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EFFICACY OF PURIFIED PECTINASE OBTAINED FROM *PAECILOMYCES VARIOTII* IN EXTRACTION AND CLARIFICATION OF JUICE FROM GRAPES AND POMEGRANATE FRUITS

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ABSTRACT

The present investigation was carried out to study the application and the competitiveness of commercial and purified pectinase obtained from *Paecilomyces variotii.* The fruit juices (grapes and pomegranate) were carried out at different enzyme concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5) and incubation time (30, 60, 90, 120 and 160 minutes) at constant temperature (50ºC) to optimize the enzymatic treatment for the yield and clarity of the juices. The optimum conditions recommended 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 50ºC. It was observed that purified pectinase obtained from pectinolytic fungus, *P. variotii* enhanced juice yield and clarity of grape and pomegranate juices and is on par with the commercial pectinase when compared to untreated juices. A maximum yield of 79% and clarity of 19.4 and 19.5% were obtained from grape juice and a significantly high yield of 74% and clarity of 4.9 and 4.8 were acheived from pomegranate juice when compared to the unclarified grape and pomegranate juices (60 and 52% respectively). There was an increase in the yield of 31.6% and 42.3% of the grape and pomegranate juices respectively when treated with purified enzyme than the untreated juices.

KEY WORDS: **Pectinase, *Paecilomyces variotii*, Grapes, Pomegranate, Yield, Clarity**

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INTRODUCTION

Enzymes are one of the important tools in modern food industry because they simplify many intermediate processes during food processing. Bulk of the industrial enzymes fall into different groups, out of which, the most important group of enzymes is pectinase, used in fruit and vegetable processing industry. Their commercial application was first observed in 1930 for the preparation of wines and fruit juices.[1] Pectinases are one of the important and imminent 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.[2] Pectinases are high molecular weight, negatively charged, acidic glycosidic macromolecules that breakdown complex polysaccharides in plant tissues into simpler molecules with extraordinary specificity, catalytic power and substrate specificity.[3] Pectinases are produced during the natural ripening process of fruits where, it splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Softening of the cell wall and increase in the yield of juice extract from the fruits takes place during this process. Fungal pectinases are mainly extracellular enzymes, prominent among them being polygalacturonase, which is also most commonly assayed to determine pectinase activity. Pectinase is produced by several fungi including *Aspergillus sp*., *Botrytis cinerea*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Trichoderma sp*., *Neurospora crassa, Penicillium* and *Fusarium*. [4] An improved knowledge of the properties of microbial pectinases is important in commercialisation of industrial production and application of these enzymes in various potential fields. Pectinases have attracted attention globally as biological catalysts in numerous industrial processes. These enzymes are used in processing agricultural and agro-industrial wastes[5] 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. As a result, today pectinases are one of the promising enzymes of the commercial sector.Alkaline microbial pectinase reveals a great significance in the current biotechnological arena with wide ranging applications in textile processing, degumming of plant bast fibers, treatment of pectic waste waters, paper making, and coffee and tea fermentations.[6]. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. Treatment of fruit pulps with pectinase also showed an increase in fruit juice volume from banana, grapes and apples.[7] 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. With this background, the present investigation was undertaken to assess the efficacy of the purified pectinase in clarification of fruit juices.

