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Research Paper

DETERMINATION OF ENZYME ACTIVITIES IN FOUR SELECTED GRAINS (RICE, MILLET, SORGHUM AND MAIZE) AT VARIOUS GERMINATION STAGES

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Abstract

The content of starch and the activities of enzymes involved in starch biosynthesis were examined in germinating grains of field grown rice, sorghum, millet and maize plants. Enzyme activities were monitored throughout the grain germinating periods for three days. The activities of most of the grain enzymes involve in starch biosynthesis increased and reached their maxima during the initial period of grain development, then gradually declined towards the later stages of grain germination.

Key words: α-amylase, β-amylase, Enzymes,*biosynthesis* , hydrolysis.

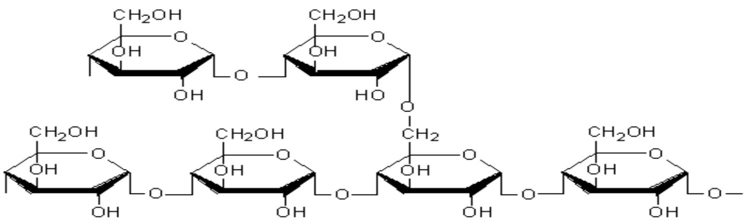
INTRODUCTION

Enzymes are biological molecules that catalyze (i.e. increases or decreases) the rate of chemical reactions. In enzymatic reactions, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the activation energy for a reaction, thus dramatically increasing the rate of the reaction. As a result, prosducts are formed faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts in that they are highly specific for their substrates. Enzymes are known to catalyze about 4,000 biochemical reactions [1].

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease or hinder enzyme activities; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, pressure, chemical environment (e.g. P^H), and the concentration of substrate. Enzymes actually act as catalyst for invivo (inside cell) reactions. A catalyst can reduce the reaction energy by taking part in such reaction. At the end, reaction convert into product but the enzymes remain unmodified. A diastase is any one of a group of enzymes which catalyzes the breakdown of larger starch (a carbohydrate) molecules into rather small maltose units.

Today diastase means any α, β or γ amylase that can break down carbohydrate. Diastase is commonly found in germinating seeds and in the digesting system of animals [2].Salivary amylase and pancreatic amylase are alpha-amylase, which act at random on alpha- 1- 4 glycosidic bonds to split starch into smaller unit (dextrins), and finally to alpha-maltose. Beta- amylases are of plant origin (almond plant, germinating seeds, etc.) which splits starch to form beta-maltose. They act on amylase to split maltose unit consecutively. Thus the enzymes start its action from one end.[3]. When beta-amylase acts on amylopectin, maltose units are liberated from the ends of the branches of amylopectin, until the action of enzyme is blocked at the 1, 6- glycosidic linkage. The action of beta- amylase stops at branching points, leaving a large molecule, called limit dextrin. The starch is represented with the reaction below:



Sorghum bicolor, commonly called sorghum and also known as durra, jowari, or milo, is a grass species cultivated in northern Africa , and is now cultivated widely in tropical and sub tropical regions. S. bicolor is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4m high. The grain is small, ranging from 3 to 4mm in diameter. Sweet sorghums are sorghum cultivars that are primarily grown for foliage; they are shorter than those grown for grain [4]. S. bicolor is the cultivated species of sorghum, its wild relatives make up the botanical genus sorghum.
Maize (*Zea mays*), known in many English- speaking countries as corn is a grain domesticated by indigenous people in Meso America in prehistoric times.The leafy stalk produces ears which contain seeds celled kernels. Though technically a grain, maize kernels are used in cooking as a vegetable or starch.
Maize is the most widely grown grain crop in the Americas, with 332 million metric tons grown annually in the United States alone. The kernel of maize has a pericarp of the fruit fused with the seed coat referred to as "caryopsis", typical of the grasses, and the entire kernel is often referred to as the "seed". The grains are about the size of peas, and adhere in regular rows round a white, pithy substance, which forms the ear.
Millets are a group of highly variable small- seeded grasses, widely grown around the world as cereal crops or grains for both human food and fodder. They do not form a taxonomic group, but rather a functional or agronomic one. Millets are important crops in the semi- arid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries. The crop is favored due to its productivity and short growing season under dry, high temperature conditions[5].The most widely grown millet is pearl millet, which is an important crop in India and parts of Africa.The millet includes species in several genera, mostly in the sub family penicoideae, of the grass family poaceae. The exception, finger millet and teff, are in the sub family chloridoideae.[4].

