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Clarification of Tomato Juice with Polygalacturonase Obtained from Tomato Fruits Infected by *Aspergillus niger*

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ABSTRACT
Two varieties of tomato fruits commonly available in Nigerian markets are the Roma VF and Ibadan local varieties of tomato fruits. The Roma VF fruits are oval in shape. It is a common type of cultivar in the Northern region of Nigeria and it is not susceptible to cracking. The Ibadan local variety of tomato fruits is a local variety commonly found on farmers fields in South-western region of Nigeria. They are highly susceptible to cracking. The Ibadan local variety was employed for this research. There are lots of benefits derived from the consumption of tomato fruits. The fruits can be made into tomato juice clarified with pectinases. Polygalacturonase is one of the pectinases used commercially in the clarification of fruit juice from different fruits. This study examined the production of polygalacturonase during the deterioration of tomato fruits by *Aspergillus niger* and the role of the purified polygalacturonase in the clarification of tomato juice. Tomato fruits of the Ibadan local variety were inoculated with mycelia discs containing spores of a 96-h-old culture of *Aspergillus niger* served as the inoculum. The organism from the stock culture was subcultured onto potato dextrose agar plates. The extraction of polygalacturonase after 10 days of incubation at 27°C was carried out by homogenizing the fruits with liquid extractant using the MSE homogenizer after the deteriorated fruits had been chilled for 30 min inside a freezer. Control fruits were similarly treated except that sterile potato dextrose agar served as the inoculum. The effect of different temperature of incubation and different volume of enzyme on the tomato juice from the tomato fruits was investigated. Extracts from the inoculated fruits exhibited appreciable polygalacturonase activity. The juice with polygalacturonase was visually clearer and more voluminous than the juice treated with water for all parameters studied. The highest volume of juice was obtained after an incubation period of 30 min for the tomato fruits. The increase in juice yield can be attributed to the hydrolysis of pectin which releases the sap inside the cells of the pulp. The occurrence of polygalacturonase in tomato tissues infected by *A. niger* coupled with the trace amount in the non-infected tissues suggests that the enzyme is of fungal origin. The role of the polygalacturonase in the clarification process was established. This study will be very useful for industrial tomato juice production.

Key words: Tomato (*Lycopersicon esculentum* MILL) fruits, *Aspergillus niger*, polygalacturonase, clarification of Juice

INTRODUCTION

The tomato is one of the most popular vegetables world wide. Global production of tomatoes is estimated at 1.26 million metric tonnes pa with China and the United States of America as the leading producers. Tomatoes are a rich source of fibre, vitamins A, vitamins C and lycopene. Epidemiological studies indicate that increased consumption

of tomatoes is coincident with a lower occurrence of cardiovascular disease (Arab and Steck, 2000; Sesso *et al*., 2003) and certain types of cancers (Giovannucci, 2002a, b; Giovannucci *et al*., 2002). The evidence supporting the health benefits of tomato consumption remains strong (Willett, 2010). Tomatoes are consumed in many ways such as the fresh fruits eaten in salads and sandwiches. The processed varieties are consumed dried or as pastes, preserves, sauces, soups and juices. In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Pectinases are of great significance with tremendous potential to offer to industry (Patil and Dayanand, 2006). They are one of the upcoming enzymes of the commercial sector, especially the juice, food industry (Kashyap *et al*., 2001) and in the paper and pulp industry (Beg *et al*., 2001; Viikari *et al*., 2001). These enzymes have a share of about 25% in the global sales of food enzymes (Kashyap *et al*., 2001). Pectinase which includes pectin methyl esterase and depolymerising enzymes finds extensive application in fruit processing industries for clarification of fruit juices and wines, in the extraction of fruit juices, in the manufacturing of pectin free starch, curing of coffees, cocoa and tobacco, refinement of vegetable fibres, scouring and as an analytical tool for the estimation of plant products (Joshi and Bhutani, 1991; Tzanov *et al*., 2001; Evans *et al*., 2002). Pectinase is produced by several fungi but *Aspergillus* has been found to be the major source of pectinase (Polizeli *et al*., 1991). The filamentous fungus *Aspergillus niger* is most often used in the commercial production of pectinase because it is classified as 'Generally Regarded As Safe' (GRAS) by the United State Food Drugs Administration (USFDA) which allows the use of its metabolites in the food industries (Pariza and Foster, 1983).

