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SCREENING AND PARTIAL PURIFICATION OF AMYLASE FROM *ASPERGILLUS NIGER* ISOLATED FROM DETERIORATED TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) FRUITS

Obafemi, Y. D., Ajayi*, A. A., Olasehinde, G. I., Atolagbe O. M., Onibokun, E. A.

Department of Biological Sciences, Covenant University, PMB 1023, Ota, Ogun State, Nigeria

***Corresponding Author: adesola.ajayi@covenantuniversity.edu.ng, +234 803-046-0901**

ABSTRACT

Amylases (EC 3.2.1.1) are cellwall degrading enzymes associated with the pathogenicity of microorganisms in the spoilage of tomato fruits. The use of amylase in many industries has made it very important to optimize production process to achieve maximum yields. Screening and partial purification of Amylase from *Aspergillus niger* isolated from tomato (*Lycopersicon esculentum* Mill.) fruits was studied. *Amylase producing fungi were isolated from fresh tomatoes kept at ambient temperature* (28±1˚C). *Isolates were characterized on the basis of their morphological and cultural techniques. Partial purification of amylase was carried out by ammonium sulphate precipitation. The enzyme activity was determined and optimum conditions were obtained. The molecular weights of the crude and partially purified Amylase were determined by SDS PAGE method. A total of five isolates were obtained using basic screening technique for amylase activity, one of the isolates (Isolate code F2) exhibited maximum amylase activity.* The fungi isolate code F2 was identified as *Aspergillus niger*. Optimum conditions for Amylase AMY F2 were ascertained at pH 6.0; temperature 30°C; substrate concentration of 0.3mg/ml, and time of heating of less than 10min. *The molecular weights of the crude and partially purified Amylase AMY F2 were found to be 55kDa and 35kDa respectively by SDS PAGE method. Microorganisms had been an encouraging means of economical production of enzymes in large scale for the food* and drug industry.

Keywords: Amylase, Partial Purification, Enzyme, Tomato Fruits

RENDEMENT ET PURIFICATION PARTIELLE DE L'AMYLASE DE ASPERGILLUS NIGER ISOLÉ À PARTIR DE TOMATE DÉTERIORÉ (LYCOPERSICON ESCULENTUM MILL.) FRUITS

*Obafemi, Y. D., Ajayi *, A. A., Olasehinde, G. I., Atolagbe O. M., Onibokun, A.E.*

Département des sciences biologiques, Université de l'Alliance, PMB 1023, Ota, Ogun State, Nigéria

** Correspondant Auteur: adesola.ajayi@covenantuniversity.edu.ng, +234 803-046-0901*

ABSTRAIT

Les amylases (EC 3.2.1.1) sont des enzymes dégradant les parois cellulaires associées à la pathogénicité des microorganismes dans la détérioration des fruits à la tomate. L'utilisation de l'amylase dans de nombreuses industries a rendu très important d'optimiser le processus de production pour obtenir des rendements maximaux. Le dépistage et la purification partielle de l'amylase d'Aspergillus niger isolés à partir des fruits à la tomate (Lycopersicon esculentum Mill.) Ont été étudiés. Les champignons producteurs d'amylase ont été isolés à partir de tomates fraîches conservées à température ambiante (28 ± 1 ° C). Les isolats ont été caractérisés en fonction de leurs techniques morphologiques et culturelles. La purification partielle de l'amylase a été réalisée par précipitation au sulfate d'ammonium. L'activité enzymatique a été déterminée et des conditions optimales ont été obtenues. Les poids moléculaires de l'Amylase brut et partiellement purifiée ont été déterminés par un procédé SDS PAGE. Au total, cinq isolats ont été obtenus en utilisant une technique de dépistage basique pour l'activité amylase, l'un des isolats (code isolé F2) présentait une activité amylase maximale. Le code isolant F2 des Fusions a été identifié comme Aspergillus niger. Les conditions optimales pour Amylase AMY F2 ont été déterminées à pH 6,0; température 30 ° C; concentration de substrat de 0,3 mg / ml et temps de chauffage de moins de 10 min. On a trouvé que les poids moléculaires de l'amylase brute et partiellement purifiée étaient respectivement de 55 kDa et 35 kDa par le procédé SDS PAGE. Les microorganismes ont été un moyen encourageant de production économique d'enzymes à grande échelle pour l'industrie alimentaire et pharmaceutique.

