Short Communication

Studies on the pH and protein content of tomato (Lycopersicon esculentum Mill) fruits deteriorated by Aspergillus niger

Ajayi, A. A.* and Olasehinde, I. G.

Department of Biological Sciences, College of Science and Technology, Covenant University, Idiroko road, Ota, Ogun State, Nigeria.

Accepted 28 January, 2009

Tomato (*Lycopersicon esculentum* Mill) fruits obtained from the local market in Sango Ota, Ogun State, Nigeria were inoculated with *Aspergillus niger* from cultures grown in the laboratory and incubated under appropriate conditions of temperature and moisture to initiate infection for seven days. Extensive lesions and subsequent collapse of the tomato fruits inoculated with *A. niger* occurred within a week of incubation. The uninoculated fruits retained the bright red color and remained firm throughout the seven days. The pH and the probable implication of the results obtained from this research work can be very useful in utilizing tomato fruits in tomato processing companies in Nigeria.

Key words: Tomato fruits, pH, protein content, *Aspergillus niger*.

INTRODUCTION

Aspergillus niger is a saprophytic fungus which is associated with rot diseases of various crops, plant, vegetables and fruit, thus having a wide range of economically important crops as host (Schmidt et al., 1995). It has been identified as one of the storage moulds of wheat, rye, barley, peas, buck wheat and mustard seeds (Fiedurek et al., 1995). Tomato (Lycopersicon esculenttum Mill) fruit, often described as a vegetable fruit is a true berry, a type of fleshy fruit characterized by its soft pulp, thin skin and many seeds (Anyanwu et al., 2003). On a worldwide scale, tomato continues to increase in importance for consumption as a fresh crop, as a major constituent in many prepared foods and also as materials for research into the fundamental principles of growth and development in plantation (McGlasson, 1993; Seymour et al., 1993). Economically, tomato tops the list in value among edible vegetables (Anyanwu et al., 2003) and it is found in most cities of the world (F.A.O., 2002). Tomato fruits contain various minerals and vitamins (Decuypere, 2006). Its stability varies

markedly as a function of environmental condition such as pH and the concentration of trace metal ions and oxygen (Robert et al., 2003). Despite all the benefits that can be derived from tomatoes, a large percentage of tomatoes produced in Nigeria are being lost to post harvest deterioration caused by micro-organisms. *A. niger* has been reported as one of the major fungi causing deterioration of tomato fruits in storage (Olutiola, 1982).

This paper therefore examined the effect of the deterioration of tomato fruits by *A. niger* on the pH and protein content of the tomato fruits over a period of time.

MATERIALS AND METHODS

Organism and culture condition

The isolate of A. *niger* used for this research work was obtained from the culture collection of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ogun State, Nigeria. The organism was grown and maintained on 1% (w/v) potato dextrose agar at room temperature (25 °C). The fungus was later subcultured onto fresh Potato dextrose agar plates to maintain the viability of the cultures.

Seventy-two hour old actively growing culture of the organism were inoculated into healthy-looking tomato (*L. esculentum* Mill)

^{*}Corresponding author. E-mail: quietasever@yahoo.com. Tel: +234803 0460 901.

Table 1. pH of the infected and uninfected tomato (Lycopersicon esculentum Mill) fruits.

| Days of Incubation | pH of infected fruits | pH of uninfected fruits |
|-----------------------|-----------------------|-------------------------|
| 1 | 4.27 | 4.75 |
| 2 | 4.32 | 4.45 |
| 3 | 4.15 | 4.41 |
| 4 | 4.30 | 4.40 |
| 5 | 4.32 | 3.92 |
| 6 | 4.32 | 3.90 |
| 7 | 4.35 | 3.90 |

Table 2. Protein content of infected and uninfected tomato (*Lycopersicon esculentum* Mill) fruits.

| Days of Incubation | Protein content of infected fruits (mg/ml) | Protein content of uninfected fruits(mg/ml) |
|--------------------|--|---|
| 1 | 8.00 | 8.25 |
| 2 | 6.92 | 7.00 |
| 3 | 6.92 | 6.92 |
| 4 | 6.75 | 6.75 |
| 5 | 6.60 | 6.60 |
| 6 | 6.82 | 6.80 |
| 7 | 6.83 | 6.82 |

fruits and employed for this research work.

