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Determination of Enzyme Activity in Four Selected Grains (Mung Beans, Chickpeas, Lentils, Green Peas) at Various Germination Stages using Colorimetry

Steffie Dmello

Department of Zoology, Wilson College, University of Mumbai
(Supervised by Ms. Neha Elizabeth Koshy)

Abstract: ***The enzyme activities of selected grains involved in starch biosynthesis were determined. Enzyme activities were monitored throughout the germinating period of the grains for 3 days. This was done using colorimetric technique. The activity of enzymes gradually declined towards the later stages of germination.***

Keywords: enzyme activity, germination, beta amylase, colorimeter

1. Introduction

Enzymes are biological catalyst that accelerate or catalyze chemical reactions. Initially the molecules at which enzymes act are called as substrates and the enzymes convert this into different molecules called as products. All the metabolic process in the cells need enzymes in order to in order to occur at faster rates to sustain life. Enzymes work by lowering the activation energy for reaction, thus dramatically increasing the rate of reaction. As a result the products are formed faster as compared to the non - catalyzed reactions.

Enzymes are known to catalyze about 4000 biochemical reactions.

A diastase is any one of the group of enzymes which catalyzes the breakdown of larger starch molecules into smaller maltose units. Diastase means α, β or γ amylase that can breakdown carbohydrate.

Diastase is commonly found in germinating seeds and digestive system of animals. α amylase is found in digestive system of animals and β amylase is found in germinating seeds which splits starch to form beta maltose amylase splits maltose units consecutively. Therefore the enzymes starts its action from one end. When beta amylase acts on amylopectin, maltose units are liberated from the ends of branches of amylopectin, until the action of enzyme is blocked at 1 - 6, glycosidic linkage. The action of beta amylase stops at branching point, leaving a large molecule called limit dextrin.

β - amylase is present in an inactive form prior to germination, whereas α - amylase and proteases appear once germination has begun. Beta amylase is used in many industrial applications such as brewing beer and liquor made from sugars derived from starch, bread making and to break down complex sugars, such as starch (found in flour), into simple sugars and so on.

The chickpea or chick pea (*Cicer arietinum*) is a legume of the family Fabaceae, subfamily Faboideae. Its different types are variously known as gram, or Bengal gram, garbanzoor garbanzo bean, Egyptian pea. [2] Its seeds are high in protein. It is one of the earliest cultivated legumes: 7, 500 - year - old remains have been found in the Middle East. They are a small legume popular in Mediterranean, Middle Eastern and Indian cookery.

The mung bean (*Vigna radiata*), alternatively known as the moong bean, green gram, or mung, is a plant species in the legume family. The mung bean is mainly cultivated in India, China, and Southeast Asia.

The lentil (*Lens culinaris*) is an edible pulse. It is a bushy annual plant of the legume family, known for its lens - shaped seeds it is about 40 cm (16 in) tall, and the seeds grow in pods, usually with two seeds in each. It is a dietary staple throughout regions of India, Pakistan, Bangladesh and Nepal. As a food crop, the majority of world production comes from Canada, India and Australia.

The pea is most commonly the small spherical seed or the seed - pod of the pod fruit *Pisum sativum*. [2] Each pod contains several peas. Pea pods are botanically fruit, [3] since they contain seeds and developed from the ovary of a (pea) flower. The name is also used to describe other edible seeds from the Fabaceae such as the pigeon pea (*Cajanus cajan*), the cowpea (*Vigna unguiculata*), and the seeds from several species of *Lathyrus*. *P. sativum* is an annual plant, with a life cycle of one year. It is a cool - season crop grown in many parts of the world; planting can take place from winter to early summer depending on location. The average pea weighs between 0.1 and 0.36 grams.

Materials and reagents: Starch, Benedict's reagent, 4 different germinating seeds, test tubes, mortar pestle, distilled water, pipettes.

2. Method

Sample collection and sterilization of apparatus:
The different germinating seeds (Mung beans, Chickpeas, Lentils, Green peas) were collected
All apparatus used were sterilized adequately with hot water at about 100 degree to avoid infection and alteration of result.