Rice is the seed of the monocot plants oryza sativa (Asia rice) or Oryzaglaberrima (Africa rice). As a cereal grain, it is the most important staple food for a large part of the world's human population, especially in Asia and the West indies. It is the grain with the second highest worldwide production, after maize (corn), according to data for 2010, since a large portion of maize crops are grown for purpose other than human consumption. Rice is the most important grain with regard to human nutrition and caloric consumed worldwide by the human species.Going by this, knowing which of the grain has the highest enzymes activities and at what germinating stage, is very importance especially for the industries which extract diastase for brewering product or in bakery that uses yeast.

Theory

The theory of this study of enzyme is related to the one began in 1902 by Adrian Brown when he reported on an investigation of the rate of hydrolysis of sucrose as catalyzed by yeast enzyme invertase
(known as β- fructofuranosidase).

$$\text{Sucrose} + H2O \longrightarrow \text{glucose} + \text{fructose}$$

When the sucrose concentration is much higher than that of enzymes, the reaction rate becomes independent of the sucrose concentration, that is, the rate is zeroth order, with respect to sucrose. Is therefore proposed that the overall reaction is composed of two elementary reactions in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzymes.

$$E + S \underset{K_2}{\overset{K_1}{\rightleftarrows}} ES \xrightarrow{K_2} P + E$$

Here, E, S, ES and P symbolize the Enzyme, Substrate, enzyme substrate complex and products respectively.

According to the model, when the substrate concentration becomes high enough to entirely convert the enzyme to the ES form, the second step of the reaction becomes rate limiting and the overall reaction rate becomes insensitive to further increases in substrate concentration.

The general expression for the velocity (rate) of this reaction is:

$$V- \frac{d\,[P]}{dt} = K2\,[ES\,]$$

The overall rate of production of [ES] is the different between the rate of the elementary reactions leading to its appearance and those resulting in its disappearance.
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MATERIALS AND METHODS

Sample Collection and Sterilization of Apparatus

The different grains used (rice, millet, sorghum and maize) were collected at Mubi . All apparatus used was sterilized adequately with hot water at about 100°C to avoid infection and alteration of result.

Methods

The method for the determination of contents of substrate is the principle of spectrophotometry. A spectrophotometer consists of two instruments, namely a spectrometer for producing light of any selected color (wavelength), and a photometer for measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, normally a galvanometer. The signal changes as the amount of light absorbed by the liquid changes, at wavelength of 550nm.

The choice of a method for estimating the enzyme activity is primarily determined by the possibility of reliable assessment of the substrate and reaction of enzyme required to turn over one mole substrate per minute under standard conditions.

Preparation of Sample

5.0g of germinating grains (rice, millet, sorghum, and maize) was individually weighed. The sample was put into a mortar and crushed with pestle.10ml of distilled water was added to the crushed grain and mixed thoroughly using a glass rod.

The solution was filtered through a filter paper to remove the larger debris for accuracy of result. The filtered solution of each of the grain was individually kept in four different test tubes and labeled accordingly. This was done after each day of germination for 3 days.

Enzyme Assay

Since starch is insoluble in water, therefore to prepare starch solution, 2g of starch was dissolved in addition with 0.2g of salicylic acid in 100ml hot distilled water. The enzyme-starch mixture was prepared in a different test tube in the ratio 3:2 by measuring 3ml of starch together with 2ml of enzyme extract (crushed grain solution). This was done for the four test tubes containing enzyme extract and also a fifth test tube containing 20ml of starch solution was kept in a water bath maintained at 37°C. As soon as all the solutions in the water bath reach 37°C,

The absorbance of the various mixtures was taken using spectrophotometer with starch solution as the standard. The absorbance was taken after every 5 minutes intervals for 1 hour. This was done for each of the day of germination for three (3) days. The absorbance for each day of germination was compared for the various grains and also at different germination days to see which of them has the highest enzyme activities. A graph was plotted for clear distinction and interpretation of the enzyme activities of the different grains at various germination stages.

RESULTS AND DISCUSSION

Results

The enzyme activities for diastase were determined for four different grains (rice, maize, millet and sorghum) at various stages of germination. The first day of germination was seen to have the highest enzyme activities with millet, while the second day was seen to have the next activity, with millet still having the highest activities. On the third day, there was no observable activity. Below is the detailed result for the four different grains at various stages of germination. For more details on the table of data, see appendix.

