THE EFFECT OF TEMPERATURE ON THE CLARIFICATION OF APPLE (*MALUS DOMESTICA*) JUICE WITH PECTINASE OBTAINED FROM *ASPERGILLUS NIGER*

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ABSTRACT

Aspergillus niger is a saprophytic fungus existing ubiquitously in the soil and on decaying vegetation. Various researchers have reported *Aspergillus niger* as frequently responsible for postharvest decay of fresh fruits such as apples, pears, grapes, melons, onions and some vegetables. The fungus has been implicated in the deterioration of apple fruits with the production of Pectinolytic enzymes during the breakdown of the plant cell wall by microbial attack. The three varieties of apple (*Malus domestica*) fruits used for this work are commonly referred to as Red, Green and Yellow varieties from their physical appearance. The fruits were obtained from a grocery store along Idiroko road, Ota, Ogun State in Nigeria. The fruits were disinfected and inoculated with a 72-h-old culture of *A. niger*. Control fruits were inoculated with sterile inoculum. The fruits were incubated at room temperature of 27^{o}C for twelve days. Extracts from the inoculated fruits exhibited appreciable polygalacturonase activity while those from the uninoculated fruits possessed only traces of the enzyme activity. The enzyme obtained from the deteriorated fruits and commercially produced pectinase were applied for the clarification of freshly ripe apple fruits under controlled experimental conditions at different temperatures (20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C and 45^{o}C) to investigate the role of pectinase in the clarification of apple juice. The temperature of incubation had different effects on the three varieties of apple fruits studied. The volume of juice was more in the cylinders with the enzyme clarification at all temperatures than that with water. The optimum temperature was at 25^{o}C for the three varieties green and red apples. The commercial pectinase produced more juice than the crude pectinase.

Key words: *Aspergillus niger,* Clarification, Apples (*Malus domestica)*

1. INTRODUCTION

Apples compared with many other fruits and vegetables may have relatively low amount of vitamin C but they are very rich source of antioxidant compounds (Eberhardt *et al.*, 2000). They are widely consumed and there are different varieties of modern apples which have resulted from natural cross-pollination involving different species (Aldwinckle, 1993). Apples are often eaten in the raw fresh forms sometimes baked or stewed and can be reconstituted for later use (Ferree *et al.*, 1999). The apple juice can be fermented to produce apple cider of different kinds (Grafton, 1996; Gunningham, 2003). Block *et al.* (1992) reported that those who consumed low amounts of fruits and vegetables were twice as likely to have cancer compared to those who ate high amounts of fruits and vegetables in one hundred and twenty eight (128) out of one hundred and fifty six (156) dietary studies carried out. They therefore reported that fruits and vegetables had a significant protective effect against a variety of cancers. Apples have been identified as one of such fruits (Ajayi *et al.*, 2011). Despite all the benefits to be derived from the consumption of apple fruits, a large number of apples undergo deterioration due to the activities of microorganisms (Ayanda *et al.*, 2013). This task is being accomplished by cell wall degrading enzymes which aids in the penetration of the hosts cell by microorganisms (Famurewa and Olutiola, 1991; Ajayi *et al.*, 2013). Cell wall degrading enzymes have also been associated with a lot of benefits in industries (Satyanarayana, 2009). Pectinases are cell wall degrading enzymes that breaks down complex pectin molecules to shorter molecules of galacturonic acid as pectinases

causes the disintegration of the cell wall and so allowing the cell sap (juice) to flow out (Ranveer, 2005). Pectins are able to form jellies such as jam but they are undesirable in fruit juices and other liquids (NCBE, 2000).This is a major reason why the biggest industrial use for pectinase is in the extraction and clarification of fruit juices (Endo, 1965). Pectins are large polysaccharide molecules, made up mainly of chains of several hundred of galacturonic acid residues (Ayanda *et al*., 2011), and they are found in the cell wall (Zubay *et al*., 2007). *Aspergillus niger* has been identified as a unique organism for higher yield in industrial processes (Satyanarayana, 2009). This study therefore examined the clarification of different varieties of apple fruits with pectinase obtained from the deterioration of apple fruits by *Aspergillus niger* with the aim of obtaining optimum temperature and the best variety of apple fruits for higher industrial yield of apple juice.

2. MATERIALS AND METHOD

Collection of Samples

Freshly ripe Green, Red and Yellow varieties of apples showing no signs of physical damage or microorganisms were employed for this research work. These apples (*Malus domestica*) fruits were obtained from the grocery section of 'Justrite' Supermarket, Idiroko road, Ota. 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 a stock 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. Commercially Produced Enzyme (Pectinase)

The enzyme, Pectinase, produced from *Aspergillus niger* for commercial use was obtained from Sigma Aldrich. The enzyme in powder form, was diluted in citrate- phosphate buffer, pH 5.0. 15 grams of pectinase was put in 500ml of citrate – phosphate buffer, and mixed thoroughly.

