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CLARIFICATION OF APPLE JUICE WITH LABORATORY-PRODUCED-PECTINASE OBTAINED FROM THE DETERIORATION OF APPLE (*Malus domestica*) FRUITS BY *Aspergillus niger*

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ABSTRACT

There are different varieties of Apple fruits which have resulted from natural cross-pollination involving different species. Apples are often eaten in a raw fresh form, sometimes baked or stewed for other uses. They have some beneficial purposes such as helping in preventing the growth of prostate cancer, improving bowel function and regulating blood sugar. The activity of enzymes is usually affected by factors such as temperature, pH, substrate concentration, and enzyme concentration. The effect of enzyme concentration on pectinase obtained from the deterioration of three different varieties of apple fruits by *Aspergillus niger* was therefore investigated. A 72-hours-old culture of *Aspergillus niger* subcultured onto fresh Potato Dextrose Agar (PDA) plates was inoculated into three different varieties of fresh Apple fruits obtained from a Supermarket along Idiroko road, Ota, Ogun State, Nigeria. The fruits were surface sterilized, inoculated with the fungi and incubated at room temperature of 27^{o}C. They were monitored for complete deterioration over twelve days. Pectinase obtained from the deteriorated fruits was used in clarifying apple juice from the different varieties of apple fruits with different volume of enzyme. Equivalent volume of water was also treated in like manner. The same experiment was carried out for commercial pectinase in comparison with the laboratory-produced – pectinase was also more effective than the commercially produced enzyme. The result of this investigation will be very useful in the production of commercial pectinase obtained from fungal deterioration.

Keywords: Apple (*Malus domestica*) fruits, *Aspergillus niger*, Pectinase, Apple juice, fungal deterioration

(I) INTRODUCTION

Apples compared to other fruits and vegetables may be considered relatively low in vitamin c content but they are very rich sources of antioxidant compounds (1). Apples are often eaten in raw fresh form, sometimes baked or stewed and can be dried and reconstituted for later use (2).There are more than seven thousand five hundred (7500) known cultivars of apples resulting in a range of desired characteristics, which vary in their yield and the ultimate size of the tree, even when grown on the same rootstock (3). The juice can be fermented to produce apple cider of different kinds but a large percentage of apples are affected by post-harvest deterioration caused by microorganisms (4, 5). Fungi have been associated with the deterioration of apples and other important fruits (6, 7, 8). Various researchers have reported members of the *Aspergillus* genus especially *A .niger* as frequently responsible

for post harvest decay of fresh fruits such as pears, peaches, citrus, grapes, tomatoes, melon, onions, yams and some vegetables (4, 9). Post harvest deterioration is however accomplished with the aid of cell wall degrading enzymes involved in the deterioration process (5). Pectinases are cell wall degrading enzymes that allows the release of apple juice from the apple fruit by breaking down the pectin which are large polysaccharide molecules found in plant cell walls (10).This research work was therefore carried out to examine the pectinase obtained from the deterioration of different varieties of apple fruits with the aim of using the pectinase for clarification of apple juice in comparison with commercially produced pectinase.

(II) MATERIALS AND METHODS

2.1. Collection of Samples

Apple (*Malus domestica*) fruits used for this research work were obtained from the fruits section of Justrite Supermarket, Idiroko road Ota. Freshly ripe Green, Red and Yellow varieties of apples showing no signs of physical damage or microorganisms were employed. They were taken to the Microbiology laboratory of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria for further laboratory work.

2.2. Organism and Cultivation Techniques

The isolate of *Aspergillus niger* employed for the research was obtained from the culture collection of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. The organism was subcultured onto fresh potato dextrose agar plates. Seventy-two-hour old culture of the organism was used as the inoculum.

2.3. Inoculation of Apple fruits

The apple fruits were surface sterilized using 10% (v/v) Sodium hypochlorite solution for thirty minutes. The fruits were later rinsed with several changes of sterile distilled water to remove the residual effect of the sodium hypochlorite solution. Tissue discs with mycelia discs containing spores of *A. niger* removed from the edge of the seventy-two-hour old culture of the organism was used in inoculating freshly ripe apple fruits. The point of inoculation was sealed with paraffin wax. The control fruits were inoculated with sterile potato dextrose agar in the same manner. Both the experimental and the control fruits were placed under separate sterile bell jars. Incubation was at room temperature of 25^{o}C for 12 days.