MATERIALS AND METHODS

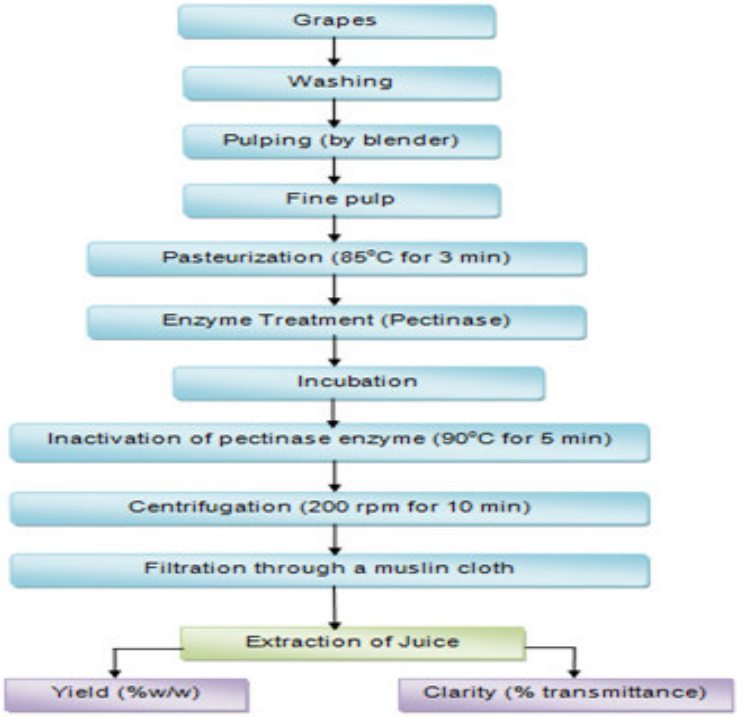

Figure 1
Flow Chart for the extraction of grape juice

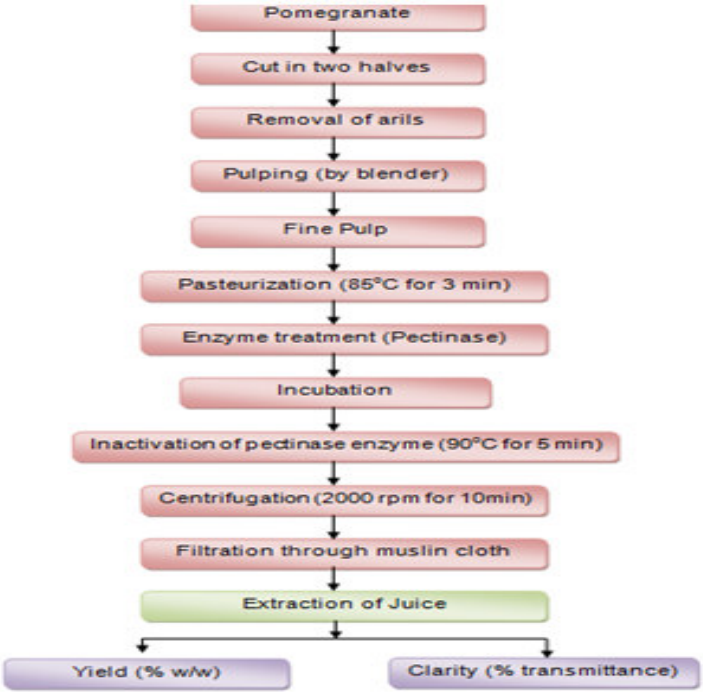

Figure 2
Flow Chart for the extraction of Pomegranate juice

Collection of fruit samples
Fully ripened fresh grape and pomegranate fruits without any visual blemishes were purchased from local market of Coimbatore, Tamil Nadu. 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 fruits were extracted from the whole pulp and the pomegranate fruit from the seeds (Figure 1 and 2).

Pre-treatment of extracted fruit pulps
The extracted fruit pulps were pasteurised at 85ºC for 3 min to inactivate the natural fruit enzymes and then cooled to 40ºC. The fruits are first cut into small pieces and then, pre-treatments like steaming, cooling or heating prior to enzymatic extraction were done to increase juice recovery.[8]

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 pectinase obtained from *Paecilomyces variotii* (crude, purified and commercial) 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 160 minutes) at constant temperature of 50ºC. At the end of enzymatic treatment, the enzyme in the sample was inactivated by heating the juice at 90ºC for 5min in a water bath.

Evaluation of Juice Yield
The treated juices extracted from the pectinase treated pulp of grapes and pomegranate were centrifuged at 2000 rpm for 10 min and the supernatant was collected and filtered through a muslin cloth spread on a glass funnel and the clear juice was collected. The juice yield was then calculated using the following formula:

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

Evaluation of juice Clarity
Clarity of the juice was determined by measuring % Transmittance at a wavelength of 660 nm using UV-VIS spectrophotometer.[9] Distilled water was used as a blank.

RESULTS AND DISCUSSION

Preliminary experiments were performed to determine the optimum conditions like enzyme concentration and incubation time for maximum yield and clarity of fruit juices. For the optimization of the enzyme treatment, 20 g pulp of grapes and pomegranate were weighed, treated with different concentrations and were incubated at a temperature of 50°C for 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
From Table 1 and 2, it was clear that with increasing enzyme concentration and incubation time, an increased juice recovery was observed. The yield of grape juice was significantly high with increasing pectinase (crude, purified and commercial) concentrations and incubation

time. The results showed significantly high yields of grape juice (69, 79 and 78%) and pomegranate juice (59, 74 and 74.5%) using 3.5 mg/ 20g pulp concentration for 180min incubation (crude, purified and commercial enzymes, respectively).A significantly high yield of guava juice using 0.15% pectinase concentration incubated for 2.5 h.[10] Similar such result was reported with the maximum juice yield of 76, 78 and 80% in guava juice, 76, 78 and 79% in jack fruit juice and 77, 80 and 81% in pine apple juice at 2.5 hrs using different concentrations of pectinase enzyme (500, 1000 and 1500 mg.kg-1).[11]

Table 1
Optimization of enzyme concentration and incubation time on Grape juice Yield (%w/w).