2.4. Inoculation of Apple fruits

Sodium hypochlorite solution the apple fruits were surface sterilized with 5% (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. Using a cork borer, holes were bored into the apples, and then inoculated with seventy-two-hour old *A. niger* culture. 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, crushed in separate mortars, and chilled for 30min inside a refrigerator. They were homogenized with a laboratory blender with addition of chilled liquid extracting buffer (1:1w/v) for 2 min at 30secs intervals.. The homogenate was allowed to percolate through four layers of Whatmans no.1 filter paper. This served as the crude enzyme solution.

2.5. Enzyme Assay

Pectinase activity was determined according to the method of Olutiola (1982) whereby the reaction mixture consisted of 1ml of 0.1% (w/v) Pectin, obtained from Sigma Aldrich in 0.01M Citrate Phosphate Buffer (pH5.0) and 0.5ml of the crude 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 3hour. The total reducing sugar was determined by the Dinitrosalicylic acid (DNSA) method (Miller, 1959). The amount of the enzyme was defined as one unit of Pectinase activity, which releases 1µmole of 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 (2006) method. Fresh apples were chopped into cubes of about five millimetres (5mm) .Triplicate samples of 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 beakers of three beakers for each variety. Varied volumes (10ml, 20ml, 30ml, 40ml and 50ml) of laboratory-produced- pectinase; commercially-produced-pectinase and water were added to the beakers. The beakers were labelled appropriately as 'Laboratory-produced -pectinase', 'Commercially-produced-pectinase and 'water'. The chopped apple pieces were covered with plastic wraps and incubated at different temperature of 20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35oC, 40^{o}C and 45 ^{o}C for fifteen minutes. The juice from the preparation was filtered using a Whatmans No.1 filter paper in funnels into a measuring cylinder. The cylinders were appropriately labelled and the amount of juice in each cylinder was measured at 5 min intervals for 30 min.

3. RESULTS

3.1 Pectinase Activity

Inoculation of freshly ripe apple fruits with *Aspergillus niger* was carried out after twelve days of incubation. The crude enzyme obtained from the deteriorated apples showed appreciable pectinase activity. The control fruits had only traces of pectinase activity.

3.2 Clarification of apple juice

The juice obtained from the clarification of apples with both the crude and commercially produced enzymes were clearer and of a higher volume than that of water (Figs. 1 – 3). The juice obtained from the clarification with the crude enzyme appeared physically clearer than the juice from the commercially produced pectinase. The crude enzyme had higher volume of juice only for green and yellow apples at 25°C (Fig. 2). The volume of juice was more with the crude enzyme clarification for green apples than the yellow apples at 25°C (Fig.2).

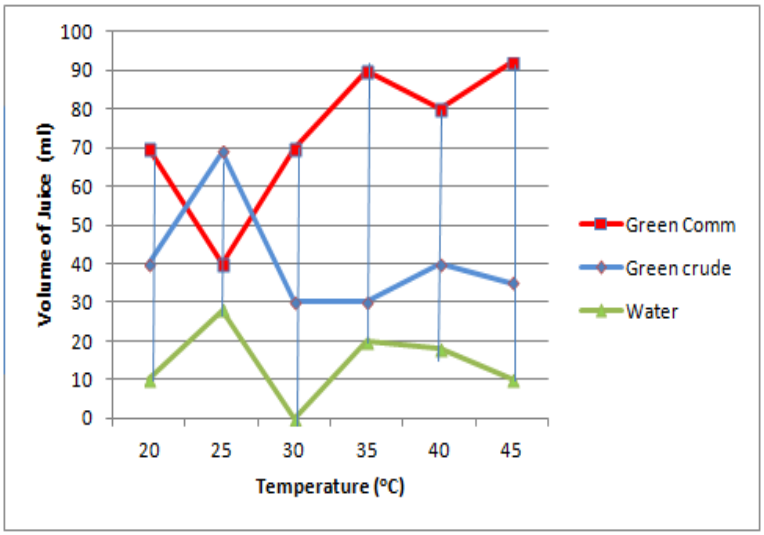

Fig 1: The Effect of Temperature on the clarification of Green apples with crude pectinase, commercially produced pectinase and water