TABLE 1: ENZYME ACTIVITIES FOR SELECTED GRAINS ON THE FIRST DAY OF GERMINATION IN RELATION TO TIME.

TIME	STANDARD	STANDARD+ MAIZE	STANDARD+ SORGHUM	STANDARD+ RICE	STANDARD+ MILLET
5	0.456	0.827	1.508	0.89	1.889
10	0.413	1.437	1.817	1.925	2.695
15	0.406	2.271	1.8	2.563	2.332
20	0.418	1.572	2.648	1.66	2.52
25	0.343	1.224	2.518	2.125	2.597
30	0.428	0.594	1.089	0.723	0.917
35	0.444	2.205	1.338	2.159	1.791
40	0.415	0.957	1.306	1.495	1.315
45	0.46	2.212	2.693	2.585	2.766
50	0.46	2.201	2.742	2.645	2.787
55	0.476	2.27	2.755	2.701	2.895
60	0.468	2.22	2.719	2.678	2.911

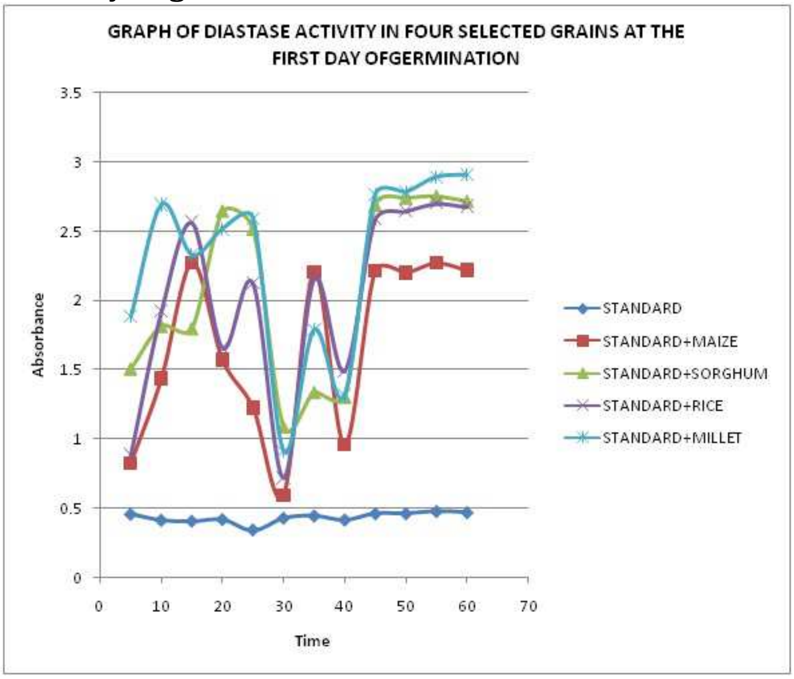
TABLE 2: ENZYME ACTIVITIES FOR SELECTED GRAINS ON THE SECOND DAY OF GERMINATION IN RELATION TO TIME.

TIME	STANDARD	STANDARD+ MAIZE	STANDARD+ SORGHUM	STANDARD+ RICE	STANDARD+ MILLET
5	0.833	2.246	0.635	0.889	1.753
10	2.451	2.632	2.686	2.304	0.782
15	1.774	2.57	2.676	2.485	2.746
20	1.758	1.873	1.714	1.928	2.157
25	1.759	1.667	1.699	1.67	1.738
30	1.762	1.69	1.745	1.72	0.787
35	1.77	1.658	1.707	1.677	1.745
40	1.782	1.684	1.743	1.707	1.786
45	1.791	1.625	1.701	1.658	1.735
50	1.807	1.616	1.693	1.642	1.728
55	1.812	1.585	1.684	1.639	1.731
60	1.816	1.561	1.686	1.627	1.721

