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Determination of amylase activity from germinated *Syzygium cumini* seed (jamun)

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Abstract
Amylase activity was determined from the extract of *Syzygium Cumini* (jamun) seed by using a Caraway Somogyi iodine/potassium iodide (IKI) method. The effect of variation in PH & temperature were also investigated. Amylase activity of the extract was measured by monitoring the amount of starch hydrolyzed by the 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 enzyme were about 6.0 & 60 ^{0}C respectively.

Keywords: *Syzygium cumini* seeds, extract, amylase activity

1. Introduction
Amylase is an enzyme that convert or breakdown starch into glucose. The use of amylase has been expanded in numerous fields such as brewing, textiles, paper and detergent industries. They are the most important of carbohydrate degrading enzymes produced by microorganisms, animals and plants. Amyalse are also, employed in starch processing industries for the hydrolysis of polysaccharides (starch) into simple sugars. Various species of bacteria, yeast, fungi& plants produced a amylase enzyme (Frangui) reported that amylases of plant origin have the highest productivity followed bythat of fungi while amylases from bacteria have relativelyless productivity. Plants and fungi have formed the basis of important amylase studies in developing countries because of their ubiquitous nature especially the fungi species *Rhizopspecies and Aspeigillus niger*. The amylase enzyme found in germinating seed. Imbibition process causes a growth of plant which stimulates the synthesis of amylase that's why germinating seed exhibit high amylase activity. In plants glucose is used for the growth of plumule and radicle when this process happen the seed are said to be undergo germination. Amylase activity is affected by many factor such as temperature, pH, enzyme concentration and substrate concentration. Amylase enzyme in the jamun seed works best at specific range of temperature. In this study, the presence of amylase activity in germinated jamun seeds was investigated.

2. Material and Methods
2.1 Collection of jamun seeds
The jamun seeds used in this study were brought locally from market, Akola. The seeds were germinated for 20 days in a pot of soil, (time period of germination: 20 days)

2.2 Extraction of Amylase from germinated seed
The germination period of *Syzygium Cumini* seed is 15 to 20 days. The seed was successfully germinated within a 20 days. After completed 20 days of germination the germinated seed remove from the soil. Firstly embryo of seeds were collected by decoated a seeds then weighed these embryo and homogenized with a volume of 0.1 M acetate buffer (PH 4.2) for 10 minutes at a very high speed (3 times). The homogenate then filtered by using a muslin cloth and then filtrate centrifuged for 10 minutes at 35000 rpm. The supernatant obtained this supernatant was collected and use as the extract for this study. From the extract the sediment was removed. Extract from germinating seed provide a good system for studying enzyme activity extraction of these enzyme is accomplished by grinding the seed in a buffer to make a pure, then removing the unwanted debris through centrifugation.

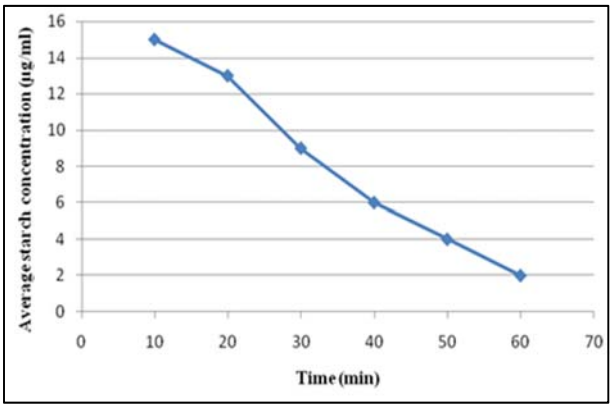

Fig 1: Time required for starch hydrolysis

2.3 Determination of amylase activity of extract

Different methods were used for determining the amylase activity of extract. In this study we used the Caraway Somogyi iodine /potassium iodide (IKI) method. The reagent iodine potassium iodide (IKI) can be used to detect the presence of starch in a solution. The first step in the assay was the gelatinization/liquefaction of the soluble commercial starch used as the substrate. This was done by adding 40ml 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 100ml with distilled water. Next, 1.0 ml of the gelatinized starch solution was further diluted to 100 ml with distilled water. Diluted gelatinized the starch solution is used as the stock solution or substrate for the assay. Take 3 test tube and add about 0.5 ml of stock solution and 3.0 ml of 0.1 M phosphate buffer (PH 5.6).The reaction mixture incubated at 37 ^{0}C after addition of 1.5 ml of amylase extract. Working indicator (Iodine potassium iodide Solution) was added about 3.0 ml then 1.0 ml of the reaction mixture was immediately transferred into another test tube containing3.0 ml of 10% HCL to terminate the reaction. After completing these steps the absorbance was read against blank at 620 nm. This was taken to be 0 h incubation time. The same procedure was repeated from the termination step at 15 min interval for 60min. The amount of starch hydrolyzed per unit time was estimated from a standard curve of starch (substrate) concentration against absorbance.