2.4. Extraction of Enzyme from Apple fruits

Twelve days after inoculation of freshly ripe apple fruits with *A. niger*, the deteriorated apple fruits were weighed and chilled for 30min inside a freezer and homogenized with a laboratory blender with chilled liquid extractant (1:1w/v) for 2 min at 30secs intervals. The extractant was 0.01M Citrate phosphate buffer pH 4.5 containing 5mM sodium azide (NaN_3) to prevent microbial contamination. The homogenate was initially allowed to percolate through four layers of sterile muslin and thereafter through Whatmanns no.1 filter paper. This was used as the crude enzyme preparation.

2.5. Enzyme Assay

Polygalacturonase activity was determined according to the method described by (7). The reaction mixture consisted of 1ml of 0.1% (w/v) Pectin (Sigma) in 0.01M citrate phosphate buffer (pH4.5) and 0.5ml of the enzyme. Each control tube contained 1ml of the substrate. The experimental and control tube were incubated in a water bath at 37^{o}C for 3h. The total reducing sugar was determined by the Dinitrosalicylic acid (DNSA) method (11). One unit of polygalacturonase activity was defined as the amount of enzyme, which released 1µmol galacturonic acid per minute.

2.6. Clarification of apple juice with polygalacturonase

The clarification of apple juice with polygalacturonase was carried out using the NCBE Method (12) method whereby fresh apples were chopped into cubes of approximately five millimeters (5mm) on a side with a sharp knife. Twenty five grammes (25g) of the chopped red apple fruits, yellow apple fruits and green apple fruits were treated separately. They were weighed into nine

separate beakers of three beakers for each of the three varieties. Different volumes (10ml, 20ml, 30ml, 40ml and 50ml) of laboratory-produced-pectinase and commercially-produced-pectinase were added to each of two different beakers and equivalent volume of water into the other beaker for each of the three varieties of apple fruits. The beakers were labeled appropriately as 'Laboratory-produced -pectinase', 'Commercially-produced-pectinase and 'water'. The chopped apple pieces were covered with plastic wraps and incubated in a water bath at room temperatures of 25^{o}C for fifteen minutes. The juice from the preparation was filtered using a Whatmanns No.1 filter paper in funnels into a measuring cylinder. The cylinders were appropriately labeled and the amount of juice in each cylinder was measured at 5 min intervals for 30 min.

(III) RESULTS

3.1 The Effect of Pectinase on the volume of juice

This investigation revealed that all the volumes of the laboratory-produced pectinase on the green variety of apple fruits produced more juice than the same volume of commercially-produced pectinase on Red and Yellow varieties of apple fruits under the same laboratory conditions. The cylinders with the laboratory-produced and commercially-produced Pectinases produced more juice than the cylinders with water for all the experiments (Figs.1-15). The green and red apples produced more juice with 10ml commercial pectinase than the laboratory-produced pectinase while the yellow produced more with 10ml crude than the commercial pectinase (Fig. 1, 2 and 3). Yellow and Green apples produced more juice with 20ml laboratory-produced pectinase while the red apples produced more with the same volume of commercially-produced pectinase (Fig.4, 5 and 6). All the varieties of apple fruits produced more juice when clarified with 30ml and 50ml of laboratory-produced pectinase than the same volume with commercial pectinase (Figs. 7, 8 and 9). Green and Red apples produced more juice with 40ml of the laboratory-produced pectinase clarification while the yellow fruits was different (Figs.10, 11 and 12).

3.2 The viscosity of the juice produced

The juice in the cylinders with pectinase was clearer and the cloudiness reduced with the enzymatic treatment than the cylinders with water.

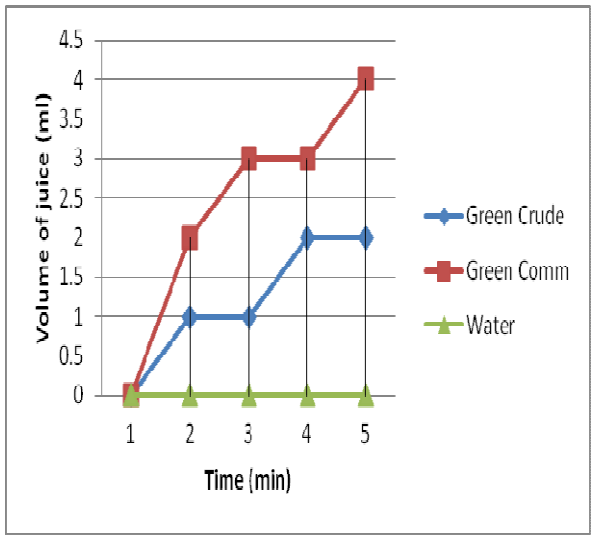

Fig 1: Clarification of Green apple juice with 10ml crude pectinase, commercially produced pectinase and water

