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Short Communication

Determination of amylase activity of crude extract from partially germinated mango seeds (*Mangifera oraphila*)

Afiukwa, C. A.[1]*, Ibiam, U. A.[1], Edeogu, C. O.[2], Nweke, F. N.[1] and Chukwu, U. E.[1]

[1]Department of Biochemistry/Biotechnology, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.
[2]Department of Medical Biochemistry, Ebonyi State University, Abakaliki, Nigeria.

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Amylase activity of crude extract from partially germinated mango seeds (*Mangifera oraphila*) was determined using Caraway-Somogyi iodine/potassium iodide (IKI) method. The effects of varied pH and temperature were also investigated. The amylase was extracted with 0.1 M acetate buffer (pH 4.2). Amylase activity of the crude extracts was measured by monitoring the amount of starch hydrolyzed by the crude extract over time. The result showed the presence of amylase activity in the extract, depicted by its ability to gradually decrease the concentration of the starch solution used as substrate. The optimum pH and temperature of the crude enzyme were about 6.0 and 60°C respectively. This study demonstrated that the abundant waste mango seeds in the south-eastern Nigeria, particularly Ebonyi state, could be exploited for production of amylase.

Key words: Mango seeds, crude extract, amylase activity.

INTRODUCTION

Amylases are enzymes that convert or breakdown starch into glucose (Schelgel, 2003). With the understanding of the nature of amylases and their hydrolytic potential, the use of amylase has been extended to various fields such as brewing, textiles, paper and detergent industries (Haq et al., 2002). They are the most important of carbohydrate degrading enzymes produced by microorganisms, animals and plants.

Amylases are employed in starch processing industries for the hydrolysis of polysaccharides (starch) into simple sugars. Amylases have been reported to be produced by various species of bacteria (Syn and Chen, 1997), yeast (Moreira et al., 2001), fungi (Tani et al., 1986) and plants (Mahmona, 1993). Frangui (2001) reported that amylases of plant origin have the highest productivity followed by that of fungi while amylases from bacteria have relatively less productivity. Plants and fungi have formed the basis of important amylase studies in developing countries because of their ubiquitous nature, especially the fungi species *Rhizopus* species and *Aspeigillus niger (*Abe et al., 1988).

Germinating seeds generally exhibit high amylase and protease activities. This is because these enzymes are synthesized during seed germination to mobilize stored food (starch and protein) for the survival of the young plant until it is capable of making its food by photosynthesis (Schramm and Loyter, 1966),

In this study, the presence of amylase activity in partially germinated mango seeds was investigated. The study was stimulated by the abundance of mango seeds in the study area, Ebonyi state, most of which is thrown away as waste plant product and hence constitute environmental nuisance.

MATERIALS AND METHODS

Collection of mango seeds

The mango seeds used in this study were collected from Abakaliki in Ebonyi state, Nigeria. The variety was identified by a botanist, Dr. O.F. A. Ibiam, of the Department of Applied Biology Ebonyi State University, as *Mangifera oraphila*. The seeds were germinated for 4 weeks in a pot of sand in the greenhouse of the biotechnology research and development centre (BRDC) of Ebonyi state university, Abakaliki.

Extraction of crude amylase

After the 4 weeks of germination, successfully germinated seeds

*Corresponding author. E-mail: afiukwa@yahoo,com. Tel.: +2347039220960.