MATERIALS AND METHODS

This experiment was carried out in the Microbiology Laboratory of the Department of Biological Sciences, Covenant University, Sango Ota, Ogun State, Nigeria.

Source of organism: The isolate of *Aspergillus niger* employed for this research work was obtained from the collection of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. The organism was sub cultured onto fresh potato dextrose agar plates for the purpose of this investigation.

Inoculation and cultivation techniques: The inoculation technique employed for this research was as described earlier (Ajayi and Olasehinde, 2009) whereby, freshly ripe tomato fruits purchased from Sango Ota market in Ogun state 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 a 72-h-old culture of the organism was used in inoculating freshly ripe tomato fruits. The point of inoculation was sealed with candle wax. The control fruits were however not treated in the same manner. Both control and experiment fruits were placed under separate sterile bell jars. Incubation was at room temperature of 25°C for ten days.

Extraction of enzyme from tomato fruits: Ten days after the inoculation of freshly ripe tomato fruits with *Aspergillus niger*, the deteriorated tomato fruits had collapsed extensively. The fruits were weighed and homogenized with a laboratory blender with liquid extractant (1.1 w/v) for 2 min at 30 sec interval each. The extract was 0.01 M citrate phosphate buffer (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 Whatmans No.1 filter paper. This was used as the crude enzyme preparation.

Enzyme assay: Polygalacturonase activity was determined according to the method described by Ajayi *et al*. (2014). The reaction mixture was 1 mL 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 contains 1 mL of substrate. The experimental and control tubes were incubated in a water bath at 37°C for 3 h. The total reducing sugar was determined by the Dinitrosalicylic acid (DNSA) method (Miller, 1959). A Genesys 10S UV-VIS spectrophotometer was used in measuring polygalacturonase activity. One unit of polygalacturonase activity was defined as the amount of enzyme, which released 1 μmol galacturonic acid per minute.

Clarification of tomato juice with polygalacturonase: The extraction of tomato juice with polygalacturonase was carried out using a modified technique whereby, fresh tomatoes were sliced into parts with a sharp knife. Twenty five grams each of the chopped parts were weighed and crushed into two separate beakers. Twenty milliliters of the enzymes were added to one beaker and 20 mL distilled water was added to the other beaker. The beaker was labeled appropriately as "polygalacturonase and water", respectively. The crushed tomato parts together with the enzyme preparation were covered with plastic wraps and incubated in water bath at 20°C and at different temperature of 25, 30, 35, 40 and 45°C for 20 min. The juices from the tomato preparation were filtered using muslin and then filter paper in funnels into a 100 mL measuring cylinder. The cylinders were labeled appropriately as "polygalacturonase and water", respectively. The amount of juice in each interval was measured at 5 min intervals for 30 min for the enzyme and water-treated tomato preparation incubated at 25, 30, 35, 40 and 45°C.

The temperature of incubation that yielded the most juice upon filtering was used as a parameter to measure enzyme activity. At that temperature, the volume of enzyme preparation used was varied. Enzyme volume used were 5, 10, 15, 20, 25 and 30 mL after which the amount of juice in each interval was measured at 5 min intervals for 30 min for the enzyme and water-treated tomato preparations.

The volume of enzyme used that yielded the most juice at the best time of temperature incubation was also linked to another parameter to measure enzyme activity. The duration of incubation was also varied. Incubation time used were 5, 10, 15, 20, 25 to 30 min after which the amount of juice in each interval was measured at 5 min interval for 30 min for the enzyme-treated and water-treated tomato preparations.