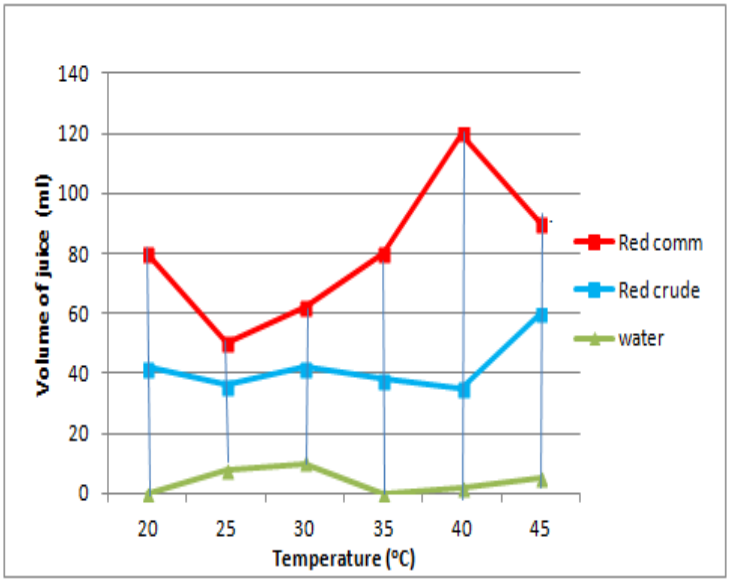

Fig 2: The Effect of Temperature on the clarification of Red apples with crude pectinase, commercially produced pectinase and water

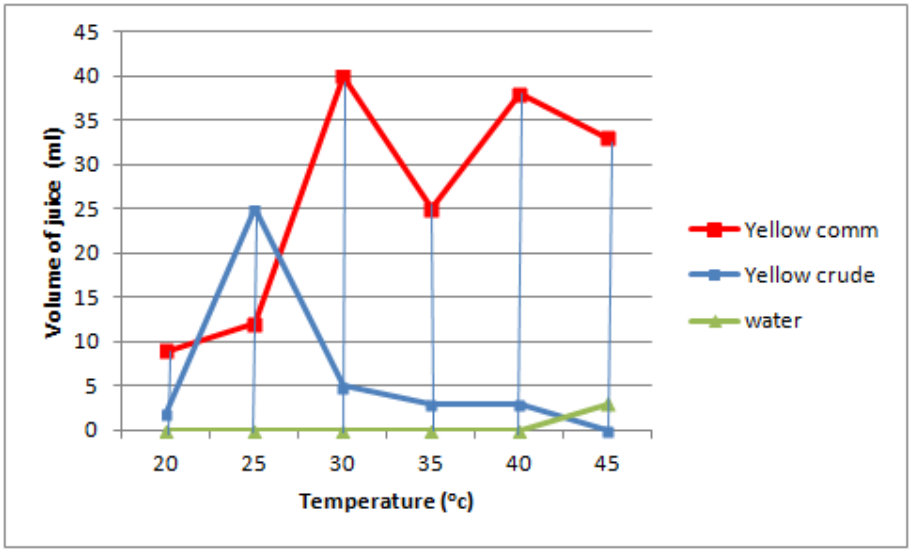

Fig 3: The Effect of Temperature on the clarification of Yellow apples with crude pectinase, commercially produced pectinase and water

4. DISCUSSION

The pectinase obtained from the fresh apple fruits deteriorated by *Aspergillus niger* was able to clarify apple juice with low viscosity. McLellan *et al*. (2006) reported similar results for the clarification of apple juice with crude enzyme and the breaking down of the pectin by pectinase had some technical advantages such as accelerating the pre-fermentation stages, clarification and pressing as well as an increased juice yield with an overall improvement in quality. Physical observation of the cylinders with the juice clarified with pectinase and water revealed reduced cloudiness of the apple juice with pectinase.
The optimum temperature for apple fruits clarified with the crude pectinase was 25^{o}C for green and yellow apples while it differs for red apples. Ajayi *et al*. (2011) reported similar results for red and green apples. The clarification process for both crude and commercial pectinase differs for the three varieties of apple fruits in terms of volume of juice production and the optimum temperature. This can be attributed to the different composition such as phenolic compounds and polyphenol oxidase (PPO) activity, found in varieties of apples (Podsedek *et al*.2000). The commercial pectinase produced more juice than the crude pectinase for the three types of apple fruits. This can be due to the fact that, the commercial enzyme has been purified and all contaminants that could inhibit its activity have been completely removed unlike the crude enzyme. (Klibanov, 1997) asserts that purified enzymes have higher yield and are more specific in their activity.