Mots-clés: Amylase, Purification partielle, Enzyme, Fruits tomates

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) has fleshy endocarp which belongs to the berry class (1). They are rich in nutrients such as vitamins, minerals, dietary fiber and protein (2). Tomato fruits have 95% water and 4.5% carbohydrate (3). In view of these, the tomato fruit is often attacked by microorganisms most especially during and after harvest, which makes tomato spoilage to occur often (4). Microorganisms which are related with the deterioration of tomato fruits can attack them after harvesting; or during the process of storage and distribution (5). The spoilage usually occurs on the fruits after a period of time (6). The plant cell wall which is a protective layer that coats the outer surface usually plays many roles, including controlling movement of water into the fruit (7). It also filters potentially damaging UV light, and limits attack by pathogens (5). In Nigeria, tomatoes are kept at the open markets; the fruits are often displayed in baskets and on benches for the prospective customers, thereby exposing them to opportunistic microbial infections especially mycotoxins (8) . Post-harvest Infections in tomato fruits could occur during storage, transportation, packaging and distribution (loading and offloading) at various point of sale at which bacteria and fungi are present (9). Adequate knowledge and careful handling procedure of the tomatoes can reduce wastage of the fruits (10). Previous study on deterioration of tomatoes by microorganism showed that post-harvest damages are mostly due to attack by fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* of up to 90% prevalence (7,11). Thus, there is need for constant research to isolate the fungi associated with tomato fruits deterioration with the view to providing suitable solutions of preserving the tomato fruits, keeping them fresh up to when they reach the consumers (12). This helps to ensure all year round availability and to protect the public health (13).Contamination of fresh tomatoes by microorganisms occurs naturally because the fruits are exposed to the environments during harvest, transportation and storage (7). Hence, the tomato normal flora and pathogen contamination often occurs at different points (14). Tomato micro flora which are mostly fungi found on fresh tomato fruits are the yeasts and molds (8). Microorganisms are present everywhere and around us (15). Pathogens such *Escherichia coli* and *Listeria monocytogenes* may be found on the cell wall of the fresh tomato fruits and this are of public health impact (7). *Enterobacteriaceae* and Gram negative bacteria of the genus *Pseudomonas* may also be found the surface of tomato fruits. Yeast of the genus *Saccharomyces* populations of 10^5-10^6 cfu/ml had been reported (8, 16). Bacterial populations ranging from 10^4 - 10^9 cfu/ml and mold from the genus *Aspergillus* and *Rhizopus* with population ranging from 10^4 - 10^9 spores/ml in fresh tomatoes had also been reported (5). Growth of pathogenic microorganisms on the intact surface of fresh tomato is not common as the microbes cannot produce cell wall degrading enzymes until they gain entrance into the tomatoes where they can get nutrients and water (17). Damage to the cell wall of the tomato fruits therefore helps the food borne pathogens to grow and multiply, most especially under room temperatures (7). Refrigerator temperature arrests the growth of microorganisms while those that could survive extremely low temperatures still grow when the cell walls are broken due to the fact that released fluids serve as nutrients for the microorganisms (8,18).

Fungi such as *Aspergillus niger* can produce different cell wall degrading enzymes that breaks down large polysaccharides into simple reducing sugars which are used up for growth and multiplication (19). These enzymes have a lot of industrial and environmental importance. For example, they are used in brewery, food processing and bioremediation of organic pollutants (19,20). **Amylases (EC 3.2.1.1) are extracellular enzymes which catalyzes starch hydrolysis (21). The enzyme catalyzes the breakdown of α-1,4-O-glycosidic bonds in polysaccharides by leaving α-anomeric configuration in the resulting compounds (22). The amylase family exists in two forms as either starch modifying enzymes or starch hydrolyzing enzymes (7).** Amylases can be recovered from different sources of plants, animals and microorganisms origin (23). **Some amylases require calcium ions (Ca^{2+}) for their optimum activity and structural stability (21). Amylases have a wide range of industrial application which ranges from production of dextrins, sugar syrup and sweeteners (23).** They are usually imported because of their application in the breweries, bakery and factories making soaps and detergents (24). **Amylase makes up to 40% of enzyme production in the world (23). However, prolonged storage of amylases often results in reduced activity (21).**

MATERIALS AND METHODS

One hundred and eighty (180) fresh tomato fruits were purchased from the Ota market, Ota, Ogun State, Nigeria. They were sorted, washed and transported to the microbiology laboratory of the Department of Biological Sciences, Covenant University, Ota, Ogun State in sterile polythene bags. Sixty (60) samples designated as P were stored in a locally made post-harvest storage system, the second set of sixty (60) fruits designated R were stored in the refrigerator and

the last set of sixty (60) fruits designated C were stored at ambient temperature in the laboratory. All samples were analyzed and sampling was done for a period of fourteen days.

Isolation of microorganisms from the tomato fruit samples

The total spore count was determined by pour plate method, each tomato fruit samples was diluted using sterile distilled water. One (1) milliliter of the dilution of 10^4 was plated onto Sabroaud Dextrose Agar (SDA) and incubated at room temperature for 3-5 days for the fungal population.**. Fungal samples were collected from the center of the growth covering the plates with the aid of sterile inoculating needle. The samples were smeared on glass slides and stained with lactophenol cotton blue (9).**

Identification of the fungal isolates

The fungi isolated in this research were identified using identification methods contained in the illustrated Handbook of Fungi (25)**.** The colony color, pattern of growth, and sporulation style were observed (6). Microscopic observation was carried out on the mature sporulating growths five days after inoculation on SDA plates. Morphological characteristics like arrangement and shape of spores, type of sporangia, and type of hyphae, presence or absence of septa on hyphae were examined under the high power objective of a compound binocular microscope (26). Microscopic examination was carried out after Gram staining the Yeast isolates, while Lactophenol blue staining was carried out on the remaining fungal isolates which are molds (27).

Preparation of fungal spore suspension for production of enzymes

A spore suspension containing 15ml of sterile distilled water and three drops of tween 80 solution was used to obtain the spore from a 120 h-old culture of each fungal isolate. The suspension was centrifuged at 4000rpm for 6 minutes. The supernatant was discarded and replaced with 10ml of sterile distilled water. The spore suspension was diluted serially from 10^{-1} to 10^{-7}. The fungal spores were counted using the Neubauer Counting Chamber according to the method of (28).