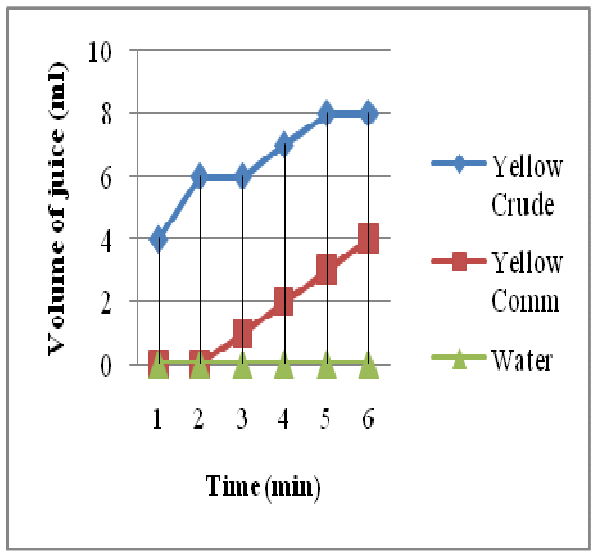

Fig 2: Clarification of Yellow apple juice with 10ml crude pectinase, commercially produced pectinase and water

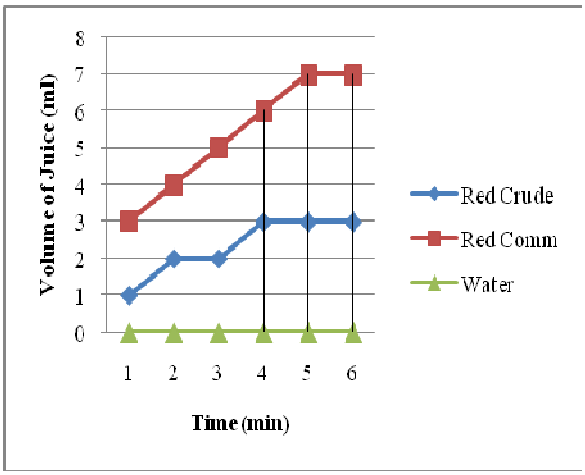

Fig 3: Clarification of Red apple juice with 10ml crude pectinase, commercially produced pectinases water

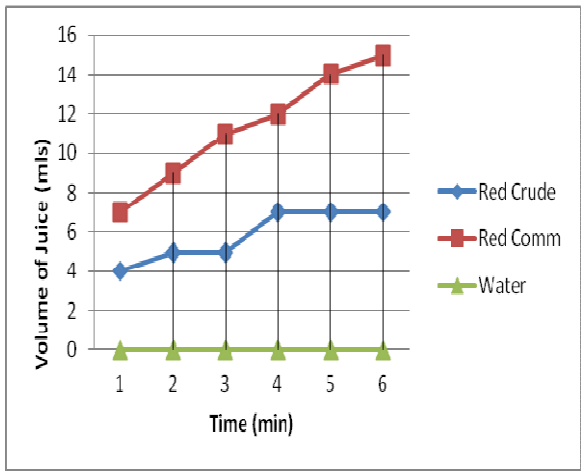

Fig 6: Clarification of Red apple juice with 20ml crude pectinase, commercially produced pectinase and water

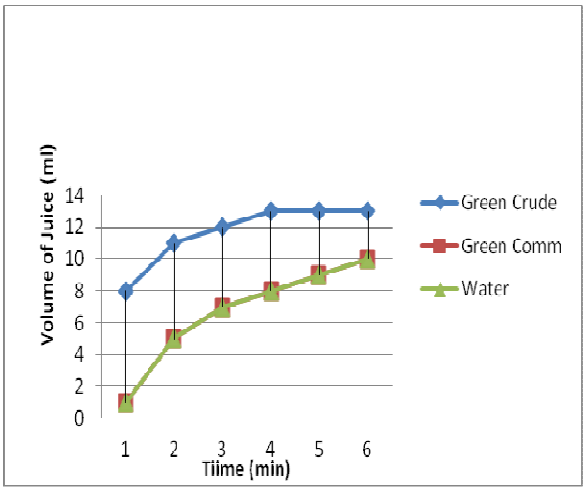

Fig 4: Clarification of Green apple juice with 20ml crude pectinase, commercially produced pectinase and water