Also commercial enzymes are known to be stable at high temperature ranges of 40^{0}C - 45^{0}C. Dettmer *et al.* (2011) reported that commercial enzymes are thermo stable hence they are suitable for industrial uses. The reason for this can be explained by the use of recombinant- DNA technique in the production of commercial enzyme. This technique involves insertion of DNA from an organism of interest into another one with a favourable character, hence optimising the culture organism in order to have high productivity, suitability and sustainability of enzyme production. At high temperatures commercial enzymes are less likely to denature unlike the crude enzymes because they have been immobilized. Immobilization of enzymes involves binding enzymes onto solid materials which restricts its mobility. This enhances easy separation of enzyme from the product and also the reuse of the enzyme. Immobilization is the key to optimizing the operational performance of an enzyme in industrial processes (Sheldon, 2007).

5. CONCLUSION

This study established the clarification of juice from three varieties of apple with an optimum temperature of 25^{o}C for Green apples which produced the highest volume of juice.

6. RECOMMENDATIONS FOR FURTHER STUDIES

- Further studies on pectic enzymes should be devoted to the understanding of the regulatory mechanism of the enzyme secretion at the molecular level and the mechanism of action of different pectinolytic enzymes on pectic substances.
- Commercial enzymes are more productive, active, sensitive and stable at high temperatures, it is of necessity to have the crude enzymes thoroughly purified and possibly immobilized in order to have highly proficient enzymes.
- The organisms or microbial sources used for the extraction of enzymes should be genetically manipulated in order to have favourable enzyme properties.

REFERENCES

[1] Ajayi, A.A., Aina, O., Olasehinde, G.I. (2011). Extraction and Clarification of Apple Juice with Polygalacturonase Obtained from Apples (*Malus domestica*) fruits Deteriorated by *Aspergillus niger*. *International Journal of Biological and Chemical Sciences* **5(3):**1047-1053
[2] Ayanda, O.I., Ajayi, A.A. Olasehinde, G.I. Dare, O.T. (2013).Isolation, characterization and extracellular enzyme detection of microbial isolates from deteriorated apple (*Malus domestica*) fruits. *International Journal of Biological and Chemical Science* **7(2):** 641 – 648
[3] Satyanarayan, C.P. (2009). Biotechnology. Arunabha Sen Books and Allied Ltd. 8/1 chintamoni Das Lane Kolkata ISBN: 9788187134909. India. Pp books@cal.vsnl.net.in
[4] Block, G., Patterson, B., and Subar, A. (1992). Fruit, Vegetables and Cancer Prevention: a review of the epidemiological evidence. Nutr Cancer, **18**:1-29
[5] Clark , D.S. (1994). Can immobilisation be exploited to modify enzyme activity?
[6] *Trends Biotechnology,* **12**:439–443
[7] Dettmer A., Ayub M.A.Z., Gutterres M. (2011). Hide unhairing and characterization of commercial enzymes used in leather manufacture. *Brazilian Journal of Chemical Engineering*.**28 (3**) :
[8] Famurewa, O. and P.O. Olutiola, 1991. Comparison of growth and cellulolytic enzyme prodsuction in Aspergillus chevalien and Penicillium steckii frommouldy cocoa beans. Folia Microbiol., 36: 330pp
[9] Klibanov , A.M. (1997) Why are enzymes less active in organic solvents than in water?
[10] McLellan, M.R., Kiimer, R.L., and Lind, L.R. (2006).Electroplasmolysis and other treatments to improve apple juice. Journal of Food Science **50(1):** 206-208
[11] NCBE (2000). An NCBE/Unilever educational guide to enzymes in fruit juice prodction "In a jam and out of juice". Science and Technology Centre, Earley Gate, University of Reading, Whiteknights. RG6 6BZ. http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/juice.html
[12] Olutiola, P.O., Patkai, T., and Onilude, A.A. (1982). Protease associated with the black mould rot of tomato fruits. *Acta Phytopathological Academia Scientarium Hungariae* **17**:53-64
[13] Podesdek, A., Wilska-Jeszka, B., Markowski, J., (2000). Compositional characteristics of some apple varieties. *European Food and Technology*. **210(4):** 268-272
[14] Ranveer, S.J., Saxena, S., and Gupta, R., (2005). Microbial pectinolytic enzymes: A review. *Process Biochemistry,* **40**:2931-2944
[15] Sheldon R.A.,(2007) Enzyme Immobilization: The Quest for Optimum Performance.Adv. Synth. Catal. **349**:1289-1307
[16] *Trends in Biotechnology* (2014), **15**:97–101
[17] Zubay GL, Person WW, Vance DE. 1995.*Principles of Biochemistry.* (8th edn) Wim. C. Brown Communications, Inc.U.S.A.; 863.