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Ritu Saini
Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

Harnek Singh Saini
Department of Biotechnology Engineering, UIET, Kurukshetra University, Kurukshetra, Haryana, India

Anjali Dahiya
Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Harnek Singh Saini
Department of Biotechnology Engineering, UIET, Kurukshetra University, Kurukshetra, Haryana, India

Amylases: Characteristics and industrial applications

Ritu Saini, Harnek Singh Saini and Anjali Dahiya

Abstract
Amylases are widely distributed in microbial, plant and animal kingdoms. They degrade starch and related polymers to yield products characteristic of individual amylolytic enzymes. Alpha amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. The pH dependence of amylase activity was assayed in a pH range of 4-11, using the standard reaction mixture. The optimum temperature for maximal Amylase activity was found to be 30-35 ^{0}C, after which the enzyme activity dropped. The addition of metals like Ca^{2+} and Mn^{2+} and Mg^{2+} increased the enzyme activity while a significant inhibitory effect on the protease activity was observed with Hg^{2+}. Amylase from apple exhibited high efficiency for the removal of chocolate stains in combination with commercial detergent (Tide). The wash performance analysis of chocolate stains on cotton fabric showed an increase in reflectance with detergent and enzyme as compared to detergent only.

Keywords: Amylases, industrial applications, characterization, apple

1. Introduction
Biotechnology is considered as a useful alternative to conventional process technology in the industrial and analytical fields because of many advantages it has over chemical catalysis. Some of the advantages may include biological systems help in ingredient substitution, less undesirable products, increased plant capacity, increased product yields and at the same time they are less energy intensive and less polluting. Amylases are amongst the most studied enzymes (Noomen *et al*., 2009) [23]. This vast diversity of amylases, in contrast to the specificity of their action, attracted worldwide attention in attempts to exploit their physiological and biotechnological applications (San-Lang Wang *et al*., 2011) [37]. Amylases are starch degrading enzymes that catalyze the hydrolysis of internal alpha 1-4 glycosidic bonds in polysaccharides with the retention of alpha anomeric configuration in the products (Takata *et al*., 1992) [40]. They are found in all forms of organisms regardless of kingdom. Amylases from plants and microbe sources have been employed for centuries in brewing industry. Fungal amylases are widely used for the preparation of oriental foods (Mabel *et al*., 2006) [18]. Amylases of bacteria, fungi and viruses are increasingly studied due to the relative ease of large scale production (low downstream cost as they are extracellular in nature) as compared to amylases from plants and animals and their importance in subsequent application at industry (Ashis *et al*., 2009) [5].

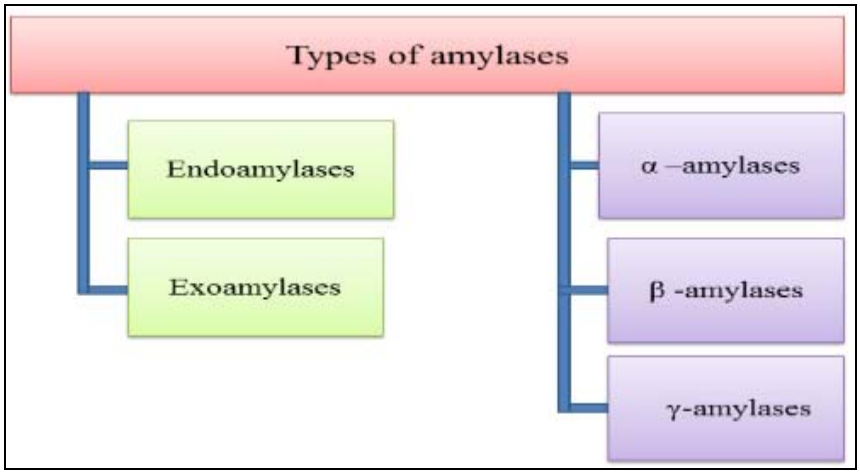


Fig 1: Different types of amylases

Alpha amylases (α-1, 4-glucan-glucanohydrolase, EC 3.2.1.1) is an extracellular enzyme (Cherry *et al*., 2004) [8]. This enzyme degrades α-1, 4-glucosidic linkage of starch and related products in an endo fashion and produce oligosaccharides (Zubeyde *et al*., 2008) [44]. β - Amylase (1, 4-α-D-glucan maltohydrolase; glycogenase; saccharogen amylase, EC 3.2.1.2) is another form of amylase, β-amylase is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1, 4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Both α-amylase and β-amylase are present in seeds. β- amylase is present in an inactive form prior to germination, whereas α-amylase and proteases appear once germination has begun. Cereal grain amylase is the key to the production of malt. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain β-amylase, although it may be present in microorganisms contained within the digestive tract.