Growth of Isolated Fungi in Basal Salt Medium Containing Starch

The Basal Salt Medium, for the growth of the isolated fungi and the enzyme production, was prepared according to the method of (29) containing (in one liter): KH_2PO_4 - 2.0g, $(NH_4)_2SO_4$ - 1.4g, $MgSO_4$.$7H_2O$ - 0.3g, $CaCl_2$ - 0.3g, Urea - 0.3g, Tween 80 - 1ml, Yeast extract - 0.4g, $FeSO_4$.$7H_2O$ - 5mg, $MnSO_4$ - 1.6mg, $ZnSO_4$ - 1.4mg, $CoCl_2$ - 2.0mg. The medium was supplemented with 1% (w/v) soluble starch. One hundred milliliters of growth medium was inoculated with 1 ml of an aqueous spore suspension containing approximately 5 x 10^6 spores/ml, 8 x 10^6 spores/ml and 10 x 10^6 spores/ml of isolated fungi F1, F2 and F3 respectively. Experimental flasks contained the inoculated sterilized medium while control flasks contained only the sterilized medium. Both experimental and control flasks were incubated without shaking according to the method of (30) for eight days at room temperature (25°C).

Crude Enzyme Extraction by Filtration

To separate the liquid enzyme mixture and the mycelia mat along with the spore bodies' filtration technique was employed. The mixture was filtered through Whatman No. 1 filter paper and then centrifuged at 5,000 rpm for 30 minutes according to the method of (31). This served as the crude enzyme.

Amylase Assay

Amylase activity was determined using the method described by (21) whereby the reaction mixture consisted of 2 ml of 0.2% (w/v) starch in 0.2M citrate phosphate buffer at pH 6.0 as substrate and 0.5 ml of enzyme. The control experiments consisted of only 2 ml of the prepared substrate. The content of the experimental and control test tubes were incubated at 35°C for 20 minutes. The reaction in each test tube was terminated with 3 ml of 1N Hydrochloric acid. The enzyme (0.5 ml) was then added to the control tube. Two milliliters of the mixture from each of the set of experimental and control was transferred into new set of clean test tube. 3ml of 0.1N Hydrochloric acid was added into the content of each new test tubes after which 0.1 ml of iodine solution was added. The optical density reading was taken at 670nm. One unit of enzyme activity was defined as the amount of enzyme which produced 0.1 percent reduction in the intensity of the blue color of starch - iodine complex under conditions of the assay. Specific activity was calculated as enzyme units per milligram protein.

Ammonium sulphate precipitation

Ammonium sulphate precipitation was done according to the method described by (31). Solid Ammonium sulphate of analytical grade was added to crude enzyme preparation to 90% saturation. The solution was then kept at 4°C for 24h. After 24 h, the precipitate was removed by centrifuging at 5000rpm for 30 minutes, the supernatant was decanted and the precipitate was then dissolved again in 1ml of 0.2M citrate phosphate buffer (pH 6.0). The enzyme solution was dialyzed overnight against five changes of the similar buffer. Dialysis was done in acetylated cellophane tubing prepared from Visking dialysis

tubing (Gallenkamp) as described by (32). The protein content of the dialysate was determined by the method of (33,34). Enzyme assay of the dialysate was also determined as described above.

Effect of Temperature on Amylase AMY F2

To determine the optimum temperature for the amylase production, the partially purified enzyme were subjected to various temperatures; 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 20 min. The enzyme activity was determined as previously described by (21)

Effect of pH on Amylase AMY F2

The effect of pH on the enzymes activity was determined by varying the pH of the substrate from 4.5 to pH 8. The substrate consisted of 0.2% (w/v) of soluble starch dissolved in 0.2M citrate phosphate buffer pH 6.0 of varying pH. Incubation was at 35°C for 20 min. Amylase activity was determined as previously described by (23).

Effect of Substrate Concentrations on Amylase AMY F2

The effect of various substrate concentrations was determined using various concentrations of starch in 0.2M citrate phosphate buffer; 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. The reaction mixture contained 1ml of starch and 0.5ml of enzyme incubated at 35°C for 20 min. Amylase activity was determined as previously described by (30).

Effect of Time of Heating on Stability of Amylase AMY F2

The effect of time of heating on the stability of Amylase enzyme was determined. Samples of partially purified enzyme was heated at 80°C for different periods of time (0, 5, 10, 15, 20, 25, 30 min) respectively. The reaction mixtures consisted of 1 ml of starch and 0.5ml of the enzyme solutions. Amylase activity was determined as previously described by (21).

Molecular weight determination of Amylase AMY F2

The crude enzyme and ammonium sulphate precipitated amylase sample were loaded on SDS-PAGE and a protein profile was obtained according to the method of (35). The molecular weight of the enzyme was determined by comparing them with the molecular weight marker. The ammonium sulphate precipitated sample was run on native PAGE. The native gel placed over the Starch agar gel was then subjected to zymogram staining as described by (35).

RESULTS

Enumeration of Fungal Population

The total fungal spore count for the tomato fruits stored in the post-harvest storage system was within the range of 1.0×10^5 – 4.0×10^6spores/ml while those fruits stored in the refrigerator had fungal spore count range of 1.0×10^5 – 3.0×10^6spores/ml and fungal spore count of 2.0×10^2 – 4.0×10^{11}spores/ml for the fruits stored at ambient temperature (Table 1).