RESULTS

Ten days after inoculation of freshly ripe tomato fruits with *Aspergillus niger*, the extracts obtained from the fruits produced appreciable polygalacturonase activity while extracts from the uninfected fruits possessed only traces of polygalacturonase activity. The tomato juice in the cylinder containing "polygalacturonase" was clearer and much more than the contents of the cylinder with "water" which was cloudy at the end of the study period at all temperatures (Fig. 1-6) and for all the volume of enzyme preparation at the optimum temperature of 25°C (Fig. 7-18). At an incubation temperature of 25°C, the highest volume of tomato juice was obtained from the enzyme treated tomato preparation (Fig. 2). The tomato juice yield was highest at an optimum temperature of 25°C when 20 mL of enzyme preparation was used (Fig. 4). When incubation was carried out at an optimum temperature of 25°C for 30 min using 25 mL of enzyme preparation, the highest volume of juice was obtained (Fig. 19). At an incubation temperature of 40°C, the highest tomato juice yield was obtained from the enzyme treated tomato preparation (Fig. 14). The tomato juice yield was highest at an incubation temperature of 25°C when 20 mL of enzyme preparation was used (Fig. 4). When incubation was carried out at an optimum temperature of 40°C for 30 min using 20 mL of enzyme preparation, the highest juice yield was obtained (Fig. 19).

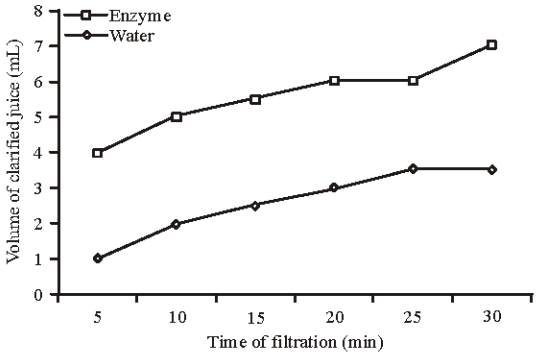

Fig. 1: Clarification of tomato juice with 5 mL of polygalacturonase and water at 25°C

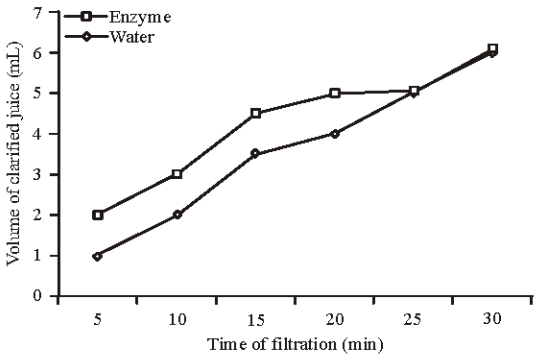

Fig. 2: Clarification of tomato juice with 5 mL of polygalacturonase and water at 25°C

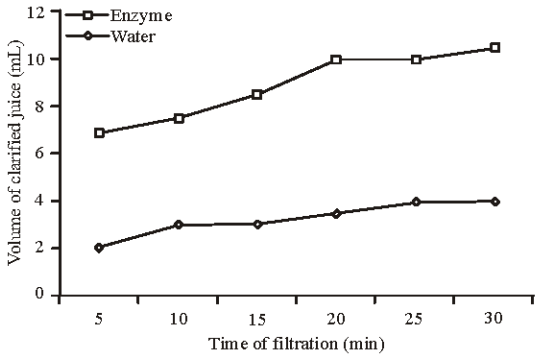

Fig. 3: Clarification of tomato juice with 10 mL of polygalacturonase and water at 25°C