Preparation of sample:
15 gm of germinating seeds were individually weighed. The sample was put into mortar and crushed with a pestle.30 ml of distilled water was added to crushed grain and mixed thoroughly using a glass rod. The solution was filtered through filter paper to remove larger debris for accuracy of result. The filtered solution was kept in 4 different test tubes and labeled accordingly. This was done after each day of germination for 3 days.

Enzyme assay:
Starch is insoluble in water therefore for preparation of starch, 2.0 gm of starch in 100 ml distilled water was kept on flame with continuous stirring of the solution to avoid burning of the starch.

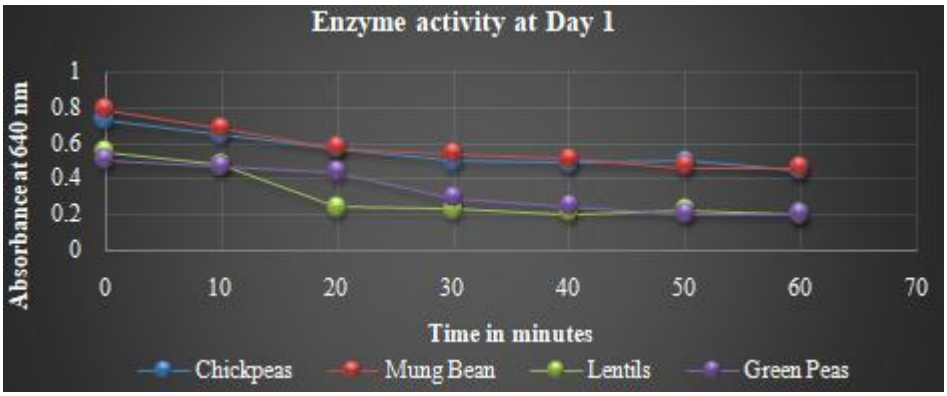
The enzyme - starch mixture was prepared in different test tubes in the ratio 3: 2, by measuring 3ml of starch solution to 2 ml of enzyme extract (crushed grain solution). This was done for 4 test tubes containing enzyme extract. From the above solution 2 ml was taken in 4 different test tubes. And to this, 4ml of benedicts solution was added. Benedict's solution reacts with the maltose which indicates the amount of maltose thereby giving the enzyme activity. Another test tube was taken (5^{th} test tube), to this 2 ml of starch and 4 ml of benedicts was added, this was considered as standard.5 test tubes were kept in boiling water bath for 2 minutes. They were cooled and again filtered through filter paper to remove the precipitate. And then the absorbance was taken.

The absorbance of various mixtures was studied using a colorimeter. The wavelength of 640 nm was used. The absorbance was taken at every 10 minutes intervals for 1 hour. The absorbance for each day of germination was compared for various grains and also at different germination days to see which has highest enzyme activity.

A graph was plotted for clear distinction and interpretation of enzyme activities of different grains at different germination stages.