γ- Amylase (EC 3.2.1.3) (Alternative names: Glucan 1, 4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase) In addition to cleaving the last α (1-4) glycosidic linkages at the non-reducing end of amylose and amylopectin, yielding glucose, γ-amylase will cleave α (1-6) glycosidic linkage. Unlike the other forms of amylase, γ-amylase is most efficient in acidic environments and has an optimum pH 3. Amylase is also used in industry. It is used in brewing and fermentation industries for the conversion of starch to fermentable sugars, in the textile industry for designing textiles, in the laundry industry in a mixture with protease and lipase to launder clothes, in the paper industry for sizing, and in the food industry for preparation of sweet syrups, to increase diastase content of flour, for modification of food for infants, and for the removal of starch in jelly production. For the ethanol production, starch is the most used substrate due to its low price and easily available raw material in most regions of the world.

2. Sources of alpha amylase

Alpha amylase can be derived from several sources such as plants, animals and microbes. The microbial enzyme meets the industrial demand and a large number of them are available commercially (Pandey *et al*., 2000) [27]. Alpha amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Fungal sources are mostly terrestrial isolates such as *Aspergillus* species. Amylases from plant and microbial sources are employed for centuries as food additives (Mabel *et al*., 2006) [18]. Barley amylases are used in Brewing industry. Fungal amylases are widely used in preparation of oriental foods (Popovic *et al*., 2009) [30]. Fungal and bacterial amylases are mainly used for industrial applications due to their cost effectiveness, consistency, less time and space requirement for production and ease of process optimization and modification (Ellaiah *et al*., 2002) [10].

Among bacteria Bacillus sp. is widely used for the production of amylases. Species like B.subtilis, B. stearothermophilus, B. licheniformis, and B. amyloliquefaciens are known to be good producers of alpha amylase. Similarly filamentous fungi have been widely used for the production of amylases for centuries. As these moulds are known to be prolific producers of extracellular proteins, they are widely exploited for the production of different enzymes including alpha amylases (Juliana *et al*., 2011) [14]. Fungi belonging to the genus Aspergillus have been most commonly employed for the production of alpha amylase. Production of enzymes by solid state fermentation using these moulds turned a cost effective production technique. Starch in cereal, root and tuber crops has been extensively characterized, but little study has been done on characterization of fruit starches and its impact on fruit texture. Apples, like many other fruit crops, accumulate starch at early stages of maturation and progressively degrade starch to increase sweetness during ripening (Parveen *et al*., 2011) [28].

3. Purification of enzyme

Purification process in downstream processing after fermentation strongly depend on the market, processing cost, final quality and available technology. Most enzymes are purified by Chromatographic techniques after crude isolation by precipitation and membrane separations (Prakash *et al*., 2009) [31]. The need for large scale cost effective purification of proteins has resulted in evolution of techniques that provide fast, efficient and economical protocols in fewer processing steps (San-Lang *et al*., 2011) [37]. Table 1 showed the results of partial purification of amylase from apple are summarized (Saini *et al*., 2016) [34].

Table 1: Purification summary of the amylase obtained from the Apple.

S. No	Purification Step	Volume	Total Enzyme Activity (units/ml)	Total Protein	Specific Activity (U/mg)	Purification Fold	Yield %
1.	Crude Extract	1000	204	800	0.2	1	100
2.	Ammonium Sulphate	200	81	200	0.4	2.2	40
3.	Gel Filtration	20	11	2.4	4.76	24	11

Safey and Ammar (2004) [33] reported approximately 5.66 fold purification of amylase from *Aspergillus Falvus var. columnaris* by ammonium sulphate precipitation 60% whereas 9.6 fold purification reported in case of gel filteration chromatography. Similarly (Shih *et al.,* 1995) [39] reported approximately 20.5 fold purification of Amylase from *Clostridium perfringens* in case of gel filteration chromatography. The purified alpha amylase exhibited an isoelectric point of 3.7, and a molecular weight of 65,000. The optimum enzyme activity was obtained at pH 5.5, and the enzyme showed stability at 40°C. The main end-products of maltohexaose, hydrolysis were glucose and maltose. Although its capability to gradually degrade some α1-6 linkages, purified enzyme ought to be classified as an alpha-amylase. (Planchot and Colonna, 2008) [29]. Downstream processing for the production of pure enzymes can generally constitute a major percentage of overall production cost especially if end purity requirements are stringent. Purification process in downstream processing after fermentation strongly depend on the market, processing cost, final quality and available technology. Most enzymes are purified by Chromatographic techniques after crude isolation by precipitation and membrane separations (Prakash *et al*., 2009) [31].

4. Characterization of Enzyme

4.1 Effect of pH on the activity of amylase

Effect of pH on alpha amylase purified from *Malus pumila* was determined by assaying enzyme at different pH ranging from 1-10 and amylase showed a pH optimum of 6.8 (Kanwal *et. al*., 2004) [15]. Similarly Gouda and Elbahloul (2008) [11] determined the effect of pH on amylase produced by halotolerent *Penicillillum sp.* Partial characterization revealed presence of 2 enzymes with pH optima at 9 and 11. For alpha amylase from *Penicillium olsonii* under the effect of some antioxidants vitamins, pH optimum was determined after incubation at different pH 3.6 to 6.8 at 30 ^{0}C for 30 minutes and maximum activity of enzyme was recorded at pH 5.6, 30 ^{0}C by (Affifi *et. al*., 2008) [1]. Safey and Ammar (2004) [33] has observed optimum pH for the activity of amylase from *Aspergilus falvas var. columnaris* by incubation at different pH viz. 5.8 to 8.0 using phosphate buffer and enzyme shown a pH optima at 6.2. Alpha amylase producing yeast strains such as S. cerevisiae and S. kluyveri exhibited maximum enzyme production at pH 5.0 (Samrat *et al*., 2011) [36].