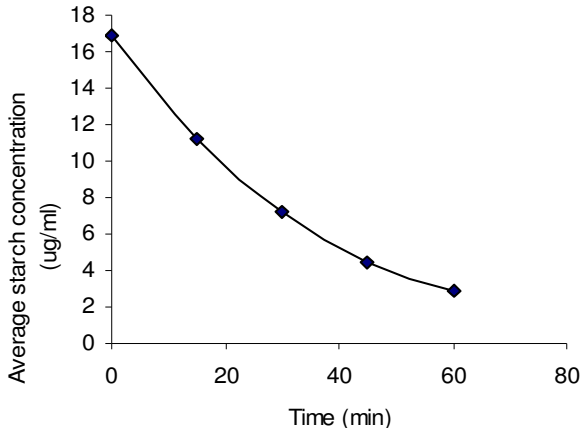

Figure 1. Time course of starch hydrolysis by crude extract from partially germinated mango seeds.

were decoated and their embryos collected, weighed and homogenized for 10 min at a very high speed with a volume of 0.1 M acetate buffer (pH 4.2) 3 times the weight of the embryo using an electric blending machine. The homogenate was then filtered by passing it through muslin cloth and the filtrate centrifuged for 10 min at 3500 rpm. The supernatant was collected and used as the crude extract while, the sediment was discarded.

Determination of amylase activity

Amylase activity of crude extracts was determined using Caraway-Somogyi iodine/potassium iodide (IKI) method (1959). The first step in the assay was the gelatinization/liquefaction of the soluble commercial starch used as the substrate. This was done by adding 40 ml of 1% soluble starch to 50 ml of gently boiling water in a beaker, while stirring. The gelatinized starch solution was allowed to cool to room temperature, after which the total volume was made up to 100 ml with distilled water.

Next, 1.0 ml of the gelatinized starch solution was further diluted to 100 ml with distilled water. This was used as the stock solution (substrate) for the assay.

Then, about 5.0 ml of the stock solution was added to each of 3 test tubes and 3.0 ml of 0.1 M phosphate buffer pH 5.6 added. About 1.5 ml of the crude amylase extract was added and the reaction mixture incubated at 37°C. After that, 1.0 ml of the reaction mixture was immediately transferred into another test tube containing 3.0 ml of 10% HCl to terminate the reaction. This was followed by addition of about 3.0 ml of the working indicator (iodine-potassium iodide solution). Finally, the absorbance was read against blank at 620 nm. This was taken to be 0 h incubation time. The procedure was repeated from the termination step at 15 min interval for 60 min. The amount of starch hydrolyzed per unit time was estimated from a standard curve of starch (substrate) concentration against absorbance.

Determination of effect of pH variation

Enzymes are generally sensitive to pH changes in their environment and have optimum pH at which they have their maximum activity, beyond which their activity decreases. The effect of pH changes on the amylase activity of the crude extract was studied using 0.1 M phosphate buffer solutions of pH ranging between 4.0 to 8.0 in increments of one pH unit.

Five identical test tubes labeled 4 - 8 were set up, with each test tube representing each of the different pH values studied. About 5.0 ml of the starch stock solution was added to each of the test tubes followed by the addition of 3.0 ml of each of the different pH buffer solutions. About 1.5 ml of the enzyme extract was added to each tube to initiate hydrolysis. The reaction was allowed to continue for 15 min by incubating at 37°C, after which 1.0 ml of the reaction mixture was transferred into another test tube containing 3.0 ml of 10% HCl to terminate the reaction. About 3.0 ml of the working indicator (iodine potassium iodide) was then added to 1.0 ml of the assay solution. Finally, the absorbance was read at 620 nm. The procedure was repeated 3 times. The rate of starch hydrolysis by the crude enzyme was calculated and plotted against pH.

Determination of effect of temperature variation

The study of the effect of temperature changes on the enzyme activity followed the same procedure as the study of the effect of pH on the amylase activity except that, instead of using buffer solutions of different pH ranges, 1 buffer solution (0.1 M phosphate buffer) of pH 5.6 was used in the assay and the reaction was monitored at different temperatures (30, 40, 50, 60 and 70°C).

RESULTS AND DISCUSSION

Determination of enzyme activity

The result of the hydrolytic ability of the crude extract from partially germinated mango seeds monitored over 60 min period at 15 min intervals is presented in Figure 1. This result shows that the concentration of the substrate decreased with time reducing from about 17 µg/ml to 2.9 µg/ml in 60 min. This shows that the crude extract from partially germinated mango seeds contained a good quantity of amylase.