TABLE 1: ENUMERATION OF FUNGAL POPULATION

Days	Post-Harvest Storage System (P) spores/ml	Refrigerator (R) spores/ml	Ambient temperature (C) spores/ml
2	1.0×10^5	1.0×10^5	1.2×10^5
3	3.0×10^5	4.0×10^5	2.0×10^5
4	3.0×10^5	2.0×10^5	4.0×10^5
5	5.0×10^5	1.0×10^5	1.0×10^6
6	3.0×10^6	4.0×10^5	2.0×10^9
7	4.0×10^6	2.0×10^6	2.0×10^9
8	3.0×10^6	3.0×10^6	5.0×10^9
9	4.0×10^6	2.0×10^6	1.0×10^{11}
10	4.0×10^6	1.0×10^6	2.0×10^6
11	4.0×10^6	1.0×10^6	1.0×10^6
12	2.0×10^6	1.0×10^6	2.0×10^6
13	1.0×10^6	NG	4.0×10^6
14	1.0×10^6	NG	2.0×10^2

KEY: NG – No growth

Identification of Fungal Isolates

The result revealed five pure fungal isolates from tomato fruits (*Lycopersicum esculentum* Mill.). Gram reaction result revealed presence of yeast (*Saccharomyces cerevisae*) while the molds were identified to be *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Aspergillus fumigatus* **(Table 2).**

Production of Amylase

The amylase produced by fungal isolate code F2 obtained from tomato fruits stored at the ambient temperature on day 7 produced amylase with a total activity of 0.266 units/ml, protein content of 0.338 mg/ml and a specific activity 0.787 units/mg proteins and a yield of 63% after ammonium sulphate precipitation (Table 3).

TABLE 2: IDENTIFICATION OF THE FUNGAL ISOLATES

Isolate code	Colony Colour	Nature of hyphae	Asexual spore	Somatic structure	Identity of Fungi Isolates
F1	Deep green colony	Septate	Globose conidia	Filamentous	*Aspergillus flavus*
F2	Blackish colony	Septate	Globose conidia	Filamentous	*Aspergillus niger*
F3	Cotton white	Non septate	Sporangiospore	Filamentous	*Rhizopus stolonifer*
F4	Light green colony	Septate	Globose conidia	Filamentous	*Aspergilus fumigatus*
F5	Shiny milky colony (moist)	No hyphae	Budding cells	Unicellular	*Saccharomyces cerevisae*

TABLE 3: PARTIAL PURIFICATION OF AMYLASE OBTAINED FROM MICROORGANISMS ISOLATED FROM TOMATO (*Lycopersicon esculentum* MILL.) FRUITS

Enzyme code	Protein step	Total Activity (units/ml)	Protein (mg/ml)	Specific activity (units/mg)	Yield (%)	Purification fold
AMY F2	**Crude extract**	**0.27**	**0.34**	**0.79**	**100.00**	**1.00**
	Ammonium sulphate precipitation	**0.17**	**0.02**	**9.33**	**63.00**	**1.86**

Characterization of Enzymes

Effect of Temperature on Amylase AMY F2

The temperature of incubation affected the amylase activity tremendously. The activity of amylase AMY F2 increased with an increase in incubation temperature until an optimum was reached (Figure 1). Subsequent increase in temperature beyond the optimum temperature led to reduction in the enzyme activity. The optimum temperature for amylase AMY F2 was 30°C.

Effect of pH on Amylase AMY F2

The pH of the reaction mixtures had effect on the activities of the amylase produced both by the fungi isolate F2. The enzyme activities increased as the pH increases and also decreased when the optimum pH value was reached. The optimum pH for amylase AMY F2 was at pH 6.0 (Figure 2).

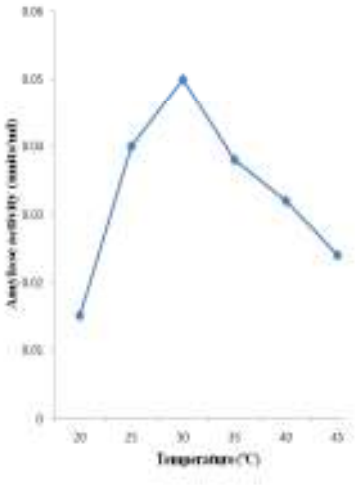

FIGURE 1: EFFECT OF TEMPERATURE ON PARTIALLY PURIFIED AMYLASE AMY F2

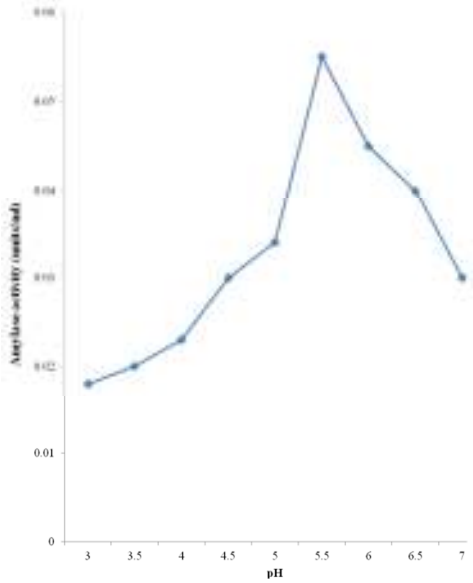

FIGURE 2: EFFECT OF PH ON PARTIALLY PURIFIED AMYLASE AMY F2

Effect of Substrate Concentration on Amylase AMY F2

The activity of the amylase produced by fungi F2 isolated from tomato fruits increases with an increase in concentration of the substrate. This continued to increase until an optimum concentration of substrate was attained (Figure 3). The optimum substrate concentration of AMY F2 was 0.30 mg/ml.