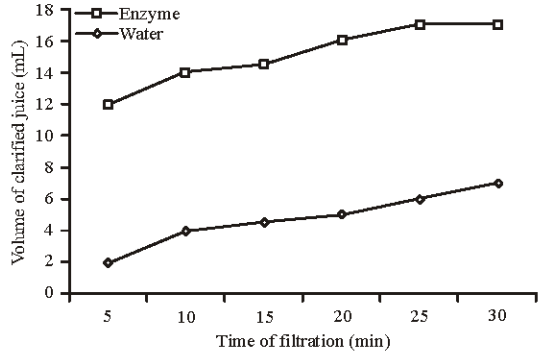

Fig. 4: Clarification of tomato juice with 15 mL of polygalacturonase and water at 25°C

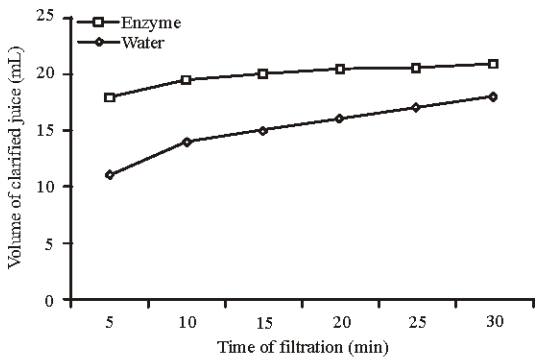

Fig. 5: Clarification of tomato juice with 20 mL of polygalacturonase and water at 25°C

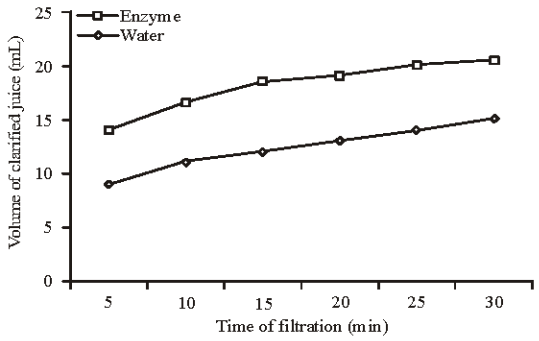

Fig. 6: Clarification of tomato juice with 20 mL of polygalacturonase and water at 25°C for 5 min

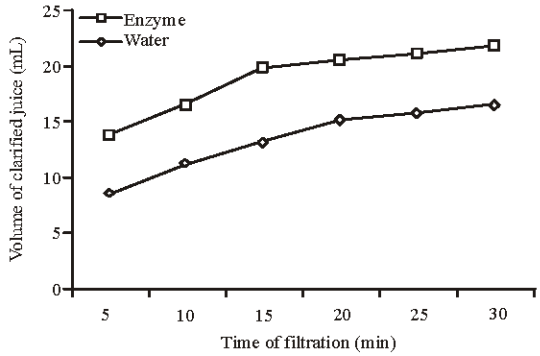

Fig. 7: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 25°C for 10 min

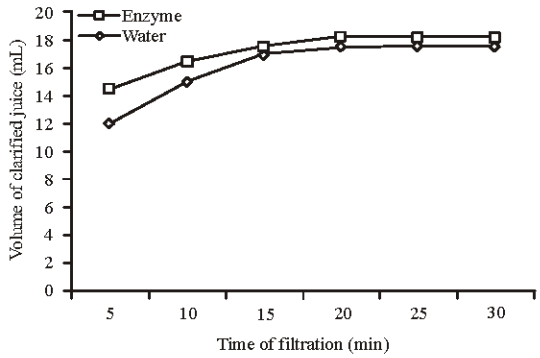

Fig. 8: Clarification of tomato juice with 20 mL of polygalacturonase and water at 25°C for 15 min

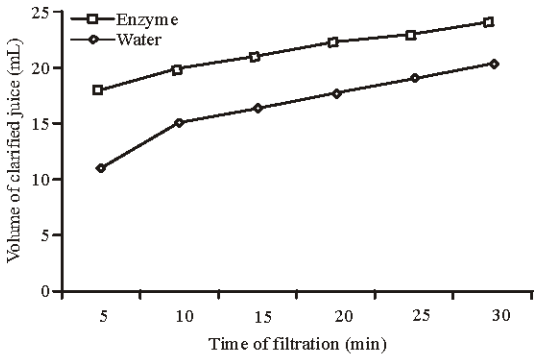

Fig. 9: Clarification of tomato juice with 20 mL of polygalacturonase and water at 25°C for 20 min

