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Extraction and clarification of apple juice with polygalacturonase obtained from apple (*Malus domestica*) fruits deteriorated by *Aspergillus niger*

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ABSTRACT

Pectinase is used commercially in the clarification and extraction of fruit juice from different fruits. Green apples and Red apples obtained from the fruits section of a supermarket, Idiroko road, Ota were surface sterilized and inoculated with *Aspergillus niger*. The stock culture was subcultured on Sabouraud Dextrose agar plates and 72-hr-old culture of *Aspergillus niger* served as the inoculum. The fruits were incubated for twenty-five days at room temperature (25 °C). Control fruits were similarly treated except that sterile inoculum was used for the inoculation. Extracts from the inoculated fruits exhibited appreciable polygalacturonase activity while those from the uninoculated fruits possessed only traces of the enzyme activity. The polygalacturonase obtained after enzyme extraction was applied to freshly ripe apple fruits under controlled experimental conditions to investigate the role of polygalacturonase in the production of apple juice. The juice in the cylinder to which polygalacturonase was added was visually clearer and more than that with distilled water. The optimum temperature of incubation for the clarification of apple fruits with polygalacturonase obtained from apple fruits deteriorated by *Aspergillus niger* was 25 °C.

Keywords: Polygalacturonase, apple fruits (*Malus domestica*), *Aspergillus niger.*

INTRODUCTION

Apple is one of the most widely cultivated tree fruits (Potter et al., 2007). Apples, compared to many other fruits and vegetables, may have relatively low amounts of vitamin C but they are very rich sources of other antioxidant compounds (Lee et al., 2003; Boyer and Liu, 2004). The fiber content helps to regulate bowel movements and may therefore reduce the risk of colon cancer, prostate cancer and lung cancer (SCC, 2008). There are modern varieties of modern apples and these are thought to have resulted from natural cross-pollination involving several species because modern varieties are heterozygous (Polomski and Reighard, 2008). Apples can be canned or juiced and they are milled to produce apple juice (Ferree and Warrington, 1999). The juice can be fermented to produce apple cider of different kinds (Juniper and Mabberley, 2006; FSA, 2005). Apples are often eaten, baked or stewed and they can be dried and reconstituted for later use. Despite all the benefits derived from apples, a large percentage of apples is lost to post harvest deterioration caused by

microorganisms especially fungi and bacteria (Fontana et al., 2005). Pectinases can be extracted from fungi during this process. *Aspergillus niger* has been identified as one of the fungi causing deterioration of apple fruits (Alexoupoulous, 1979). One of the most studied and widely used commercial pectinases is polygalacturonase (Singh, 2009). Polygalacturonase is useful because pectin is the jelly-like matrices which helps cement plant cells together and in which other cell wall components such as cellulose fibrils are embedded (Acquaah, 2004). They are therefore used in processes such as speeding up the extraction of fruit juice from fruits (Glick and Pasternak, 1998; Shewfelt, 1986) which involves the degradation of plant materials.

This investigation was therefore carried out to examine the extraction of polygalacturonase, a pectinase from the deterioration of apple fruits by *Aspergillus niger* under laboratory conditions. The polygalacturonase was also used for the clarification of apple fruits with a view of examining its role in the production of fruit juice. Some factors were also varied in the experimental process to examine their effects on the results obtained.

MATERIALS AND METHODS

Organism

The isolate of *Aspergillus niger* employed for this research work was obtained from the culture collection of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. The organism was subcultured from the stock culture and plated onto fresh Sabouraud Dextrose agar plates for the purpose of this investigation. Seventy-two-hour old culture of the organism was used as the inoculum.

Inoculation and cultivation

The inoculation technique is as described by Ajayi and Olasehinde (2009) whereby healthy ripe apple fruits obtained from the fruits section of a supermarket on Idiroko road, Ota, were surface sterilized using 10% sodium hypochlorite solution for 30 min. 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 *Aspergillus niger* removed from the edge of the seventy-two- hour old culture of the organism was used in inoculating apparently healthy and freshly ripe apple fruits. The point of inoculation was sealed with paraffin wax. The control fruits were inoculated with sterile Sabouraud Dextrose agar (SDA) 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 twenty-five (25) days.

