Study of Amylase (alpha & beta) Activity and its Isozymic Analysis During Germination of Buckwheat (Fagopyrum Esculentum Moench) Seeds

DR. SAPNA DESHWAL

College of Applied Education and Health Sciences, Meerut.
E-mail: deshwalsapna@gmail.com

Abstract

This study investigated the total amylase,alpha and beta amylase activity,moisture content of seed and purification of amylases using polyacryl amide gel electrophoresis during germination of Buckwheat seeds. Commom Buckwheat(Fagopyrum esculentum Moench) seeds were taken and germinated in dark at room temperature. Moisture content and amylase activity were determined throughout the seed germination period. Results observed that moisture increased from 10% to 36% (from 0 to 192 hours) of seed germination and the activity of alpha and beta amylase increased from 0 to day 4 of germination and then decreased gradually up to 192 hours. When calculated, the specific activity of total alpha and beta amylase was found highest at 72 hours,120 hours and 72 hours. For purification of amylase, native poly acrylamide electrophoresis is used.

Key Words: Buckwheat seed(Fagopyrum esculentum Moench), alpha and beta amylases, specific activity

Introduction

Buckwheat is classed as a pseudocereal as it resembles cereal in structure, chemistry and edibility. In recent years, Buckwheat has regained interest as an alternative crop for organic cultivation and as a health food because of its high nutritive value. Buckwheat contains protein of high biological value (due to high amino acid scores) and also contains several anti oxidants. Buckwheat does not contain glutenin and has a potential to be used as a raw material for the production of gluten free beer. It is an abundant source of minerals and contains large amounts of soluble and insoluble dietary fibers.

Various enzymes are known to be involved in the regulation of metabolic activity in plants but amylases play a major role in the metabolism as it is involved in the degradation of starch during seed germination. Amylase is an enzyme that catalyzes the breakdown of starch into sugars. All amylases are glycosidic hydrolases and act on alpha-1,4-glycosidic bonds.

The alpha amylases are calcium metalloenzymes,completely unable to function in the absence of calcium. Along with starch chain, alpha-amylase breaks down the long chain of carbohydrates. Because it can act anywhere on the substrate, alpha-amylase tends to be faster acting than beta-amylase. In animals, it is a major digestive enzyme at a pH of 6.7-7.0. In humans, both salivary and pancreatic amylases are alpha-amylases.

Beta-amylase is also synthesized by bacteria, fungi and plants. Beta-amylase acts from the non-reducing end and catalyzes the hydrolysis of second alpha-1,4 glycosidic bond, cleaving of two glucose units at a time. Beta amylases are also present in seeds in inactive form prior to seed germination

The aim of the present study is focused on studies on amylases during germination of seeds in Buckwheat(Fagopyrum esculentum Moench) seeds. The objectives are to determine moisture content in germinated seeds and measurement of alpha and beta amylase activity from 0 to 92 hours. Purification of alpha amylase is done by PAGE technique.

Material and Methods

The common Buckwheat seeds were brought from the local market and the chemicals used were of Anala R or molecular biology graded, manufactured in India. Instruments manufactured by Indian companies were used. Glasswares used were procured from Borosil, India and Schott Duran, Germany. Micro pipette of different stages were obtained from Eppendorf, Germany.

Each of 2gm seed were washed with distilled water followed by soaking in 0.1% $HgCl_2$ for 3 minutes for sterilization. Then, again the seeds were thoroughly washed under running water for 15 minutes to remove all traces of the sterilizing agent. The seeds were then placed in sterilized petri dishes in dark for germination.

After germination, endosperm of seeds were macerated with little amount of distilled water and 5ml of phosphate buffer(50m/M). Then, this mixture was centrifuged at 8000pm for 15 minutes and supernatant was taken in falcon tubes for enzyme activity.

To determine the moisture content ,for every 24 hours interval 1gm of moisture endosperm were weighed and kept in an oven at 120 degree centigrade for 12 hours. Moisture content is then calculated by subtracting final weight from initial weight and then the percentage of moisture is determined.

During Biochemical analysis, total amylase activity was measured by reduction of 3,5 di-nitro salicylic acid.

Quantification of total protein was done by performing Lowry's method for enzyme extract and recording the absorbance at 610nm with the help of calorimeter.

Purification of amylase is done by PAGE Method. Electrophoresis is widely used to separate and characterize charged molecules such as amino acids and proteins under an applied electric field. Depending on the pH of the medium, various proteins assume different charges and at a pH above the iso-electric point, the proteins have a negative charge. The rate of migration is governed by total charge and mass ratio. Poly acrylamide gel electrophoresis is more convenient as it is possible to alter the pore size of gel in PAGE and better resolution of protein bands can be obtained.

Result and Discussion

In this study, total amylase, alpha and beta amylase activity was determined in germination of seeds. The sample was taken from petri plates firstly at 0 hour and then after every 24 hours until 192 hours. At 0 hour, a low amylase activity was found. Initially, the amylase activity gradually increased from 24 hours to 96 hours and was maximum at 96 hours. After 96 hours, the total amylase activity started decreasing and became very low at 192 hours.