Enzyme concentration mg/ 20g pulp	Crude pectinase					Purified pectinase					Commercial pectinase				
	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min
0.5	64	63.5	64.5	64.5	65.5	71.5	71	71.5	72	73	71	71.5	72.5	74	75
1.0	64	64	64	65.5	65.5	72	72.5	72	73	73	71	73.5	73	74	76
1.5	65	65	65	66.5	66.5	72	73.5	72	72.5	74	71.5	73.5	73.5	74.5	76
2.0	65.5	65.5	66	66.5	67	73	73	73	74	74	71.5	74	74.5	75	77
2.5	65.5	66.5	66.5	67.5	67.5	73.5	73	74	74.5	75	72	74	74.5	75	78
3.0	66	66.5	67.5	67.5	67	74.5	75.5	76	76.5	77.5	72.5	74	76	76.5	78
3.5	66.5	67	67.5	67	69	75.5	77	78	78.5	79	73	75.5	76.5	77	79

Table 2
Optimization of enzyme concentration and incubation time on Pomegranate juice Yield (%w/w).

Enzyme concentration mg/ 20g pulp	Crude pectinase					Purified pectinase					Commercial pectinase				
	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min
0.5	53	54.5	55.5	56	57	67	68.5	68.5	69	69	67.5	67.5	68	68	69
1.0	53	54	55.5	57	57.5	67	67.5	68	68.5	69	67.5	68.5	68.5	69	69
1.5	53.5	54.5	56	56.5	57	68	68	69	69	70	69	69	69	69	70
2.0	54	55.5	56	57	58	69.5	69	70	70	71	70.5	69.5	70	70.5	71
2.5	54.5	54.5	56	57.5	58.5	69.5	69.5	71	70.5	72	70	71.5	71.5	72	72
3.0	54.5	55	56	57	58.5	71.5	70.5	72	72	73.5	71.5	72	72	73.5	73.5
3.5	55	55.5	56	57.5	59	72	72	73	73	74.5	72	72.5	72.5	74.5	74.5

Effect of enzyme concentration and incubation time on Grape and Pomegranate juice Clarity

It may be inferred 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 5.2, 19.4 and 19.5 % and 2.7, 4.9 and 4.7 % were obtained at an incubation time of 180 min and pectinase (crude, purified and commercial) enzyme concentration of 3.5 mg/ 20g 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, thus reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and eventually settled out.[12] When litchi pulp was added with 500 ppm of pectinase it resulted in maximum transmittance of 80% at 660 nm which coincides with our findings.[13] Similar view was expressed with maximum clarification of about (92.5±0.26%) at 40°C and 150 minutes of incubation by pectinase from *Aspergillus foetidus*.[14] A 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/ 25 g was observed in Bael fruit juice sample when compared to the untreated sample (17.4%) which was similar to our result.[15] The minimum clarity of 6.3% was obtained when the pulp was treated with 0.05ml/50g crude enzyme for 210 min at 35°C whereas maximum clarity was observed at 0.10 ml/50g for 375 min at 45◦C using *Alu bukhara.* [16] The optimized enzyme treatment by adding 869.36 ppm pectinase in guava mash incubated for 71.27 min was on par with our study.[17]

Table 3
Optimization of enzyme concentration and incubation time on Grape juice Clarity (%T)

Enzyme concentration mg/ 20g pulp	Crude pectinase					Purified pectinase					Commercial pectinase				
	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min
0.5	2.1	2.1	2.2	2.3	2.4	16.1	16.1	16.5	16.4	16.5	15.8	16.2	17.0	17.3	17.6
1.0	2.8	2.7	2.7	2.8	2.9	16.3	16.2	16.3	16.4	16.6	16.1	17.1	17.4	17.5	17.6
1.5	3.0	3.2	3.2	3.3	3.2	16.5	16.5	16.7	16.8	16.8	16.4	17.3	17.4	17.4	17.6
2.0	3.7	3.5	3.7	3.8	3.9	16.5	16.7	16.8	16.8	17	16.4	17.3	17.7	17.8	18.2
2.5	4.1	4.2	4.2	4.4	4.3	16.9	16.9	17.2	17.1	17.3	16.4	17.4	17.8	18.1	18.7
3.0	4.3	4.4	4.4	4.7	4.8	17	17.4	17.8	17.8	17.9	17.1	17.3	17.9	18.1	19.2
3.5	4.6	4.6	4.8	4.9	5.2	17.6	17.7	17.8	18.5	19.4	18.2	18.3	18.8	19.4	19.5

Table 4
Optimization of enzyme concentration and incubation time on Pomegranate juice Clarity (%T).