Effect of Heat on Amylase AMY F2

The activity of amylase on heating at 80°C decreased with an increase in the time of heating. When the amylase was subjected to heat for 2 minutes, activities of approximately was lost. Amylase AMY F2 was completely inactivated after 10 minutes of heating (Figure 4).

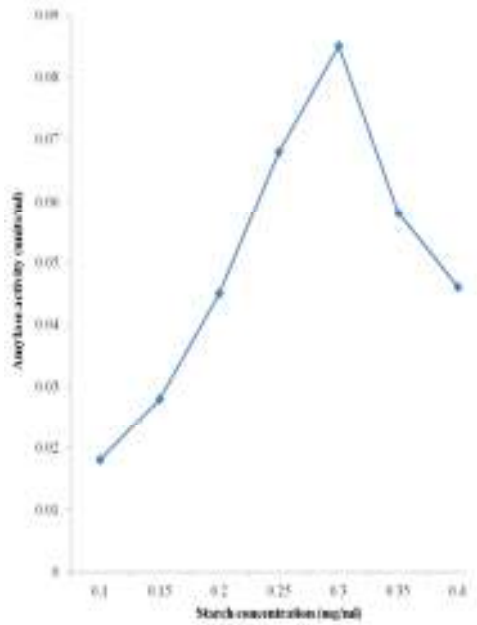

FIGURE 3: EFFECT OF SUBSTRATE CONCENTRATION ON PARTIALLY PURIFIED AMYLASE AMY F2

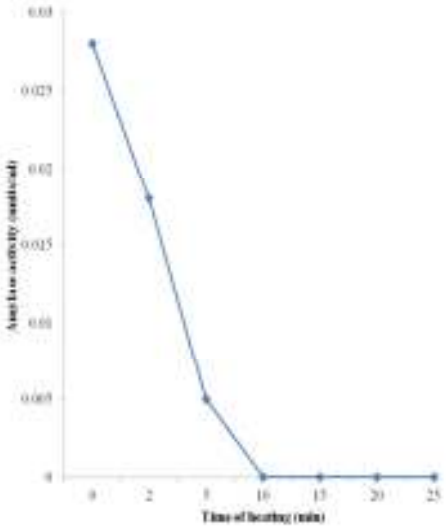

FIG. 4: EFFECT OF HEAT (80°C) ON PARTIALLY PURIFIED AMYLASE AMY F2

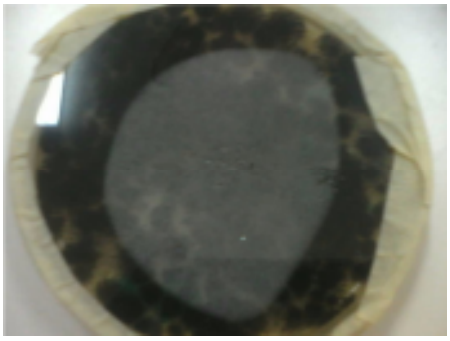

PLATE 1: ASPERGILLUS NIGER ISOLATED FROM TOMATO (LYCOPERSICON ESCULENTUM MILL.) FRUITS

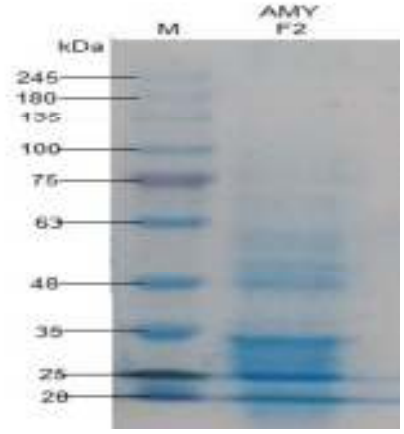

PLATE 2: SDS PAGE OF THE CRUDE AMYLASE AMY F2

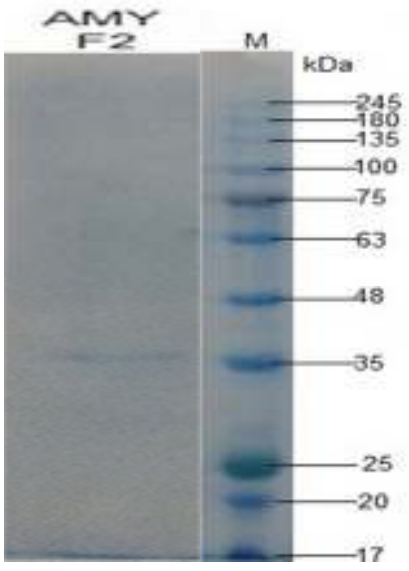

PLATE 3: SDS PAGE OF THE PARTIALLY PURIFIED AMYLASE AMY F2

Molecular Weight Determination of Amylase AMY F2

Molecular weight of the crude Amylase and partially purified Amylase AMY F2 are 55KDa and 35KDa respectively (Plate 2 and 3).