2.4 Determination of effect of pH variation

Enzymes are generally sensitive to pH changes in their environment and have optimum pH at beyond which their activity decreases. The effect of pH changeson the amylase activity of the extract was studied using 0.1 M phosphate buffer solutions of pH ranging between 4.0 to 8.0in 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 for15 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.

2.5 Determination of effect of temperature variation

The effect of varied temperature on the activity of enzyme followed the same procedure as the effect of pH variation on the activity of amylase, but in case of determining the effect of temp variation the buffer solution was used with a PH of 5.6(0.1M Phosphate buffer) and the reaction was monitored at different temperature (20, 30, 40, 50, 60 & 70 ^{0}C)

3. Results

3.1 Determination of enzyme activity

The hydrolytic ability of the extract of germinated *syzygium cumini* seed showed a result in a figure which monitored over 60 minutes period at 15 minutes interval, so thy showed that the concentration of the substrate decreased with time reducing from about 15µg/ml to 2 µg/ml in 60 min. This shows that the extract from germinated jamun seeds contained a good quantity of amylase. So, instead of allowing the seeds of jamun fruits to waste, they could be utilized for amylase production. This information is important for industrialists, who may be looking for a cheap source the enzyme.

3.2 Effect of varied pH on the amylase activity

The activity of enzyme is depending on pH. The increase and decrease in pH has marked influence of activity of enzyme. The enzyme is active at its optimum pH. The increase and decrease in pH may result in denaturation of enzyme and hence its activity gets lost. Figure 2 represents the effect of pH variation on the amylase activity of extract A narrow pH range (3.0, 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. The result showed that the amylase activity initially increased with increase in pH until it reached its optimum pH at about pH 6.0. Beyond this pH value, the activity decreased slowly. This is a basic property of all enzymes and is probably due to concomitant alteration in the conformation of the enzymeprotein caused by changes in pH of its environment.

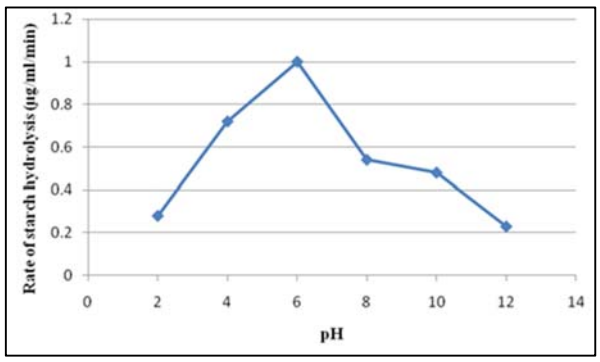

Fig 2: Effect of pH on the rate of starch hydrolysis

3.3 The effect of varied temperature on amylase activity

We know the enzymes are protein in nature and hence the temperature has marked effect on its catalytic activity. Increase in temperature so increases the activity of enzyme. It has been observed that every 10^0 rise in temperature so the activity of enzyme is doubled. but the enzyme being protein in nature are thermo labile and gets denatured beyond certain temperature. The effect of varied temperature condition on

the amylase activity of the extract as investigated is showed 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.

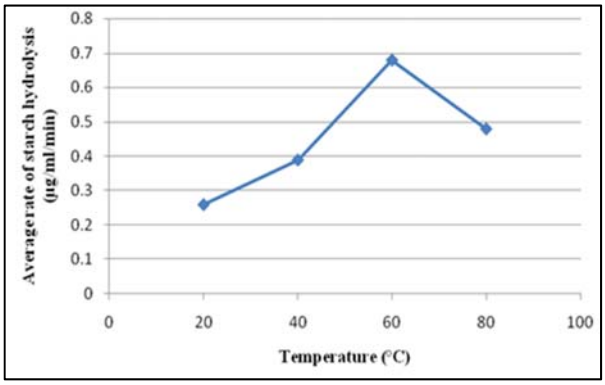

Fig 3: Effect of temperature on the rate of starch hydrolysis

4. Conclusion

This study reached to the conclusion that germinated *Syzygium Cumini* seeds, like many other seeds studied such as wheat, chana, ground nut seeds contain amylase. Moreover, its ability to hydrolyze gelatinized starch, the extract exhibited the pattern of variation in enzyme activity at different pH values and temperatures common to typical enzymes. Thus, the waste jamun 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 *S. Cumini* seeds.

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