Inoculation of tomato fruits

The tomato fruit used for this research work is the Roma VF type which is high yielding. The fruits are oval in shape and they have smooth and unridged surface. It is a common type of cultivar in the Northern region of Nigeria where large acreages of tomato are grown under irrigation. It is not known to be susceptible to cracking. Fresh type of the tomato fruits were obtained from the Sango Ota main market. The tomato fruits were surface sterilized by soaking them in 5% sodium hypochlorite solution for 30 min. The tomato fruits were then rinsed thoroughly using several changes of distilled water to remove the residual effect of sodium hypochlorite solution. A sterile cork borer (4 mm) was used to remove a tissue disc from each tomato fruit prior to inoculation with A. niger. Disc (4 mm) obtained from the edge of a three day old plate culture was used to inoculate each tomato fruit. The point of inoculation was sealed with molten wax. The control fruits were treated similarly except that sterile potato dextrose agar disc served as the inoculum. The experimental and control fruits were then placed inside sterile Petri dishes under separate surface sterilized bell jars. The rims of the bell jars were sealed with Vaseline. Both the experimental and control tomato fruits were incubated at room temperature for seven days.

Determination of pH

Each day, tomato fruits of approximately the same weight were picked and crushed gently using the laboratory mortar and pestle.

The pH was taken using the Jenway 3505 pH meter. This was done for seven days.

Estimation of protein content

This was carried out using the Lowry's technique (Lowry et al., 1951). The optical density of each of the sample was taken at 600 nm with the Jenway 6051 Colorimeter. Serial dilutions of egg albumin powder (Sigma) were treated in the same manner and used to plot a standard graph. The unknown value of protein in each test sample was extrapolated from the standard graph.

RESULTS

The freshly ripe tomato fruits inoculated with *A. niger* were extensively degraded by the seventh day of incubition. The extracts from the infected tomato fruits and the uninfected tomato fruits gave the following pH results (Table 1). The pH of the inoculated tomato fruits became more acidic with days of incubation while the acidity of the infected fruits slightly reduced with days of incubition (Table 1). The protein content of the infected and uninfected tomato fruits decreased with days of incubition but the decrease was more profound on the first day of incubation and it decreased for some time till the sixth day when there was slight increase (Table 2).

DISCUSSION

The results of this present investigation revealed that the pH of the tomato fruits were acidic. The inoculated fruits became more acidic than the uninoculated fruits with days of incubation. This difference in results of the inoculated and uninoculated fruits showed that the differrence in pH value is of fungal origin. Shewfelt (1986) reported that anthocyanins' destruction is pH dependent and it is greater at higher pH values. They also form complexes with metals such as Al, Fe, Cu and Sn. These complexes generally results in a change in the color of the pigment. The bright red color of the tomatoes actually changed with days of incubation while the uninfected fruits retained the initial color and was still firm throughout the period of incubation. The effect of pH on the activity of the enzyme may be explained in terms of its protein constituents since enzymes are proteins (Prescott et al., 2003). pH changes will affect the ionic characteristics of the amino and carboxylic acid groups on the protein as well as the catalytic site and conformation of the enzyme (Robert et al., 2003).

A. niger caused deterioration of the apparently freshly ripe tomato fruits within eight days of incubation. It has been reported from previous researches that during this period of deterioration, the organism causing the deterioration secretes proteins which exhibited polygalacturonase and cellulase activities (Ajayi et al., 2003; Jan and Chen, 2003; Kalogeris et al., 2003). Other workers have implicated cellulases and polygalacturonases in the

infection of plant tissues (Famurewa and Olutiola, 1991; Fiedurek et al., 1995; Lang et al., 1997; Kollar, 1998; Sakar and Upadhyay, 1994; Bakai-Golan and Karadavid, 1991). A major problem in tomato fruit marketing is premature ripening and softening during transport (Adegoke and Moyosade, 1987). These changes in the tomato fruits are part of the natural aging process of the fruits (Glick and Pasternak, 1998). The results of this present study also revealed that the protein content increased and this is probably due to the ripening process. It has been postulated that by interfering with the expression of one or more of the genes responsible for ripening which are due to the enzymatic activities of polygalacturonase and cellulase, the ripening process may be delayed. This can be done by creating transgenic plants with antisense RNA-producing versions of these genes introduced into tomato plants (Glick and Pasternak, 1998). This genetically engineered tomato will lower polygalacturonase production thereby inhibitting fruit ripening in tomatoes and this would permit tomatoes to ripen on the vine instead of being harvested while they are still green.