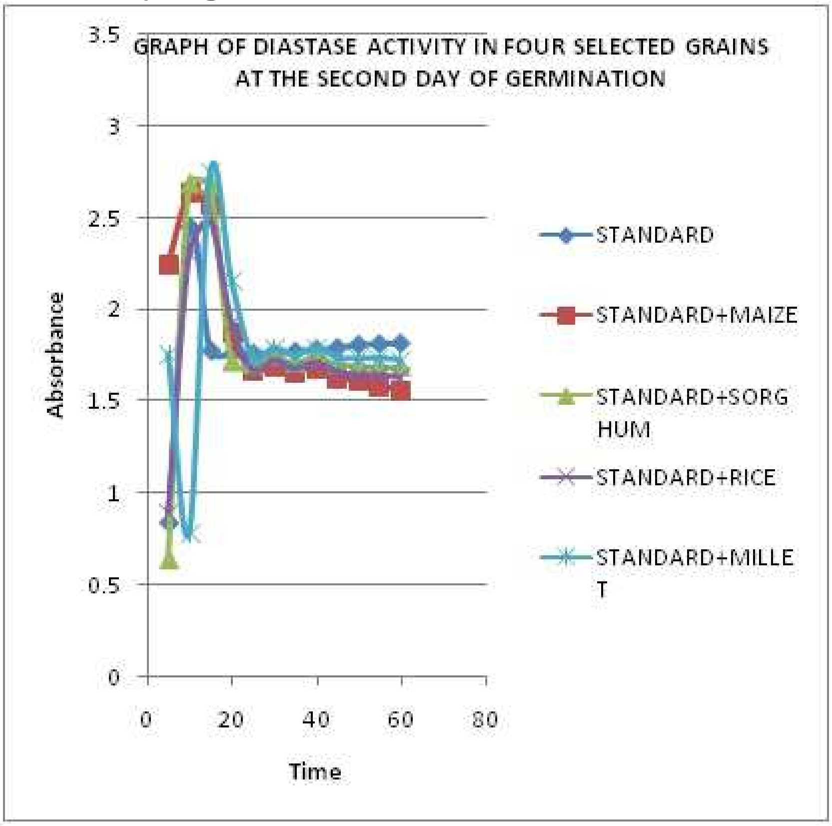
TABLE 3: ENZYME ACTIVITIES FOR SELECTED GRAINS ON THE THIRD DAY OF GERMINATION IN RELATION TO TIME.

TIME	STANDARD	STANDARD+ MAIZE	STANDARD+ SORGHUM	STANDARD+ RICE	STANDARD+ MILLET
5	1.818	1.794	1.74	1.753	1.514
10	1.816	1.774	1.721	1.725	1.762
15	1.821	1.787	1.731	1.721	1.725
20	1.825	1.782	1.717	1.714	1.747
25	1.834	1.781	1.732	1.699	1.735
30	1.724	1.774	1.686	1.686	1.74
35	1.825	1.781	1.667	1.684	1.732
40	1.825	1.787	1.67	1.667	1.714
45	1.802	1.782	1.636	1.67	1.713
50	1.812	1.774	1.536	1.622	1.627
55	1.83	1.767	1.605	1.592	1.676
60	1.72	1.765	1.546	1.585	1.672