Extraction of enzyme from apple fruit

Twenty-five days (25 days) after inoculation of freshly ripe apple fruits with *Aspergillus niger*, the deteriorated apple fruits were weighed and chilled for thirty minutes inside a freezer and homogenized with a laboratory blender with chilled liquid extractant (1.1w/v) for two minutes (2 mins) at thirty seconds (30 secs) interval. The extractant was 0.01M citrate phosphate buffer with pH 4.5 containing 5 mM sodium azide (NaN_3) to prevent microbial contamination. The homogenate was initially allowed to percolate through four layers of sterile muslin and thereafter through Whatmann No.1 filter paper. This was used as the crude enzyme preparation.

Enzyme assay (Polygalacturonase)

Polygalacturonase activity was determined according to the method described by Olutiola 1982. The reaction mixture was one milliliter (1ml) of 0.1% (w/v) Pectin (Sigma) in 0.01 M citrate phosphate buffer (pH 4.5) and 0.5 ml 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 three hours (3 hrs). The total reducing sugar was determined by the Dinitrosalicylic acid (DNSA) method (Miller, 1959). One unit of

polygalacturonase activity was defined as the amount of enzyme, which released 1µmol galacturonic acid per minute.

Extraction of apple juice with pectinase

The extraction of apple juice with pectinase was carried out using a modified method of the NCBE (2006) method whereby fresh apples were chopped into cubes of approximately five millimeters (5 mm) on a side with a sharp knife. Twenty five grammes (25 g) each of the chopped apples (red apple fruits were treated separately from the green apple fruits) were weighed into four separate beakers. Twenty milliliters (20 ml) of the enzyme was added to one beaker and 20ml of distilled water to the other beaker for each of the varieties of apples. The beakers were labeled appropriately as "Pectinase" and "Water". The chopped apple pieces were covered with plastic wraps and incubated in a water bath at room temperature of 25 ^{o}C, 30 ^{o}C and 40 ^{o}C for 20 minutes. The juice from the apple preparations was filtered using muslin and then filter paper in funnels into a 100ml measuring cylinder. The cylinders were labeled appropriately as "Pectinase" and "Water". The amount of juice in each interval was measured at five minutes intervals for thirty minutes (30 min) for apples incubated at 25 ^{o}C, 30 ^{o}C and 40 ^{o}C.

RESULTS

Twenty-five days (25 days) after the inoculation of freshly ripe apple fruits with *Aspergillus niger*, extracts obtained from the fruits produced appreciable polygalacturonase activity while extracts from the uninfected fruits possessed only traces of Polygalacturonase activity.

The apple juice in the cylinder containing "Pectinase" was clearer and more than the contents of the cylinder with "Water" which was cloudy at the end of the study period, for both green and red apples at all temperatures. The volume of juice for green apples in the pectinase cylinder at 25 ^{o}C was more than that of the water cylinder while the water cylinder had a little less than that in the pectinase cylinder (Table 1). The volume of pectinase was more than the volume obtained from that with water for green apple fruits at 30 ^{o}C (Table 2). The volume obtained from the measuring cylinder with pectinase was a little more than that in the water cylinder (Table 3), but this volume was more than the volume of juice obtained from green apples after thirty minutes (30 mins) (Table 4). The volume of juice from the water cylinder was more than that of the volume of juice obtained from pectinase at 40 ^{o}C for green apples (Table 5) while the volume of juice from the pectinase cylinder was more than that of the juice from the water cylinder for red apples at the same temperature (Table 6).