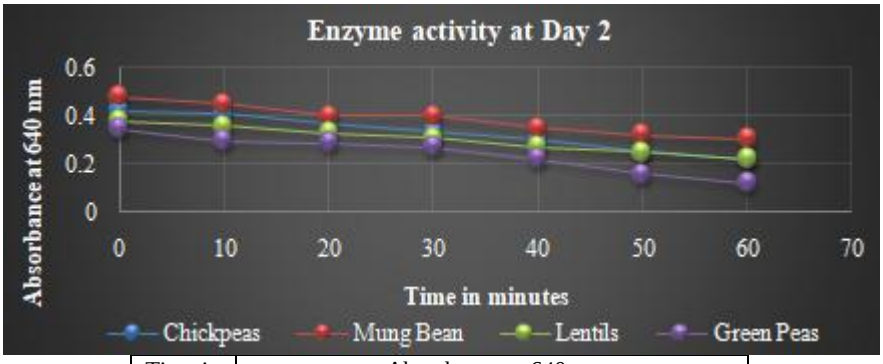
3. Results

3.1 Enzyme activity at day 1:



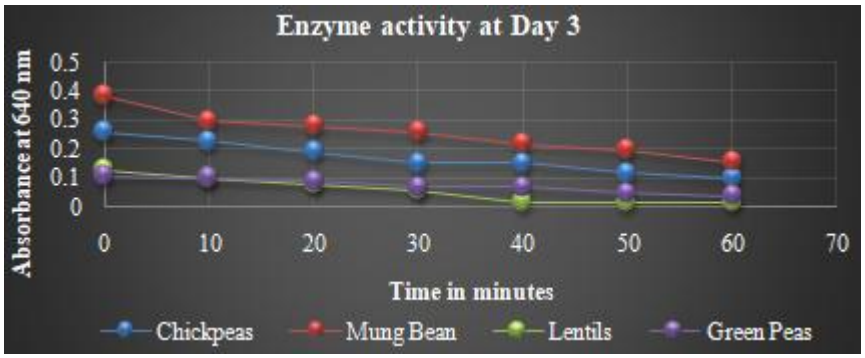
Time in minutes	Absorbance at 640nm			
	Chickpeas	Mung Beans	Lentils	Green Peas
0	0.73	0.79	0.55	0.51
10	0.65	0.68	0.48	0.47
20	0.57	0.57	0.24	0.44
30	0.5	0.54	0.23	0.29
40	0.49	0.51	0.21	0.25
50	0.5	0.47	0.22	0.2
60	0.45	0.46	0.2	0.2

Enzyme activity at day 2:



Time in minutes	Absorbance at 640nm			
	Chickpeas	Mung Beans	Lentils	Green Peas
0	0.42	0.48	0.38	0.35
10	0.41	0.45	0.36	0.3
20	0.37	0.4	0.33	0.29
30	0.33	0.4	0.31	0.27
40	0.3	0.35	0.27	0.22
50	0.25	0.32	0.25	0.16
60	0.22	0.31	0.22	0.12

Enzyme activity at day 3:



Time in minutes	Absorbance at 640nm			
	Chickpeas	Mung Beans	Lentils	Green Peas
0	0.26	0.39	0.13	0.11
10	0.23	0.3	0.1	0.1
20	0.19	0.28	0.08	0.09
30	0.15	0.26	0.06	0.07
40	0.15	0.22	0.02	0.07
50	0.12	0.2	0.02	0.05
60	0.1	0.16	0.02	0.04

4. Discussion

First day of germination:
The activity of enzyme on first day of germination was seem to be very high. Mung beans was seen to have maximum activity. Chickpeas was seen to follow mung beans followed by lentils and green peas which seem to have lowest activities. There was decrease in the enzyme activity with time, probably because the substrate is being used up

Second day of germination:
On the second day mung beans was seen to have the highest enzyme activities, though as not as high as first day followed by chickpeas, lentils and green peas

Third day of germination:
At the third day of germination, the activity of the enzyme was greatly reduced. Mung beans was seen to have the lowest activity followed by chickpeas, lentils and green peas. The possible reason for the reduction when compared to the first day is the inability of an enzyme to perform, as the germination proceeds. One of the reason for decrease in enzyme activity is attributed to their lower granule bound starch synthase and starch debranching enzyme activities at early and midphase of germination. When the germination proceeds the stored starch has been exhausted because immediately the leaves start to develop and the nutrients are no longer taken from roots and cotyledon but they start the process of photosynthesis by absorbing photon from light, thereby reducing enzyme activities

5. Conclusion

The activities of enzyme were determined for 3 days of germination and mung beans was seen to have highest activity of all for 3 consecutive days. Therefore, the amylase production is highest in mung beans and thus the beta amylase which is present can also be used for industrial applications such as making food additives, detergents and so on. Amylase hydrolysis starch and therefore it can be used as dough raising leaven in baking high quality bread, as well as alcoholic fermentation starters in manufacture of beer. Mung bean have the highest activity and therefore it can be more effective in their application.

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