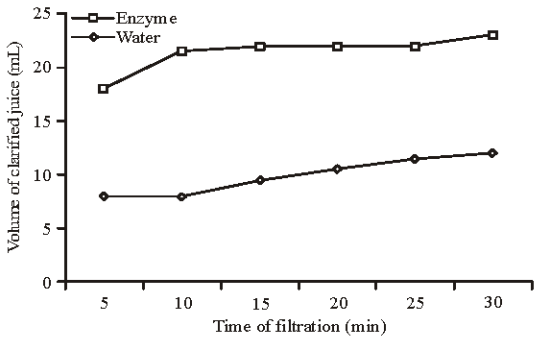

Fig. 10: Clarification of tomato juice with 20 mL of polygalacturonase and water at 20°C

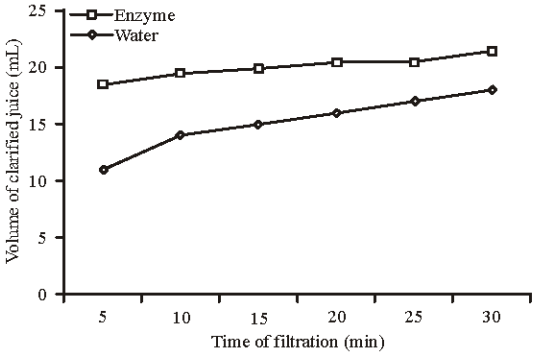

Fig. 11: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 25°C

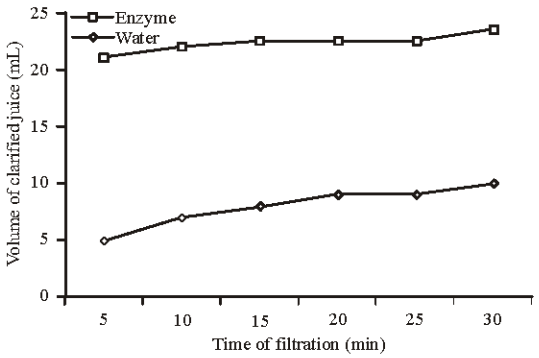

Fig. 12: Clarification of tomato juice with 20 mL of polygalacturonase and water at 30°C

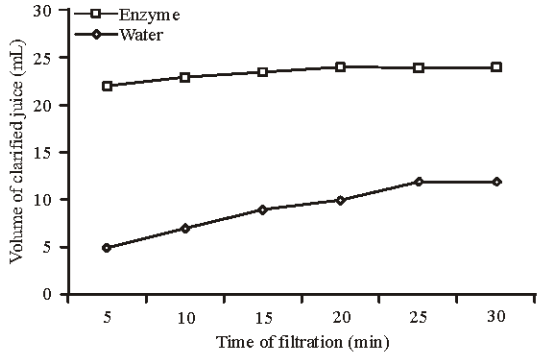

Fig. 13: Clarification of tomato juice with 20 mL of polygalacturonase and water at 35°C

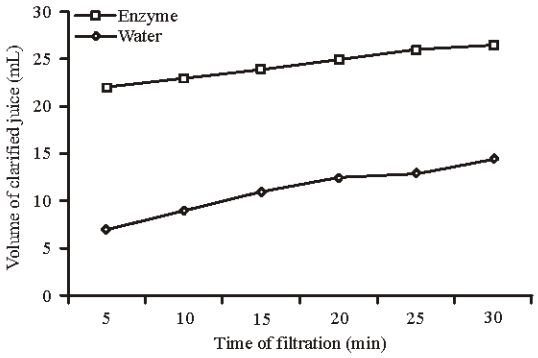

Fig. 14: Clarification of tomato juice with 20 mL of polygalacturonase and water at 40°C