Such trend in amylase activity was found because starch was the only source of energy for the germinating seeds. As amylases start degrading the starch present in seed embryo after 24 hours and this activity became maximum at 96 hours. But after 96 hours, the seedling starts emerging out and the concentration of starch in the seed embryo starts decreasing, so the amylase activity also shows degradation.

The alpha amylase activity was also studied separately and it was observed that alpha amylase was the main contributor in the total amylase activity in the Buckwheat seed. It was found that the alpha

amylase activity first started at 24 hours and became maximum at 96 hours and then showed a decrease in activity as the days pass and becomes very low at 192 hours.

Same trend was found for beta amylase activity but its activity was very low in comparison to alpha amylase activity. At 24 hours, Beta amylase activity increases after 24 hours, decreases at 2nd day and again increases at 3rd day and same at 4th day and then decreases till 192 hours. The total, alpha and beta amylase activity were expressed in units per ml.

Table 1 Total amylase , Alpha amylase and Beta amylase activity in units per 30 minutes

hours	*Total amylase units/Micromoles/30 minutes*	*Alpha amylase units/micromoles/30 minutes*	*Beta amylase units/micromoles/30 minutes*
0	2.42	1.5	0.9
24	2.9	1.6	1.3
48	3.82	2.72	1.1
72	4.26	2.86	1.4
96	4.8	3.4	1.4
120	4.42	3.22	1.2
144	3.74	2.44	1.3
168	3.05	1.95	1.1
192	2.72	1.92	0.8

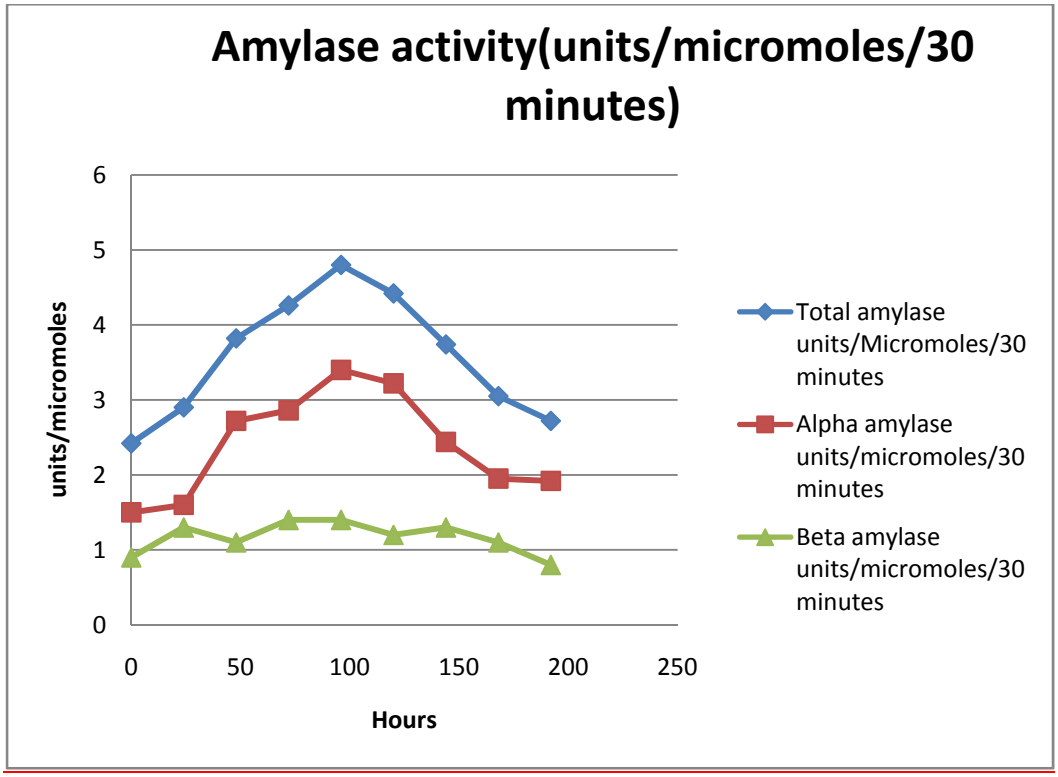
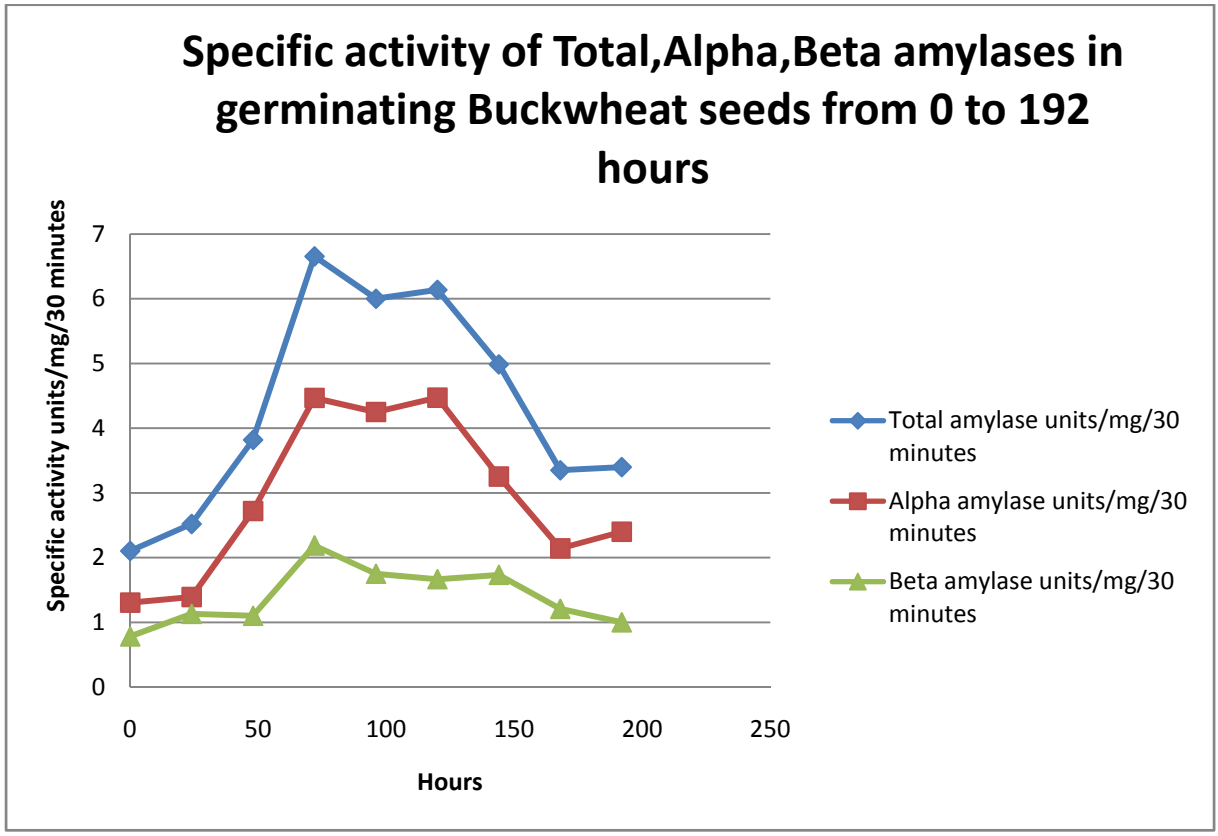