Enzyme concentration mg/ 20g pulp	Crude pectinase					Purified pectinase					Commercial pectinase				
	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min	30 min	60 min	90 min	120 min	180 min
0.5	0.6	0.6	0.7	0.7	0.7	2.6	2.8	2.8	2.7	2.9	3.1	3.0	3.4	3.4	3.4
1.0	0.8	0.9	1.1	1.1	1.0	2.7	2.7	2.8	2.9	2.9	3.2	3.3	3.4	3.6	3.6
1.5	1.3	1.4	1.4	1.5	1.5	2.7	2.9	2.9	3.1	3.0	3.4	3.5	3.6	3.7	3.8
2.0	1.4	1.5	1.6	1.7	1.7	2.9	3.1	3.1	3.4	3.4	3.3	3.6	3.6	3.8	4.0
2.5	1.7	1.7	1.8	1.9	2.1	3.6	3.6	3.7	3.8	3.8	3.7	3.7	3.8	4.1	4.3
3.0	1.8	1.9	2.0	2.0	2.3	4.1	4.1	4.3	4.4	4.5	3.9	3.9	4.2	4.3	4.3
3.5	2.0	2.1	2.1	2.4	2.7	4.3	4.4	4.5	4.7	4.9	3.9	4.2	4.4	4.7	4.7

Evaluation of the enzymes for the yield and clarity of fruit juices (grapes and pomegranate)

Table 5
Yield and Clarity of Grape juice from treated and untreated fruit pulps

Grape juice	Volume of pulp	Volume of juice	Yield (%w/w)	Clarity (%T)
Untreated	20	12.87 ± 1.97	60	0.06
Crude	20	13.07 ± 1.01	69	5.2
Purified	20	15.53 ± 1.12	79	19.4
Commercial	20	15.47 ± 0.76	79	19.5
SEd		1.0601		
CD (p<0.05)		2.4447		

Values are mean ± SD of three samples in each column

Table 6
Yield and Clarity of Pomegranate juice from treated And untreated fruit pulps

Pomegranate juice	Volume of pulp	Volume of juice	Yield %	Clarity%
Untreated	20	10.40 ± 0.80	52	0.08
Crude	20	11.63 ± 0.76	59	2.7
Purified	20	12.63 ± 2.10	74	4.9
Commercial	20	14.77 ± 0.71	74.5	4.7
SEdCD (p<0.05)	-	1.0124 2.3347	-	-

Values are mean ± SD of three samples in each column

From the Tables 5 and 6, it is clear that, purified pectinase obtained from pectinolytic fungus, *P. variotii* enhanced juice yield and clarity of grape and pomegranate juices and is on par with the commercial pectinase when compared to untreated juices. A maximum yield of 79% and clarity of 19.4 and 19.5% were obtained from grape juice and a significantly high yield of 74% and clarity of 4.9 and 4.8 were achieved from pomegranate juice when compared to the unclarified grape and pomegranate juices (60 and 52% respectively). There was an increase in the yield of 31.6% and 42.3% of the grape and pomegranate juices respectively when treated with purified enzyme than the untreated juices. 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 was on par with our findings.[15] The maximum volume of 23.7 ml was obtained by pectinase and amylase combination and maximum activity of pectinase enhanced the yield of apple juice upto 34ml/50gm and 25ml/50gm at 5.5 pH and at temperature (45-50°C) respectively.[18] The crude pectinase enzyme treatment from *Bacillus* sp. MBRL576 increased the juice volume of 40ml in apple and banana and 50ml in carrot compared to untreated (30, 25 and 40ml) apple, banana and carrot juice respectively and of 25ml in commercial pectinase which was similar to our work.[19]

CONCLUSION

Thus 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. Thus, the present study showed that the usage of purified pectinase obtained from pectinolytic fungus, *P. variotii* 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.

CONFLICT OF INTEREST

Conflict of interest declared none.

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