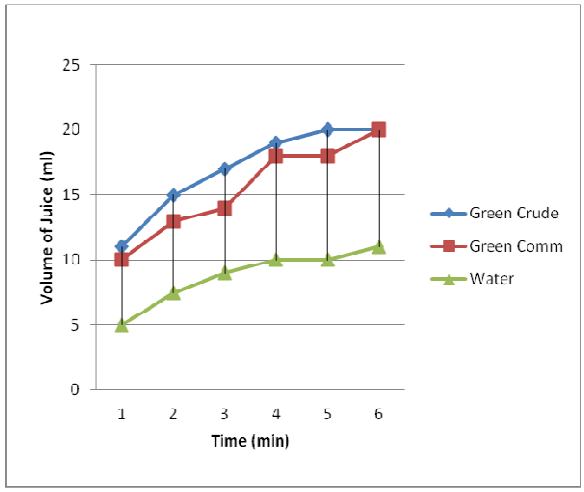

Fig 7: Clarification of Green apple juice with 30ml crude pectinase, commercially produced pectinase and water

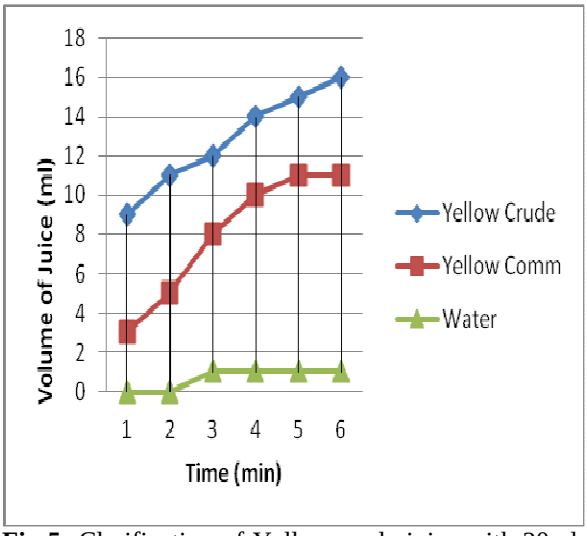

Fig 5: Clarification of Yellow apple juice with 20ml crude pectinase, commercially produced pectinase and water

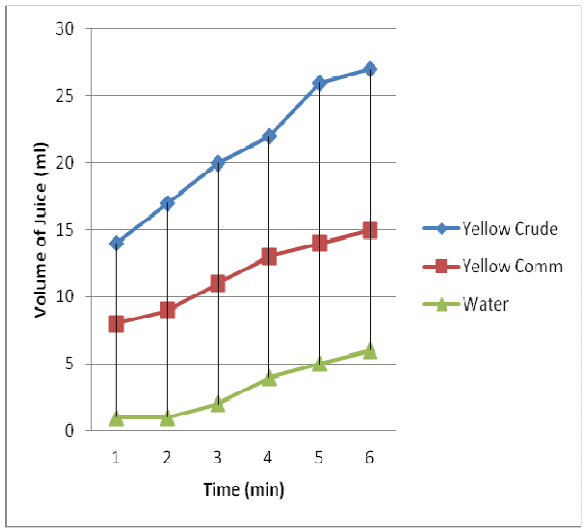

Fig 8: Clarification of Yellow apple juice with 30ml crude pectinase, commercially produced pectinase and water

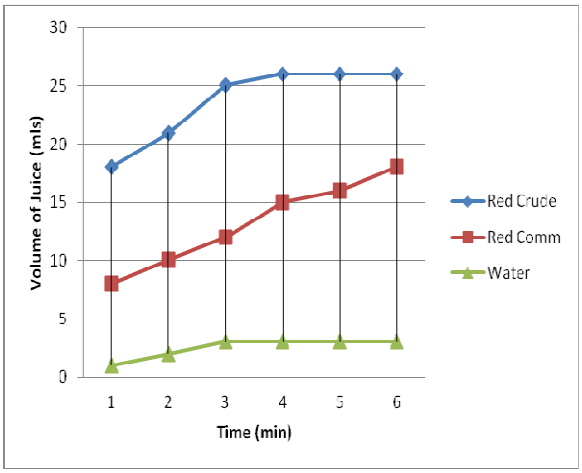

Fig 9: Clarification of Red apple juice with 30ml crude pectinase, commercially produced pectinase and water

