity

Enzyme concentration (mg/20g pulp)	Crude pectinase (%T)					Purified pectinase (%T)					Commercial pectinase (%T)				
	Incubation time (min)					Incubation time (min)					Incubation time (min)				
	30	60	90	120	180	30	60	90	120	180	30	60	90	120	180
0.5	1.5	1.6	1.6	1.7	1.8	10.1	10.2	10.5	10.6	11.0	17.1	17.5	17.4	18	18.2
1.0	1.6	1.7	1.8	1.9	2.0	10.2	10.5	11	11.5	11.7	17.3	17.2	17.4	18.9	19.0
1.5	2.0	2.1	2.4	2.5	2.6	10.5	10.7	11.2	11.5	11.7	17.5	17.5	17.5	18.1	18.4
2.0	2.3	2.5	2.8	2.9	3.1	10.5	10.7	11.5	12.0	12.2	17.5	17.7	18	18.5	18.6
2.5	2.8	2.9	3.0	3.2	3.4	10.7	10.7	11.4	12.0	12.2	17.7	17.8	18	18.5	19
3.0	3.0	3.2	3.3	3.5	3.7	10.9	11	11.2	12	12.2	17.9	18	18.2	18.4	18.8
3.5	3.1	3.4	3.4	3.5	3.7	11	11.5	11.5	12.5	13.0	18.2	18.2	18.5	18.4	18.6

Table 4. Optimization of enzyme concentration and incubation time on pomegranate juice clarity

Enzyme concentration (mg/20g pulp)	Crude pectinase (%T)					purified pectinase (%T)					Commercial pectinase(%T)				
	Incubation time (min)					Incubation time (min)					Incubation time (min)				
	30	60	90	120	180	30	60	90	120	180	30	60	90	120	180
0.5	0.3	0.3	0.4	0.4	0.5	0.6	0.8	1.0	1.2	1.5	2.0	2.2	2.5	2.6	2.7
1.0	0.5	0.6	0.6	0.7	0.8	0.8	1.0	1.4	1.4	1.7	2.1	2.3	2.5	2.7	2.8
1.5	1.0	1.1	1.3	1.5	1.7	1.0	1.2	1.6	1.8	1.8	2.3	2.5	2.6	2.8	3.0
2.0	1.2	1.4	1.4	1.5	1.7	1.5	1.4	1.5	1.7	1.8	2.7	3.0	3.2	3.5	3.6
2.5	1.4	1.6	1.7	1.8	2.0	1.7	1.7	1.8	2.0	2.2	2.9	3.0	3.1	3.1	3.2
3.0	1.6	1.7	1.8	1.8	2.0	2.0	2.2	2.6	2.8	3.0	3.0	3.5	3.7	3.8	3.8
3.5	1.8	1.9	1.9	2.0	2.0	2.2	2.4	2.6	2.8	3.0	3.3	3.3	3.5	3.6	3.6

A critical evaluation of the tables 5 and 6 explains that purified pectinase obtained from *A. niger* increased juice yield and clarity of grape and pomegranate juices and was on par with the commercial pectinase when compared to untreated juices. The figure 2 depicts the clarity of the treated and untreated juices. A maximum yield of 62% and 65.5% and clarity of 3.4 and 3.7% were obtained from grape juice treated with purified and commercial pectinase respectively and a significantly high yield of 68% and 72.5%and clarity of 2.7% and 3.6% were achieved from pomegranate juice treated with purified and commercial pectinase respectively when compared to the untreated grape and pomegranate juices.

The present results are on par with Singh *et al.* (2012) who reported an increase of 17.5% in bael fruit juice yield from untreated sample at an enzymatic concentration of 20mg/100g pulp, incubation time of 425 min and temperature of 47°C. The present findings coincide with the work of Sandri *et al.* (2011) who demonstrated the use of crude pectinase and commercial enzyme in the clarification process increased fruit juices like juice apple, butia palm fruit, blueberry, and grape juices by *A. niger* and *A. oryzae*.