DISCUSSION

The result of this research had the total fungal population ranges between 1.0 x 10^5 and 4.0 x 10^6spores/ml for the tomato fruits stored in the post-harvest storage system, those in the Refrigerator ranges between 1.0 x 10^5 and 3.0 x 10^6spores/ml while the tomato fruits stored at ambient temperatures had fungal population range of 2.0 x 10^2 and 4.0 x 10^{11}spores/ml. This results is similar to the finding of (36) having an average fungal counts ranging between 1.3 x 10^3 and 2.0 x 10^3spores/ml and identified them to be *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporium, Saccharomyces cerevisiae, Alternaria alternate, Penicillium digitatum* and *Geotrichum candidum.* (37) also reported an average fungal count of 5.4 x 10^5spores/ml and 2.0 x 10^3spores/ml for *Penicillium notatum* and *Aspergillus flavus* at ambient temperature for tomato samples from new Benin Market in Edo State, Nigeria.

The fungi isolated from the tomato fruits were members of the yeast family *Saccharomyces cerevisae* and the mold family *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer.* (21) reported *Aspergillus* sp. and *Saccharomyces cerevisae* as the fungi associated with spoilage of tomato fruits. (38) also reported two fungi, *Aspergillus flavus* and *Rhizopus stolonifer* after five days of storage of fresh tomatoes at ambient temperatures.

The result of this research revealed the ability of some fungal isolates to produce Amylase. (31) and (39) reported production of amylase from fresh tomato fruits. The production from fungi and yeasts has also been reported by (21). The production of amylase from microorganisms isolated from other sources were also reported from other fruits and vegetables (40), bread (28), Irish potato (30) and even soil (41).

Members of the genera *Aspergillus*, *Rhizopus* and *Saccharomyces* were reported by (7,42,43) for their potentials to secrete a number of cell wall degrading enzymes.

The optimum temperature of amylase produced in this study was 30°C. This is in support of the reports of (23,39). (28) had earlier reported that the optimum temperature for amylase production by *Penicillium citrinum* ranges from 30°C - 35°C depending on source. However, this report contrasts with earlier

reports by (23) and (44) which reported that amylase production by was optimum at 37°C. Optimum pH was identified as 6.0 for Amylase AMY F2. This result confirmed that of previous researchers that who reported that amylase was most active between pH 5.0 and 6.0 (43). The optimum substrate concentration for the starch was 0.25mg/ml. (23) reported optimum substrate concentration of 0.20mg/ml for amylase. In this research, the effect of time of heating was investigated at 80°C over a period of 30min revealed loss of enzyme activity within 10min of heating. This confirmed earlier reports by (23,28,45). (30) revealed that amylase was active for over 5min of heating at 80°C. There was continuous reduction in the enzyme activity as heat was applied. After 10min of heating, enzyme activity was completely lost in all enzymes as was also revealed by (21) .

This research work recommends that tomato fruits could be potential source of amylase enzyme needed for various industrial processes. The Potential use of amylase has made it very important to optimize production process to achieve maximum yields.

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REFERENCES

1. Esua OJ, Chin NL, Yusof YA, Sukor R. Postharvest deterioration of tomato (*Solanum lycopersicum*) and associated effects on antioxidant bioactive contents. Acta Hortic [Internet]. 2017 Mar [cited 2017 Sep 7];(1152):327–34. Available from: http://www.actahort.org/books/1152/1152_44.htm

2. Ilupeju EAO, Akanbi WB, Olaniyi JO, Lawal BA, Ojo MA, Akintokun PO. Impact of organic and inorganic fertilizers on growth, fruit yield, nutritional and lycopene contents of three varieties of tomato (Lycopersicon esculentum (L.) Mill) in Ogbomoso, Nigeria. African J Biotechnol. 2015;14(31):2424–33.

3. Chika E, Ifeanyichukwu I, Carissa D, Thomas A, Okoro O, Joshua E, et al. Occurrence of Metallo-Beta-Lactamase-Producing Enterobacteriaceae from a Local Poultry Farm in Abakaliki, Nigeria. Int J Appl Pharm Sci Res [Internet]. 2016 Jun 12 [cited 2017 Sep 8];1(2):70–5. Available from: http://www.sierrajournals.com/direct/index.php/IJAPSR/article/view/17

4. Agbabiaka TO. Microbial Deterioration of Tomato Fruit (Lycopersicon esculentum) Sold in Three Popular Markets in Ilorin, Kwara State, Nigeria. Fountain J Nat Appl Sci [Internet]. 2015 [cited 2017 Sep 7];4(1). Available from: http://fountainjournals.com/index.php/fujnas/article/view/54

5. Udoh IP, Eleazar CI, Ogeneh BO, Ohanu ME. Studies on Fungi Responsible for the Spoilage / Deterioration of Some Edible Fruits and Vegetables. Adv Microbiol. 2015;5(April):285–90.

6. Amadi JE, Nwaokike P, Olahan GS, Garuba T. ISOLATION AND IDENTIFICATION OF FUNGI INVOLVED IN THE POST-HARVEST SPOILAGE OF GUAVA (Psidium guajava) IN AWKA METROPOLIS. 2014 [cited 2017 Sep 7];4(10). Available from: http://eaas-journal.org/survey/userfiles/files/v4i1002 Biological Engineering.pdf

7. Al-Hindi RR, Al-Najada AR, Mohamed SA. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. African J Microbiol Res. 2011;5(4):443–8.

8. Chinedu SM, Emmanuel E. Original Research Article Isolation of Microorganisms associated with Deterioration of Tomato (Lycopersicon esculentum) and Pawpaw (Carica papaya) Fruits. Int J Curr Microbiol Appl Sci. 2014;3(5):501–12.