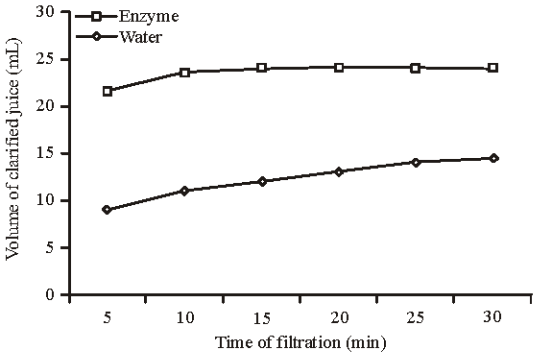

Fig. 15: Clarification of tomato juice with 20 mL of polygalacturonase and water at 45°C

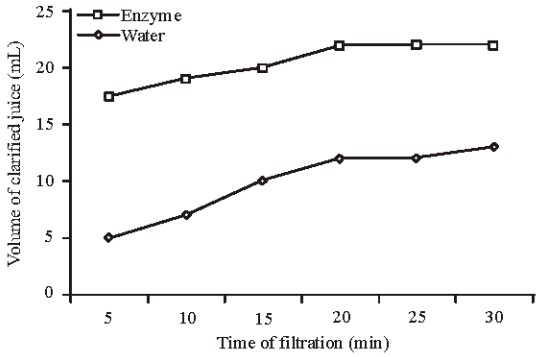

Fig. 16: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 40°C for 5 min

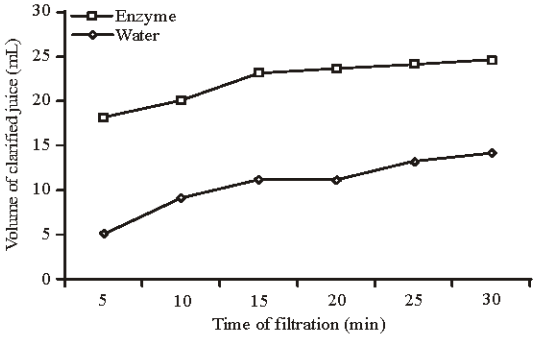

Fig. 17: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 40°C for 10 min

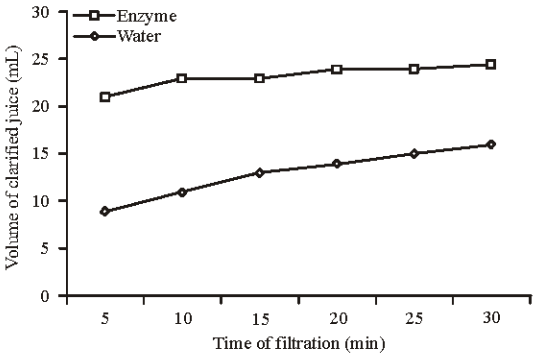

Fig. 18: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 40°C for 15 min

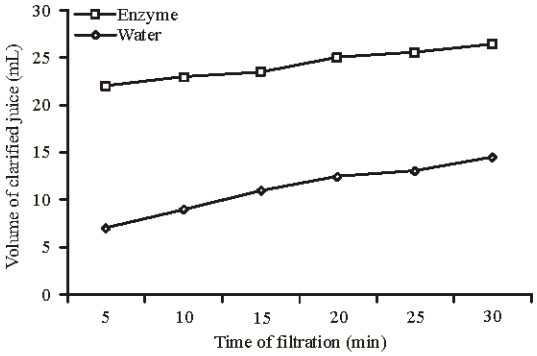

Fig. 19: Clarification of tomato fruits with 20 mL of polygalacturonase and water at 40°C after 20 min