REFERENCES

- Adegoke GO, Moyosade JO (1987). Spoilage microflora of tomatoes and onions in a tropical marketing system. Nig. Food J. 5: 80-84.
- Ajayi AA, Olutiola PO, Fakunle JB (2003). Studies on Polygalacturonase associated with the deterioration of tomato (Lycopersicon esculentum Mill) Fruits infected by Botryodiplodia theobromae PAT. Science Focus, 5: 68-77
- Anyanwu CO, Komolafe BT (2003). Agricultural Science for Schools and Colleges. Longman Publishers, Ibadan, Nigeria.
- Bakai-Golan R, Karadavid R (1991). Cellulolytic activity of *Penicillum digitatum* and *Penicillum italicum* related to fungal growth and pathogenesis in citrus fruit. J. Phytopathol. 131: 65-72.
- Decuypere JD (2006). Food and industrial value of tomato. United State Department of Agriculture (USDA) food and Nutrition centre. Annual Publication, pp. 100-106.
- Famurewa O, Olutiola PO (1991). Comparison of growth and cellulolytic enzyme production in *Aspergillus chevailieri* and *Penic illum steckii* from mouldy cocoa beans. Folia Microbiol. 36: 347-352.
- FAO (2002). Production of Nigeria, Principal Crops, pp. 26-28.
- Fiedurek J, Szczodrak J, Roalski J (1995). Seeds as natural matrices for immobilization of Aspergillus niger mycelium producing pectinase. J.Appl.Bacteriol. 78: 409–412.
- Glick BR, Pasternak JJ (1998). Principles and Applications of Recombinant DNA. ASM, Washington D.C. p. 683.
- Jan H-D, Chen K-S (2003). Production and characterization of thermostable cellulases from Streptomyces transformant T3-1. World J. Microbiol. Biotec. 19: 263-268.

- Kalogeris E, Christakopeulos P, Katapodis P, Alexiou A, Vlachou S, Kekos D, Macris BJ (2003). Production and characterization of cellulolytic enzymes from the thermophilic fungus Thermoascus auranticus under solid state cultivation of agricultural wastes. Process Biochem. 38: 1099-1104.
- Kollar A (1998). Characterization of an endo-polygalacturonase produced by the apple slab fungus, *Venturia inaequalis*. Mycological Res. 102: 313- 319.
- Lang C, Cornelia G, Milan P, Ulf S (1997). Optimization of fungal polygalacturonase synthesis by *Saccharomyces cerevisiae* in fedbatch culture. Chem. Engine. J. 65: 219-266.
- Lowry OH, Rosebrough NJ, Parr AL, Randal RJ (1951). Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 219: 13-25
- McGlasson B (1993). Tomatoes in Encyclopedia of Food Science Technology and Nutrition. Academic Press. 7: 4579-4589.
- Olutiola PO (1982). Extracellular polygalacturonase enzyme complex from *Penicillium citrinum*. Thom. Associated with internal mouldiness of cocoa (Theobroma cacao) beans Acta phytopathological Academiae Scientiarum Hungaricae 17: 239-247.
- Prescott LM, Harly JP, Klein DA (2003). McGraw Hill Higher Education, New York, pp. 221-248.
- Robert KM, Daryl KG, Peter AM, Victor WR (2003). Harper's illustrated Biochemistry twenty sixth edition. 2003 by the McGraw-Hill companies Inc. Printed in London. pp. 12-21.
- Sakar A, Upadhyay SN (1994). Purification and properties of cellulase from *Micrococcus roseus*. World J. Microbiol. Biotechnol. 10: 1081-1086
- Schmidt O, Angermann, Frommhold TI, Hope K (1995). Experimental and theoretical investigations of submerged fermentation and synthesis of pectinolytic enzymes by *Aspergillus niger*. Appl. Microbiol. Biotechnol. 43: 420-430.
- Seymour GB, Taylor TE, Tucker GA (1993). Biochemistry of fruit ripening 1: 405-440.
- Shewfelt RL (1986). Postharvest treatment for extending the shelf-life of fruits and vegetables. Food Technol. 40 (5): 70-89.