So, instead of allowing the seeds of mango fruits to was-te, they could be harnessed for amylase production. This information is important for industrialists, who may be looking for a cheap source the enzyme.

Effect of pH variation

The effect of varied pH on the amylase activity of the crude extract is presented in Figure 2. A narrow pH range 4.0, 5.0, 6.0, 7.0 and 8.0) was chosen for this study, because it has been reported that amylases act better within this range (Tindall, 1996). The result showed that the amylase activity initially increased with increase in pH until it reached its optimum at about pH 6.0. Beyond this pH value, the activity declined progressively. This is a basic property of all enzymes and is probably due to concomitant alteration in the conformation of the enzyme protein caused by changes in pH of its environment (Hagerman and Reeners, 1964).

Effect of temperature variation

The effect of varied temperature condition on the amy-

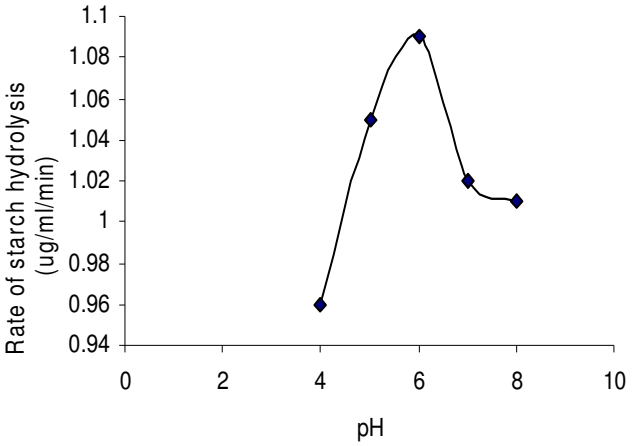

Figure 2. Effect of pH on the rate of starch hydrolysis by crude extract from partially germinated mango seeds.

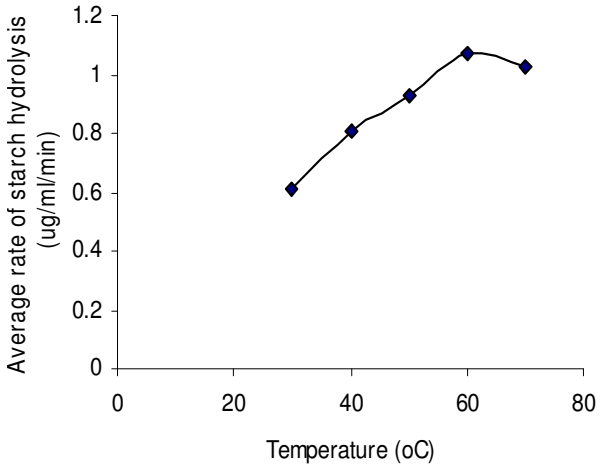

Figure 3. Effect of temperature on the rate of starch hydrolysis by crude extract from partially germinated mango seeds.

lase activity of the crude extract as investigated is presented in Figure 3. The result showed that the enzyme activity increased with increase in temperature and attained optimum at about 60°C. Beyond 60°C, the activity of the crude extract started to decrease. This may perhaps be accounted for by denaturation of the enzyme protein at temperatures higher than 60°C. Denaturation of enzyme proteins leads to loss of activity (Schweigert et al., 2007).

Conclusion

This study has demonstrated that partially germinated mango seeds, like many other seeds studied such as pawpaw, cashew and avocado seeds (Delgado et al., 2006), contain amylase. In addition to its ability to hydrolyze gelatinized starch, the crude extract exhibited the pattern of variation in enzyme activity at different pH values and temperatures common to typical enzymes. Thus, the waste mango seeds could be converted to wealth by developing a standard method of producing amylase and other useful enzymes that may be present in it. This would not only provide income and employment opportunities, but also help reduce the environmental pollution caused by the waste mango seeds.

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