DISCUSSION

The result of this present investigation reviewed that the pectinase used for clarifying tomato juice reduces the cloudiness of the juice. This cloudiness could be attributed to the presence of suspended pieces of cell which was clarified using the pectinase as physically shown in the content of the cylinder labeled "pectinase" that was obviously clearer than the content of the cylinder labeled "water" at all temperatures. The result also revealed the viscosity of the juice obtained from all enzymes treated tomato fruit was lowered as evidence from the rapid juice yield compared to a much slower juices. It decreases filtration time up to 50% (Blanco *et al*., 1999). Clarity of juice treated with enzymes improved because of removal of colloidal and suspended particles in the juice for fruits for plum, peach, apricot and apple (Joshi *et al*., 1991; Joshi and Bhutani, 1991). Similar result was obtained from previous studies employing 3 different varieties of apple fruit juice (Ajayi *et al*., 2014).

The juice obtained in the cylinder labeled "pectinase" was more voluminous than that in the cylinder labeled "water" at all temperatures. Treatment of fruit pulps with pectinase also revealed an increase in fruit juice volume from banana, grapes and apples. The volume of juice obtained from crushed tomato pulp incubated at 25°C was more compared to other incubation temperatures. This suggest that 25°C is optimum for the incubation of tomato fruits investigated under laboratory

conditions as exceeding this temperature reduced enzyme reaction rates as exhibited in terms of reduced juice yield as the incubation temperature increased. After incubation at 45°C, the volume of juice obtained was at its lowest and was almost similar to the volume of juice obtained when the same amount of crushed tomato pulp was clarified with water. This suggests that incubation at 45°C, most of the pectinase enzymes were beginning to lose their catalytic effect. At incubation temperature of 50°C and above, complete or near complete denaturation is expected. A reduction of temperature stability for fungal enzymes after 50°C had been reported (Lea, 1995). Optimum temperature for various pectinases varied between 20 and 50°C (Kollar, 1998; Mishra and Das, 2001; Huang and Mahoney, 1999).

Treatment with enzyme preparation at all volumes resulted in an increase in juice recovery when compared to the volume obtained by equal volumes of water with both treatment taking place at the already determined optimum temperature of 25°C for enzymatic reaction. The volume of extracted juice increased gradually as the volume of enzyme preparation was applied after which a further increase in volume of enzyme resulted in the fall of extracted juice. The increase in juice yield had been attributed to the hydrolysis of pectin which releases the sap inside the cells of the pulp (Broeck *et al*., 1999; Wang and Thomas, 1989). This can be explained by an increase in enzyme concentration which increases the amount of available active sites into which the substrate pectin binds to and are broken down into simpler forms which ultimately results in an increase in juice yield. However, a point is reached when at extreme enzyme concentration, all the substrate is rapidly dissociated as there are increasingly more available enzyme active sites than the available substrate pectin eventually leading to a gradual fall in juice yield.

The duration of incubation of crushed tomato pulp with enzyme preparation and water at an optimum temperature of 25°C enzymatic action and a fixed volume of 25 mL was also considered. The juice yield obtained from enzymatic treatment increased as the duration of incubation increased and the volume of juice obtained was more than the volume of juice obtained from the water treated tomato pulp at all tried duration of incubation. The highest volume of juice was obtained after incubation duration of 30 min. During incubation, enzymes reach their activation state, bind to its specific substrate through its active sites and carry out its catalytic effect. Therefore the longer the duration, the higher the number of enzymes that will get to their activated state and carry out catalysis which in this case results in more substrate (pectin) being transformed and subsequently more juice yield. During the breakdown of 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 (Jyothi *et al*., 2005). The juice produced from enzymatic treatment of the tomato fruits was not tested to determine whether it was sweeter compared to the juice obtained from water treated tomato fruits. This was due to safety reasons as the enzymes were not treated aseptically during the research.

CONCLUSION

It is evident from this study that tomato fruits can serve as substrate for the extraction of pectinase enzymes. Pectinases could be employed for the extraction of juices as well as for clarification. Some reports are available on the application of pectinases in other industries. 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.

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