9. Firas AA, Brent SS, Anne MA. Postharvest diseases of tomato and natural products for disease management. African J Agric Res [Internet]. 2017 Mar 2 [cited 2017 Sep 7];12(9):684–91. Available from: http://academicjournals.org/journal/AJAR/article-abstract/B78AB8662982

10. Schneider KR, De J, Li Y, Sreedharan A, Goodrich Schneider R, Danyluk MD, et al. Microbial evaluation of pre- and post-

processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control [Internet]. 2017 [cited 2017 Sep 22];73:511–7. Available from: http://www.sciencedirect.com/science/article/pii/S0956713516304881

11. Chu EH, Shin EJ, Park HJ, Jeong RD. Effect of gamma irradiation and its convergent treatment for control of postharvest Botrytis cinerea of cut roses. Radiat Phys Chem. 2015;

12. Zoellner C, Venegas F, Churey JJ, Dávila-Aviña J, Grohn YT, García S, et al. Microbial dynamics of indicator microorganisms on fresh tomatoes in the supply chain from Mexico to the USA. Int J Food Microbiol [Internet]. 2016 Dec [cited 2017 Sep 7];238:202–7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168160516304858

13. Ogundipe FO, Bamidele FA, Adebayo-Oyetoro AO, Ogundipe OO, Tajudeen OK. Incidence of Bacteria with Potential Public Health Implications in Raw Lycopersicon esculentum (Tomato) Sold in Lagos State, Nigeria. Niger Food J. 2012;30(2):106–13.

14. Schneider KR, De J, Li Y, Sreedharan A, Goodrich Schneider R, Danyluk MD, et al. Microbial evaluation of pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control [Internet]. 2017 [cited 2017 Sep 7];73:511–7. Available from: http://www.sciencedirect.com/science/article/pii/S0956713516304881

15. Valdramidis V. Predictive microbiology. In: Modeling in Food Microbiology: From Predictive Microbiology to Exposure Assessment. 2016.

16. Agriculturae S, Bello OB, Olawuyi OJ, Azeez AH, Adebisi OS, Owoade TA. Microorganisms causing post harvest tomato (solanum lycopersicum l.) fruit decay in nigeria. 2016 [cited 2017 Sep 22];13(2016):93–6. Available from: http://pscipub.com/Journals/Data/JList/Scientia Agriculturae/2016/Volume 13/Issue 2/5.pdf

17. Olasehinde GI, Okolie ZV, Oniha MI, Adekeye BT, Ajayi AA. In vitro antibacterial and antifungal activities of Chrysophyllum albidum and Diospyros monbuttensis leaves. J Pharmacogn Phyther [Internet]. 2016 Jan 31 [cited 2017 Sep 7];8(1):1–7. Available from: http://academicjournals.org/journal/JPP/article-abstract/98A32D856891

18. Larsen MH, Koch AG, Ingmer H. *Listeria monocytogenes* efficiently invades Caco-2 cells after low-temperature storage in broth and on deli meat. Foodborne Pathog Dis. 2010;

19. Ajayi, AA, Adedeji OM . Modification of Cell Wall Degrading Enzymes from Soursop (Annona muricata) Fruit Deterioration for Improved Commercial Development of Clarified Soursop Juice (A Review). Med Aromat Plants. 2014;4(1):1–5.

20. Koyani RD, Rajput KS. Journal of Environmental Science , Computer Science and Engineering & Technology Fungal Enzymes : Dynamic Strategy and Significance for Remediating Chlorinated Compounds. 2016;5(1):10–30.

21. Adejuwon AO, Adejuwon MA, Ajayi AA, Bamkefa BA, Omololu-Aso J, Alao OO, et al. Effect of some nitrogen sources of growth medium on α-amylase production by Penicillium solitum and Aspergillus rubrum isolated from yam (Dioscorea alata). Researcher [Internet]. 2013 [cited 2017 Sep 7];5(2). Available from: http://www.sciencepub.net/researcher

22. Mari M, Bautista-Baños S, Sivakumar D. Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. Postharvest Biol Technol [Internet]. 2016 Dec [cited 2017 Sep 7];122:70 81. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925521416300734

23. Matpan Bekler F, Güven K. Isolation and production of thermostable α-amylase from thermophilic Anoxybacillus sp. KP1 from Diyadin hot spring in Ağri, Turkey. Biologia (Bratisl). 2014;

24. Awojobi KO, Adejuwon MA, Ologbosere O. 7 1*, 2, 3. 2013;5(6):10–7.

25. Reddy PL, Sreeramulu A. Isolation, identification and screening of pectinolytic fungi from different soil samples of Chittoor District. Int J Life Sci Biotechnol Pharma Res. 2012;1(3):186–93.

26. Vandewoestyne M, Goossens K, Burvenich C, Van Soom A, Peelman L, Deforce D. Laser capture microdissection: should an ultraviolet or infrared laser be used? Anal Biochem [Internet]. 2013 Aug 15 [cited 2017 Sep 22];439(2):88–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23643622

27. Xu A, Pahl DM, Buchanan RL, Micallef S a. Comparing the Microbiological Status of Pre- and Postharvest Produce from Small Organic Production. J Food Prot. 2015;

28. Adejuwon AO, Oluduro AO, Agboola FK, Olutiola PO, Segal SJ. Expression and characterization of α -Amylases from penicillium citrinum with bread as growth substrate. 2015;9(August):2136 51.