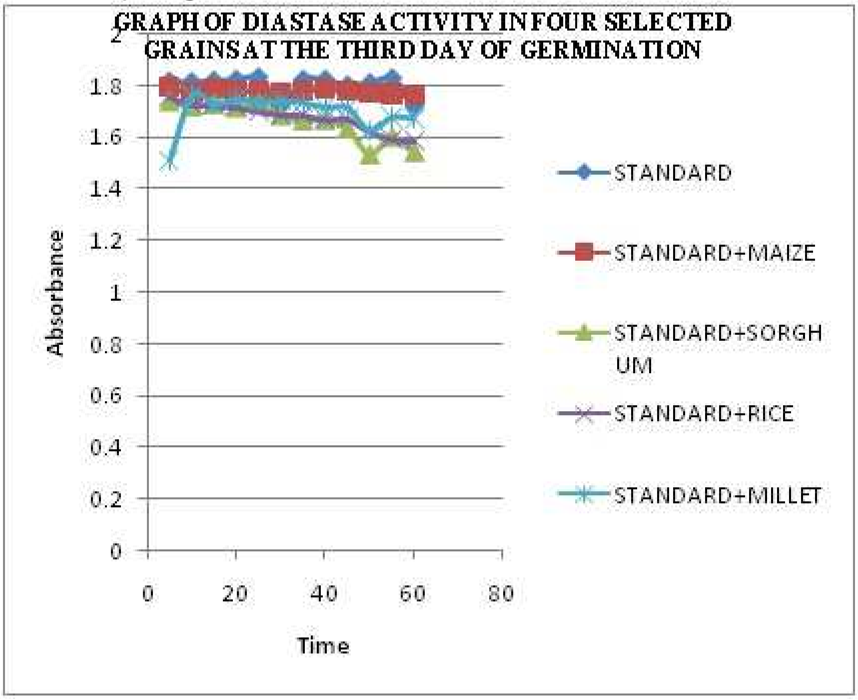
First day of germination



Second day of germination



Third day of germination



DISCUSSION

First Day of Germination

The activity of the enzyme on the first day of germination was seen to be very high. Millet was seen to have the highest enzyme activity and there is closed interaction between other grains. Sorghum was seen to follow millet closely in the activities, then rice and maize was seen to have the least enzyme activities. On the first day, the starch was clearly seen to have constant enzyme activities. The activities of all the enzymes were seen to be irregular until the 45th minute where the activities were at equilibrium which later increase with time, probably because of the change in temperature.

Second Day of Germination:

On the second day of germination, millet was seen to have the highest enzyme activities, though not as high as the first day, follow by Sorghum, maize and then rice. At the 23rd minutes, the activities of the enzymes was seen at equilibrium, showing that there was a stable enzyme activities on the second day of germination on the 23rd minute which later decrease with time. The starch was seen to have a higher activity on the second day when compared with the first day, as a result of change in temperature.

Third Day of Germination:

At the third day of germination, the activities of the enzymes greatly reduced, infact there is no activities at all when compare to the starch solution without the enzyme solution. Millet was seen to have the lowest enzyme activities, followed by sorghum, rice and maize respectively. The possible reason for the drastic reduction in the enzyme activities compared to the first day is because of the in ability of the enzyme to perform as the germination proceeds. When the substrate concentration reaches a saturation threshold, the activity of enzyme no longer increases. The substrate enhances the enzyme stability and facilitates the adoption of an appropriate conformation by the active centre of the enzyme. Another reason for the decreased enzyme activity is attributed to their lower granule bound starch synthase and starch debranching enzyme activities at early and mid phases of germination. When the germination proceeds, all the stored starch has been exhausted because immediately the leaves starts to developed, the nutrients is no longer gotten from the root and cotyledon but start photosynthesis by absorbing photon from sunlight, thereby reducing enzyme activities.

Summmary

The first day of germination has the highest enzyme activities with millet taking the highest among the grains, followed by sorghum, rice and maize respectively. The enzyme is very active at the first day of germination.

The second day of germination was characterize by long equilibrium activities starting from the 23rd minute, and having millet as having the highest activities, followed by sorghum, maize and rice respectively. The third day of germination was not having any enzyme activities because of the temperature and the concentration of the substrate. During the third day of germination, the activities of enzymes was denatured at it was seen to be in equilibrium.

CONCLUSION

Putting everything under consideration, the activities of enzymes was determined for the three days of germination and millet was seen to have the highest enzyme activity on the first and second day of germination, with the first day having the highest activities.TheRate amylase production in selected seed are high in millet sorghum,rice and maize respectively. Hence millet is reacher in amylase than the rest seeds and can be selected for industrial sources of amylase.

Recommendation(s)

Based on the observed phenominum, I hereby recommend for industries using enzymes, example amylase preparation capable of accelerating starch hydrolysis, are used as dough-raising leaven in baking high-quality bread, as well as alcoholic fermentation starters in the manufacture of beer, that millet at the first day of germination has highest activities and that it will be most effective in their application. Also amylase that is used as food additives and also for dish washing will find it interesting when germinating millet is adopted at the first day of germination.

REFERENCES

1. A.L.Smith. (Ed) (1997). Oxford dictory of biochemistry and molecular biology. Oxford (oxford shire): Oxford university press. ISBN 0-19-854768-4 The catalystic site Atlas at The European Bioinformatics institute. Retrieved 4th April ,2007.
2. M. Grisham, Charlse and H .Reginard Garrett (1999). Biochemistry. Philadelphia: Saunders College Pub. Pp. 426-7. ISBN 0-03-022318-0.
3. T. Cech, .(2000). “structural biology. The ribosome is a ribozym”. Science 289 (5481): 878-9.
4. W.Crawford, Gary. (1983). Paleoethnobotany of the Kameda Peninsula. Ann Arbor. Museum of Anthropology, University of Michigan. ISBN 0-932206-95-6
5. W .Crawford, Gay . (1992). “Pre historic plant Domestication in East Asia”. In Cowan C.W., Watson P.J. The origins of Agriculture: An international perspective. Washinton: Smithsoniar institution Press. Pp. 117-132. ISBN 0-87474-990-5.