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Role of an Antioxidant Beta Amylase in Industries as well as Seed Germination and Starch Degradation

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Amylases [which includes the enzymes alpha-amylase, Beta-amylase, and glucoamylase (GA)] are among the most significant enzymes in modern biotechnology. Because of its broad range of possible applications, the amylase family of enzymes is extremely important. In 1894, an amylase from a fungal source was the first enzyme produced industrially, and it was employed as a pharmacological aid for the treatment of digestive diseases which was known as Beta amylase.

Industrial Role

β-Amylase not only used as digestive aid but also due to its saccharogenic activity it plays important role in food industries. Anhydrous crystalline maltose is utilized to absorb moisture as well as for the treatment of dry eyes. To produce DPT (*diphtheria-pertussis-tetanus*) vaccine high purity maltose is used as a major exclusive carbon source in pharmaceutical industries. Saccharogenic activity of *β*-Amylase is used during mashing and malting process in brewing and baking industries for the production of beer and breads.

High maltose syrup (HMS), which contains approximate 50-55% maltose as its carbohydrate constituent is an economical and major source of fermentable solids that makes it a good choice in food. Due to unique properties such as sweetness, density control ability, low hygroscopic, high thermal stability and prevent crystallization at storage, it is used for the production of jellies, bread, canned fruits and wine. Owing to low allergenicity of maltose rich syrup, saccharose is substituted by maltose in child foodstuff. HMS also acts as a stabilizer and decreases freezing point thereby improves the shelf life of the product. Maltitol is sugar alcohol, when it hit to our digestive system it gives only one molecules of glucose. That is why, maltitol is sweet but sweetness is lesser than sugar and has almost half the calories. Owing to these unique properties maltitol is used as a lower caloric diet, which can help to lose weight and manage diabetes.

It has been demonstrated that if the number of glucose units in an amylolytic product is less than 12, re-association between the glucose chains does not occur and thereby retro-gradation is inhibited. It has been demonstrated that *β*-amylosis products of maize and potato had 2-6 glucose units. That is why, *β*-amylase is usedto retard bread staling in baking industries. Researchers showed, when the surface of starch was treated with *β*-amylase, thinning effect was observed on the starch particles situated on the outer most layers. Thinning effect decreases external long chains and increases degree of polymerization values 2-5 units. These surface modified starch nano-particles (30-110 nm) shows resistance to enzymatic hydrolysis and can be used as carrier to deliver functional molecules.

β-Amylase gene is a nuclear encoded gene, which is conserved and used for phylogenetic analysis. Nucleotide sequence analysis in Ipomoea series Batatas revealed that exonic parts had no variation, whereas intron sequences showed variability. These variability in intronic sequences are used to understand evolutionary relationship.

Recently, it has been demonstrated that *β*-amylase from peanuts act as antioxidant and protects the cells from oxidative damage by neutralizing free radicals.

Role of β-Amylase in germinating seeds

Germination is a process in which a plant embryo resumes its metabolic activity in a sequential manner, resulting in growth of radicle and plumule**.** In an un-germinated seed embryo stay on quiescent state and onset of germination the quiescent state becomes metabolically active by absorbing water. This metabolically active embryo uses stored ATP to get energy by hydrolyzing stored lipids, protein or carbohydrates. Further, active amylases present in seeds hydrolyze substrates which provides energy and carbon skeleton to the growing seedling until it becomes photo-synthetically active. Comparative analysis of *α*- and *β*-amylases during rice seed germination revealed greater importance of *β*-amylase over *α* -amylase.

It has been demonstrated by western blot analysis that the molecular mass of the *β*-amylase present in germinated and un-germinated seeds was differ. In case of un-germinated seed it was higher (64 kDa) with relative low enzyme activity compare to germinated seed (59 kDa). These results indicate that the steric hindrance between substrate and active site is removed by proteolytic cleavage during germination of the enzyme .

During germination of barley seed it was found that there was an enhancement in enzyme activity. This enhancement in enzyme activity might be due to release of free *β*-amylase from bound form during germination process. This bound form was released by breaking the di-sulphide bond by cysteine endopeptidases such as papain. These results was confirmed by using leupeptin which inhibit -SH proteinases , thereby no liberation of bound was observed Barley seeds, which had previously been demonstrated to have free and bound *β*-amylase, released extra quantity of Barley*β*-amylase when was treated with SDS and β-mercaptoethanol (ME). This extra fraction of *β*-amylase was concerned as latent *β*-amylase. Thus,

$$\text{Total } \beta-\text{amylase} = \text{Free} + \text{Bound} + \text{Latent } \beta-\text{amylase}$$

However, it have also been demonstrated using Northern blot analysis and incorporation of radioactive isotopes (in maize and rice) that ungerminated *β*-amylase is not long-lived and it is degraded during germination and denovo synthesis of *β*-amylase occurs. further, eukarytoic protein synthesis inhibitors (cyclohexamide) and labeling with deuterium oxide followed by cesium chloride density gradient centrifugation techniques have also been employed to confirm the denovo synthesis of the enzyme during germination.

Role of β-Amylase in starch degradation

Starch is a polysaccharide, which is major source of carbon and energy and present in granular form in leaves, fruits, seeds, roots and stems. These granules have specific size and morphology, which are unique for a particular plant. Starch is made up of two type's polymers namely amylose and amylopectin. Amylose (25% of total glucose units) is considered as a linear polymer of dextrose, which are connected via *α*-(1,4)-glycosidic linkage having less than 0.8% of branched molecules formed by *α*-(1,6)- linkage, whereas remaining 75% glucose units are present in the form of amylopectin, which has 5% [*α*-(1,6)] branched molecules. To enhance beneficial characteristics and eliminate negative attributes chemical, physical or enzymatic methods can be used to modify the starch granules. Among these methods enzymatic hydrolysis is frequently used at industrial scale.

During enzyme hydrolysis of starch, longer chain of amylose and amylopectin are broken and glucose, maltose, fructose and maltodextrin are generated. Generally the enzyme hydrolysis is carried out upon geletinized starch. When starch is heated in excess amount of water, starch granules absorb water and become hydrates, which break the helical structure, consequently hydrogen bonding are ruptured and finally due to so much swelling of granules, granular form is lost and amylose and amylopectin are exposed. These exposed chains increase the rate of enzyme hydrolysis.

Dextrose equivalent (DE) values are used to a measure the total reducing capacity of the molecules with respect to dextrose on a dry mass basis. For instance maltose is disaccharide and its molecular weight is 342 g/mol. DE for maltose was calculated using following formula and it was found to be 52.

$$DE = 100X180/\text{Molecular weight of test sample}$$

where 100 and 180 is DE and molecular weight for dextrose.

It has been demonstrated that β-amylase play a crucial role in starch metabolism. To confirm this, transgenic potato plants having chloroplast-targeted β-amylase isoform were repressed by antisense technique. Results revealed that plant leaves shows reduced activity of the enzyme along with starch excess phenotype. Study of transgenic and mutant plants in *Arabidopsis thaliana*lacking β-amylase have demonstrated the importance of the enzyme in starch hydrolysis**.**