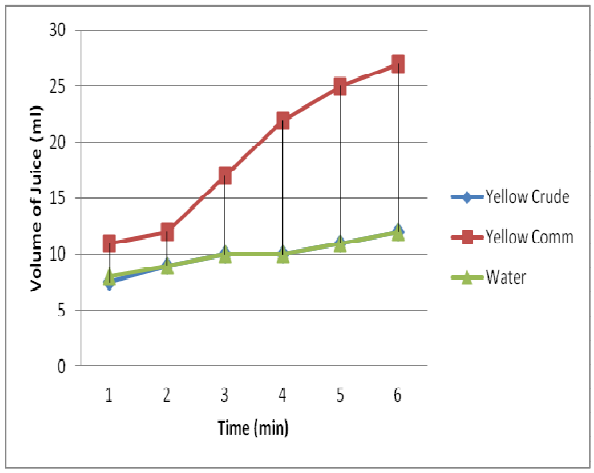

Fig 12: Clarification of Red apple juice with 40ml crude pectinase, commercially produced pectinase and water

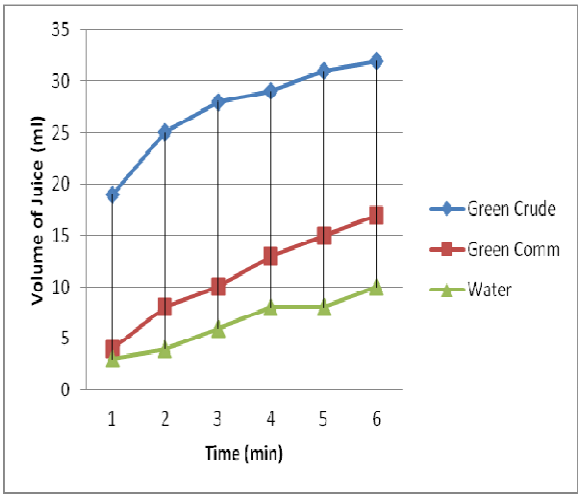

Fig. 10: Clarification of Green apple juice with 40ml crude pectinase, commercially produced pectinase and water

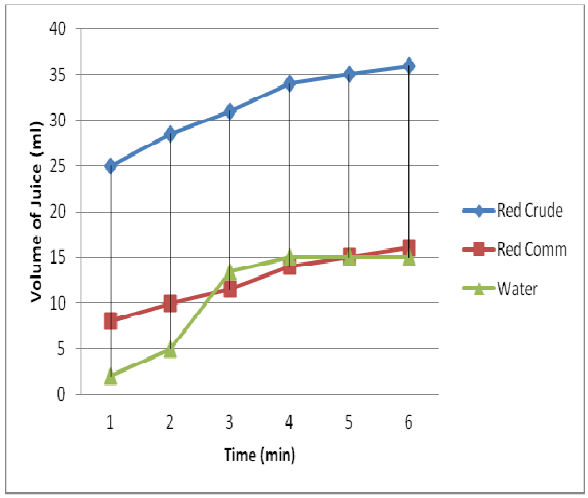

Fig. 13: Clarification of Green apple juice with 50ml crude pectinase, commercially produced pectinase and water

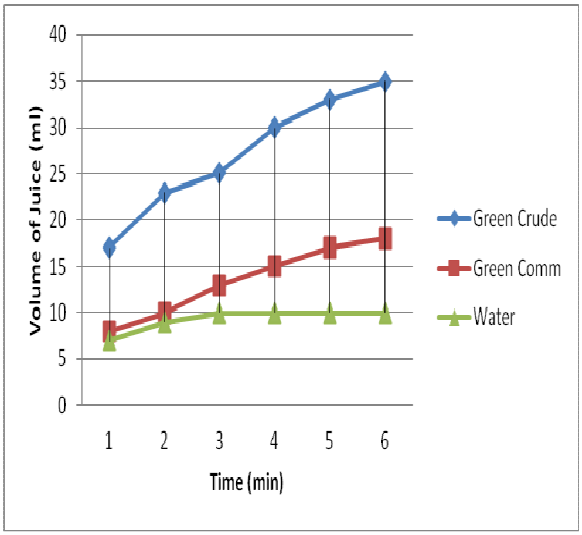

Fig. 11: Clarification of yellow apple juice with 40ml crude pectinase, commercially produced pectinase and water