Table 1: The volume of juice obtained from Green apples incubated at 25 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	11	7
2	10	13	9
3	15	14	10
4	20	14	11
5	25	15	11
6	30	15	12

Table 2: The volume of juice obtained from Red apples incubated at 25 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	3	4
2	10	4	5
3	15	5	6
4	20	5	7
5	25	7	8
6	30	8	9

Table 3: The volume of juice obtained from Green apples incubated at 30 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	2	0
2	10	2	1
3	15	2	1
4	20	3	1
5	25	3	2
6	30	4	2

Table 4: The volume of juice obtained from Red apples incubated at 30 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	2	0
2	10	3	1
3	15	3	1
4	20	3	1
5	25	4	2
6	30	5	2

Table 5: The volume of juice obtained from Green apples incubated at 40 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	0	2
2	10	0	2
3	15	1	3
4	20	1	4
5	25	1	5
6	30	2	5

Table 6: The volume of juice obtained from Red apples incubated at 40 ^{o}C and clarified with pectinase and water against time for thirty minutes.

S/N	Time (Min)	Volume of Juice (ml) with pectinase	Volume of Juice (ml) with water
1	5	1	1
2	10	2	1
3	15	3	1
4	20	3	1
5	25	3	2
6	30	4	2

DISCUSSION

The result of this investigation revealed that pectinase obtained from the deterioration of apple fruits infected with *Aspergillus niger* reduced the cloudiness of apple juice which is caused by suspended pieces of cell wall and therefore clarified the apple juice physically as shown in the contents of the cylinder with the "Pectinase" that was obviously clearer than the contents of the cylinder with water at all temperatures and also for both green and red apples. This corroborates the finding of Mclellan et al. (2006). The juice in the pectinase cylinder was more than that in the water cylinder at all the temperatures but the volume of juice obtained for apples incubated at 25 ^{o}C was more than at 30 ^{o}C and 40 ^{o}C. This suggests that 25 ^{o}C is optimum for the incubation of the apple fruits investigated. The enzyme activity was obviously denatured at 40 ^{o}C for Green apples because the volume of juice produced for water was more than that with pectinase but the same temperature produced more juice from the pectinase cylinder for red apples than in the water cylinder. The optimum temperature for red apples seems to be at 40 ^{o}C but the volume of juice produced was not as much as what was produced at 25 ^{o}C where the volume of water was slightly more than that of pectinase. Temperature increases enzyme activity by increasing the kinetic energy within the molecules for faster reactions but at extreme temperatures, enzymes is denatured (MLA, 2010). Fruit juice companies use a variety of different treatments and enzymes to maximize their yield (SAPS, 2010; Kashyap et al., 2001). The red apples produced more juice at 30 ^{o}C than the Green apples. This could be attributed to the composition, makeup or texture of two varieties of apple fruits. Pectinases acts in different ways on the pectins found in the primary cell wall and in the middle lamella (SAPS, 2010). The enzyme which are used in fruit juice companies are designed to break down the cell walls within the fruits and release the liquids and the sugars, which make up the fruit (Singh, 2005; NCBE, 2006). Pectinases are one of the enzymes, which break down different structures of the fruit cells and therefore affect the extraction process in different ways (Glick and Pasternak, 1998). During breakdown of the fruit cells, a variety of polysaccharides are found within the juice extract, these can cause the juice to become cloudy (Kashyap et al., 2001). Pectinases can break down these insoluble compounds releasing soluble sugars, which clarify the juice producing a clearer and sweeter product (Singh, 2009). The juice produced in this experiment was not tested and therefore, it was not known whether it was sweeter than the one without the pectinase because the concentration of pectinase used will be much higher than is used in commercial juice production and the apple fruits and enzyme have not been handled aseptically. The juice produced in the pectinase cylinder was just a little more than that of water at each of the temperatures

suggesting that the yield was not much. Different reasons could be attributed to this because enzymes have different factors affecting their activities (Dixon and Webb, 1971; Zubay et al., 1995). The results also revealed that the viscosity of the juice was lowered because the juice in the pectinase cylinder runs more than that in the water cylinder during the breakdown of the cell wall at all temperatures.

Conclusion

The optimum temperature for the clarification of juice by polygalacturonase obtained from apple juice deteriorated by *Aspergillus niger* under laboratory conditions was 25 ^{o}C but further studies need to be carried out to take into consideration so many variations such as the different concentrations of the enzyme, the period of incubation, the pH of the extractant and some other factors that could possibly increase the yield of apple juice produced in this way.

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