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Research Paper

Effect of Substrate Concentration on the Amylase Activity in Germinating Buckwheat Seeds

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Abstract - *Common Buckwheat (Fagopyrum esculentum Moench) seeds were taken and germinated in dark at room temperature. Dialysis was used for the partial purification of amylases and then, the effect of substrate concentration on amylases was determined. The Km of amylases was 9mg starch and Vmax was 14.30 µ moles of maltose per 30 min. In conclusion, the amylases from the Buckwheat showed high level of activity during seed germination, has low Km and low Vmax. These characterization meets the prerequisite needs of the food industry and useful in the malting of Buckwheat seed for the production of various products.*

Keywords: Buckwheat seed (*Fagopyrum esculentum Moench*), Dialysis, Km, Vmax.

Introduction

Common Buckwheat (*Fagopyrum esculentum Moench*) is a pseudocereal as it is not related to wheat. It is a traditional crop grown in Central and Eastern Europe and Asia. It is a short duration crop and requires a moist and temperate climate to grow. The presence of pollinators greatly increases the yield.

The Buckwheat achenes (fruits) consist predominately of starch, they are edible and they have a starchy endosperm and a non-starch aleurone layer. Buckwheat contains protein of high biological value due to relatively high amino acid scores and high levels of lysine and tryptophan, when compared to cereals. Buckwheat also contains lectin (antioxidant), which has a hypertensive effect and is claimed to strengthen capillary blood vessels and suppresses the internal bleeding.

The embryo in a Buckwheat seed is located in the centre of the endosperm and possesses two cotyledons. The endosperm cells have thin cell walls and consist mainly of starch. Buckwheat seeds are usually processed into flour. This flour is mainly used on Hindu fasting days (Navaratri, Shivratri and Janmastami). So Buckwheat has its aesthetic value also.

Material and Methods

The present study was carried out in the Department of Biochemistry, College of Applied Education and Health Sciences, Meerut. The experiments carried out in the present study were designed to study the kinetic characterization of alpha amylase during germination of seed in Buckwheat.

The seeds were placed in sterilized pteridishes containing moistened filter paper at 27+-2 C in dark for germination.

The purification of enzymes is done by the dialysis technique. The dialysis of the crude extract was carried out with regular change of buffer solution during desalting. The enzyme activity was measured by Bernfeld method. The protein was estimated by the Lowry's method.

The graph was plotted V v/s (S) and 1/V v/s 1/(S).

Results and Discussion

Buckwheat seeds were germinated on filter papers in pteriplates. Samples were drawn for amylase assay and other studies at 24 hours intervals from 0 to 192 hours. The enzyme extract that was prepared by macerating the seed endosperm in phosphate buffer and after centrifugation was used for the partial purification of amylases. For partial purification, dialysis was performed. This result in the partial purification of amylases which were used for the study of enzyme kinetics such as for the determination of Vmax and Km values by studying the effect of substrate concentration on the enzymes.

During the study of the effect of substrate concentration, the velocity of enzyme was increased initially with the increase in the substrate concentration but at a certain point, velocity of enzyme was constant with increase in substrate concentration. The Km and Vmax was determined by double reciprocal plot (Lineweaver Burk Plot) and the Vmax and Km was found to be 14.3 µ moles of maltose produced per 30 min and 9 mg starch respectively.

Conclusion

The present study was conducted on the kinetic characterization of amylases in Buckwheat (*Fagopyrum esculentum Moench*) seed during germination. The crude extract was prepared after germination of seeds and then centrifuged. The supernatant was used for enzyme assays through DNS method and using Lowry Method.

Amylases was partially purified through the process of dialysis and used for the study of enzyme kinetics. The effect of substrate concentration was observed on the enzyme and values of Km and Vmax were determined. The Km was found to be 9mg starch and Vmax was 14.3 µ moles of maltose produced per 30min.

The present kinetic study provides the basis for the further study on amylases in the germinating seed of Buckwheat.

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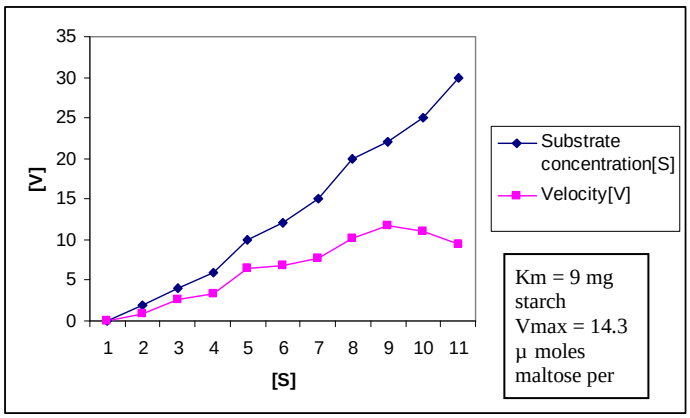

Figure 1: Effect of substrate conc. for amylase activity V v/s (S)

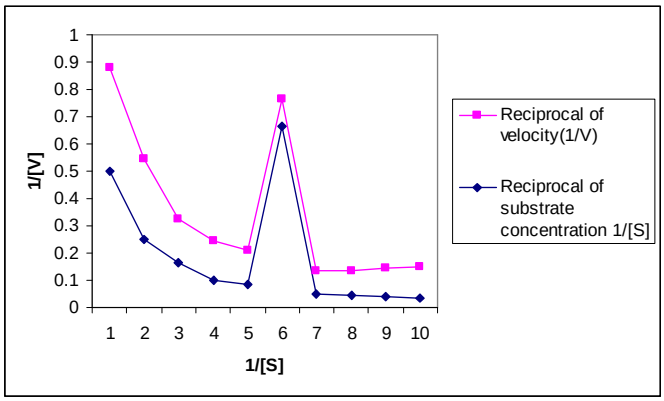

Figure 2: Determination of Km and Vmax for amylase activity by Double reciprocal Plot (Lineweaver Burk Plot)