29. Sternberg D. A method for increasing cellulase production byTrichoderma viride. Biotechnol Bioeng [Internet]. 1976 Dec [cited 2017 Sep 23];18(12):1751 60. Available from: http://doi.wiley.com/10.1002/bit.260181209

30. Adejuwon AO, Ladokun OA. Effect of Carbon Source of Growth on α -Amylase Production by a Strain of Penicillium funiculosum Thom . Isolated from Irish Potato (Solanum tuberosum Linn): Comparative Studies 1 . Introduction α -Amylases are starch degrading enzymes that catalyze th. 2015;3(1):1 11.

31. Adedeji OM AA. Modification of Cell Wall Degrading Enzymes from Soursop (Annona muricata) Fruit Deterioration for Improved Commercial Development of Clarified Soursop Juice (A Review). Med Aromat Plants [Internet]. 2014;4(1):1–5. Available from: http://www.omicsgroup.org/journals/modification-of-cell-wall-degrading-enzymes-from-soursop-annona-muricata-fruit-deterioration-for-improved-commercial-development-of-clarified-soursop-juice-a-review-2167-0412.1000178.php?aid=40924

32. WHITAKER DR, HANSON KR, DATTA PK. Improved procedures for preparation and characterization of myrothecium cellulase. 2. Purification procedures. Can J Biochem Physiol [Internet]. 1963 Mar [cited 2017 Sep 23];41:671–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14000266

33. Gebauer W. Protein Quantitation: Lowry Protocol. In: Encyclopedia of Life Sciences [Internet]. Chichester: John Wiley & Sons, Ltd; 2003 [cited 2017 Sep 23]. Available from: http://doi.wiley.com/10.1038/npg.els.0003754

34. PROTEINS (LOWRY) PROTOCOL. [cited 2017 Sep 23]; Available from: http://web.itu.edu.tr/~dulekgurgen/Proteins.pdf

35. Gopinath SM, Shareef I, Ranjit S. Isolation , Screening and Purification of Cellulase from Cellulase Producing Klebsiella variicola. Int J Sci Res. 2014;3(6):1398–403.

36. Onuorah and Orji (2015). Onuorah and Orji (2015) - Google Search [Internet]. [cited 2017 Sep 23]. Available from: https://www.google.com/search?q=Onuorah+and+Orji+%282015%29+&oq=Onuorah+and+Orji+%282015%29+&gs_l=psy-ab.3...6710.10724.0.13375.31.3.0.0.0.0.1476.1476.7-1.2.0....0...1.1.64.psy-ab..29.0.0.0...1969.PmBG_b0d7ac

37. Md W, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits, Lycopersicum esculentum, sold in markets in Benin City, southern Nigeria. Sch Acad J Biosci Sch Acad J Biosci [Internet]. 2014 [cited 2017 Sep 23];2(7):2321–6883. Available from: www.saspublisher.com

38. Bello OB, Bello IS, Aminu D, Olawuyi OJ, Afolabi-Balogun NB, Lawal AO, et al. Antibiotic sensitivity of bacterial and fungal isolates from tomato (Solanum lycopersicum L.) fruit. ISSN [Internet]. 2016 [cited 2017 Sep 22];3(1):2349–9265. Available from: www.tropicalplantresearch.com

39. Adejuwon A. Alpha Amylases from Fungi Species of Aspergilli and Penicilli. LAP LAMBERT Academic Publishing; 2013.

40. Godheja J, Shekhar SK, Modi DR. Advances in Molecular Biology Approaches to Guage Microbial Communities and Bioremediation at Contaminated Sites. 2014;2(4):167–77.

41. Sethi S, Datta A, Gupta BL, Gupta S. Optimization of Cellulase Production from Bacteria Isolated from Soil. ISRN Biotechnol [Internet]. 2013 Feb 19 [cited 2017 Sep 23];2013:1–7. Available from: http://www.hindawi.com/journals/isrn/20

13/985685/

42. Heerd D, Tari C, Fernández-Lahore M. Microbial strain improvement for enhanced polygalacturonase production by Aspergillus sojae. Appl Microbiol Biotechnol [Internet]. 2014 Sep 4 [cited 2017 Sep 23];98(17):7471–81. Available from: http://link.springer.com/10.1007/s00253-014-5657-z

43. Atolagbe OM, Ajayi AA, Edegbo O. Characterization of α-Amylase from Soursop (Annona muricata Linn.) Fruits Degraded by Rhizopus stolonifer. Pakistan J Biol Sci [Internet]. 2016 Feb 1 [cited 2017 Sep 23];19(2):77–81. Available from: http://www.scialert.net/abstract/?doi=pjbs.2016.77.81

44. Fernández Núñez EG, Barchi AC, Ito S, Escaramboni B, Herculano RD, Mayer CRM, et al. Artificial intelligence approach for high level production of amylase using *Rhizopus microsporus* var . *oligosporus* and different agro-industrial wastes. J Chem Technol Biotechnol [Internet]. 2017 Mar 1 [cited 2017 Sep 22];92(3):684–92. Available from: http://doi.wiley.com/10.1002/jctb.5054

45. Shinde S, Soni R. " Production and Partial Purification of α-Amylase from Bacterial Strains " Int J Genet Eng Biotechnol [Internet]. 2014 [cited 2017 Sep 23];974(1):57–62. Available from: http://www.irphouse.com