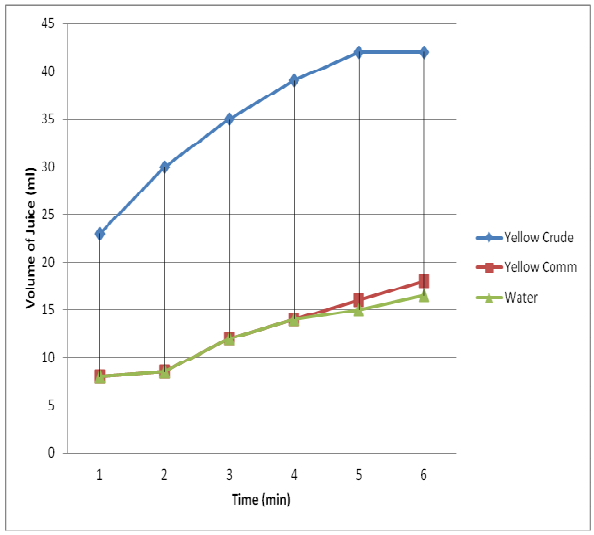

Fig.14: Clarification of Yellow apple juice with 50ml crude pectinase, commercially produced pectinase and water

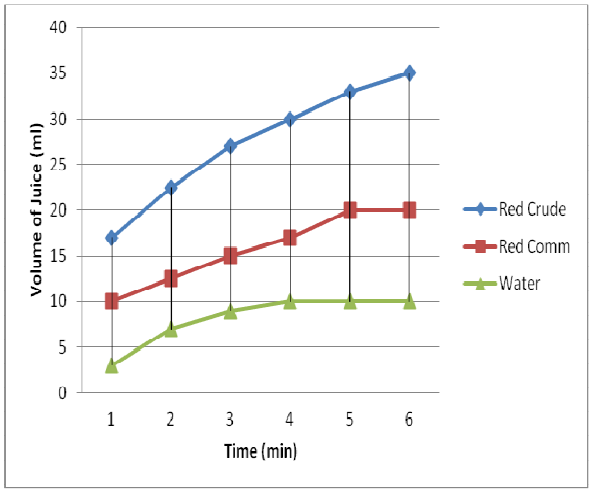

Fig.15: Clarification of Red apple juice with 50ml crude pectinase, commercially produced pectinase and water

(IV) DISCUSSION

The results of this investigation revealed that the volume of juice increased with increased volume of enzyme for the three varieties of apple fruits. The crude pectinase was also more effective than the commercial pectinase in the clarification process and the cylinders with water in all cases produced lesser juice than the cylinder with laboratory-produced-pectinase and the commercially- produced- pectinase. This result reveals the role of enzymes in the clarification process. The water had little or no effect on the apples in the water cylinder. Several reasons have been attributed to this. The juice in the pectinase cylinder was more than that in the water cylinder at all temperatures studied for green and red apples (5). The volume of juice produced at 50ml was more for yellow apples with the crude enzyme than other varieties. This can be attributed to the composition, make and texture of the different varieties. SAPS (13) reported that pectinases acts in different ways on the pectins found in the primary cell wall and in the middle lamella. The efficacy of absolutely purified xylanase was studied on juice enrichment of apples and the treatment with purified *Bacillus pumilus* SV-85S xylanase had an increased juice yield (14). Previous researchers have reported a decrease in the turbidity and viscocity of fruits as well as the increase in yield and clarity in apple fruit juices using hydrolytic enzymes (15). The enzymatic treatment employed for the clarification of fruit juice resulted in the improvement of two fold in the release of reducing sugars and 52.97% in juice yields, whereas 35.34% reduction in turbidity was observed for clarification of citrus fruit juice using xylanase from *Bacillus stearothermophilus* under batch fermentation (16).

(V) CONCLUSION

This study revealed that the minimum volume of laboratory-produced pectinase needed to produce more juice than the commercially-produced pectinase was 30ml for all the three different varieties of apple fruits.

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