Table 2: Specific activity of Total, Alpha and Beta amylases in germinating Buckwheat seeds from 0 to 192 hours

HOURS	*Total amylase units/mg/30 minutes*	*Alpha amylase units/mg/30 minutes*	*Beta amylase units/mg/30 minutes*
0	2.104	1.304	0.782
24	2.521	1.391	1.13
48	3.82	2.72	1.1
72	6.656	4.468	2.187
96	6	4.25	1.75
120	6.138	4.472	1.666
144	4.986	3.253	1.733
168	3.351	2.142	1.208
192	3.4	2.4	1



Summary and Conclusion

The present study was conducted on the amylases in Buckwheat(Fagopyrum esculentum Moench) seed during germination. The seeds of Buckwheat were germinated and the embryo was taken for preparation of crude extract. This extract was then centrifuged and the supernatant was used for enzyme assays through DNS method and using Lowry's method for protein estimation.

The moisture content of the seed was also calculated and it was found low at 0 hours ,i.e, 10% and maximum at 92 hours,i.e., 36%. The total amylase activity in germinating seeds was also calculated.

It was observed that amylase activity was low at 0 hours and gradually starts increasing and becomes maximum at 96 hours and starts decreasing again and becomes low at 192 hours. Alpha and Beta amylase activity was also calculated separately.

Specific activity of total amylase, alpha amylase and beta amylase was also calculated. The specific activity of total amylase, alpha amylase and beta amylase was found maximum at 72 hours,120 hours and 72 hours. Amylase was also purified by the process of poly acryl amide gel electrophoresis. The bands observed were clear and prominent and bands of amylase were found maximum at stage of 72 hours.

Buckwheat has major potential as a food ingredient especially for the functional food industry. Starch and fibre are present in similar amounts and buckwheat also contains a high level of poly unsaturated essential fatty acids such as linoleic acid, several vitamins B,C and E are present in abundance in comparison to cereals. Buckwheat protein is of high nutritional quality due to its relatively high level of lysine. Alpha amylase contributed a large account to total amylase activity which plays an important role in starch metabolism in developing as well as germinating seeds which can be used for drug discovery and treatment of diseases like diabetes, polycystic ovary syndrome, bowel upset, constipation,etc.

Fagopyrum Buckwheat contains various phytochemical compounds viz. phenols, flavanoids, terpenoids, steroids and fatty acids, which provide Buckwheat a convincing medical potential. It shows anti-allergic, anti-fatigue, anti-oxidant and anti-diabetic activity.

On the other hand, the amylases present in the seeds of Buckwheat play a very important role in the germination of seeds as they help in utilization of starch present in the endosperm of Buckwheat seed.

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