Table 5. Yield and clarity of grape juice from treated and untreated fruit pulps by *A. niger*

Grape juice	Volume of pulp (gm)	Volume of juice (ml)	Yield (%)	Clarity (%T)
Untreated	20	10.0^{d}±0.001	50	1.0
Crude	20	11.50^{c}±0.001	57.5	1.8
Purified	20	12.40^{b}±0.001	62	3.4
Commercial	20	13.10^{a}±0.001	65.5	3.7
S.E ± Anova $p \leq 0.005$				

Table 6. Yield and clarity of pomegranate juice from treated and untreated fruit pulpsby *A. niger*

Pomegranate juice	Volume of pulp (gm)	Volume of juice (ml)	Yield (%)	Clarity (%T)
Untreated	20	11.50^{d}±0.001	57.5	1.5
Crude	20	12.10^{c}±0.001	60.5	2.0
Purified	20	13.60^{b}±0.001	68.0	2.7
Commercial	20	14.50^{a}±0.001	72.5	3.6
S.E ± Anova $p \leq 0.005$				

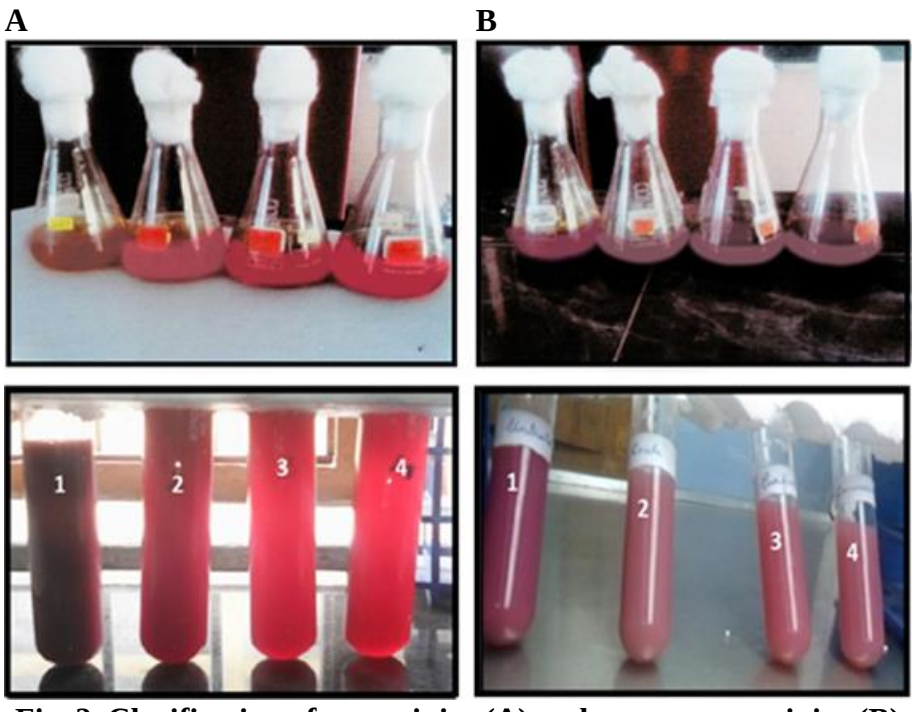

Fig. 2. Clarification of grape juice (A) and pomegranate juice (B) with the help of extracted and commercial pectinases

1. Untreated juice; 2. Crude pectinase treated; 3. Purified pectinase treated; 4. Commercial pectinase treated

Similar view was suggested by Srivastava and Tyagi (2013) who observed that the maximum volume of 23.7ml was obtained by pectinase and amylase combination and maximum activity of pectinase increased the yield of apple juice up to 34ml/50gm and 25ml/50gm at pH 5.5 and at temperature (45-50°C) respectively. *Penicillium oxalicum* endopolygalacturonase improved the light transmission of papaya pulp by 29.5% (Cheng *et al.,* 2016).

4. CONCLUSION

It was observed that with an increasing enzyme concentration and incubation time, the yield of the juice increased and also the treated juice became more clear and transparent. The juice yield increased on enzyme treatment as degradation of pectin led to reduction in the water holding capacity of pectin, thus releasing free water into the system and the clarity is due to extended contact between enzyme and substrate. Therefore, the present investigation showed that the usage of purified pectinase obtained from pectinolytic fungus, *A. niger* enhanced juice yield and clarity when compared to control and also indicated the equal effectiveness and competitiveness of the purified enzyme to that of commercial one.

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