4.2 Effect of temperature and metal ions on the activity of amylase

The influence of temperature on amylase production is related to the growth of the organism (Pandey *et al*., 1990) [26]. The temperature stability for the alpha amylase obtained from *Malus pumila* was determined by keeping at various temperature ranges 5 ^{0}C to 75 ^{0}C and optimum temperature for efficient growth and activity was 37 ^{0}C as reported by (Kanwal *et al*., 2004) [15]. For alpha amylase from *Penicillium olsonii* under the effect of some antioxidants vitamins, pH optimum was determined after incubation at different pH 3.6 to 6.8 at 30 ^{0}C for 30 minutes and maximum activity of enzyme was recorded at pH 5.6, and at 30 ^{0}C temperature (Affifi *et. al.,* 2008) [1]. Alpha amylase producing yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces kluyveri* exhibited maximum enzyme production at pH 5.0 (Samrat *et al.,* 2011) [36]. The addition of Ca^{+2}, Mn^{+2} and Mg^{+2} increased the enzyme activity A significant inhibitory effect on the protease activity was observed with Hg^{2+} (0% relative activity). Other metal ions which had a negative impact included Na^{+}, K^{+}, Cu^{+2}, Fe^{+2} and Zn^{+2}. Addition of Calcium chloride to the fermentation media increased the enzyme production (Arthur *et al*., 1996) [4].

Fig 2: Properties of α-amylase (Saini *et al*., 2016) [34].

5. Industrial application of amylase

Amylase, a starch degrading enzyme have gained importance in various industrial process like pharmaceutical, food, brewing, paper, textile and chemicals. It is extensively used in pharmaceutical industries in digestive tonics, for hydrolysis of starch to produce different sugars like glucose and maltose which have several applications. The most widespread applications of α-amylases are in the starch industry, which are used for starch hydrolysis in the starch liquefaction process that converts starch into fructose and glucose syrups (Nielsen and Borchert, 2000) [22].

5.1 Manufacture of high fructose containing syrup and maltose

High fructose containing syrups (HFCS) 42 F (Fructose content equal to 42%) is prepared by enzymic isomerization of glucose with glucose isomerase. The starch is first converted to glucose by enzyme liquefaction and saccharification. Maltose is a naturally occurring disaccharide. Its chemical structure has 4-0-α-D-glucopyronosil-D-glucopyranose. It is the main component of maltose sugar syrup (Yakup *et al*., 2010) [43]. Maltose is widely used as sweetener and also as intravenous sugar supplement. It is used in food industry because of low tendency to be crystallized and is relatively non-hygroscopic (Sameh *et al*., 2011) [35]. Corn, potato, sweet potato and cassava starches are used for maltose manufacture (Uma *et al*., 2007). The concentration of starch slurry is adjusted to be 10-20% for production of medical grade maltose and 20-40% for food grade. Thermostable alpha amylase from *B. licheniformis* and *B. amyloliquefaciens* are used (Archana *et al*., 2011) [3].

5.2 Manufacture of oligosaccharides mixture

Oligosaccharides mixture (Maltooligomer mixture) is obtained by digestion of corn starch with alpha amylase, beta amylase and pullulanase. Maltooligomer mix is a new commercial product. Its composition is usually as follows: Glucose, 2.2%; maltose, 37.5%; maltotriose, 46.4%; and maltotetrose and larger malto oligosaccharides, 14% (Marc *et al*., 2002) [19].

Maltooligomer mix powder obtained by spray drying is highly hygroscopic. Therefore it serves as a moisture regulator of the food with which it is mixed (Takata *et al*., 1992) [40]. Maltooligomer mix tastes less sweet than sucrose. It has lower viscosity than corn syrup because of its low content of glucose. Maltooligomer mix is mainly used as a substitute for sucrose and other saccharides. It is also used for preventing crystallization of sucrose in foods (Vander *et al*., 2002) [42].

5.3 Removal of starch sizer from textiles (Desizing)

In textiles weaving, starch paste is applied for warping. This gives strength to textiles at weaving. It also prevents the loss of string by friction, cutting and generation of static electricity on the string by giving softness to the surface of the string due to laid down wrap. After weaving the cloth, the starch is removed and the cloth goes to scouring and dyeing. The starch on cloth is usually removed by application of alpha amylase (Allan *et al.,* 1997) [2]. Textile industries are extensively using alpha amylases to hydrolyze and solubilize the starch, which then wash out of the cloth for increasing the stiffness of the finished products. Fabrics are sized with starch. Alpha amylase is used as desizing agent for removing starch from the grey cloth before its further processing in bleaching and dyeing. Many garments especially the ubiquitions' Jean' are desized after mashing. The desired fabrics are finally laundered and rinsed (Iqbal *et al.,* 1997) [12].

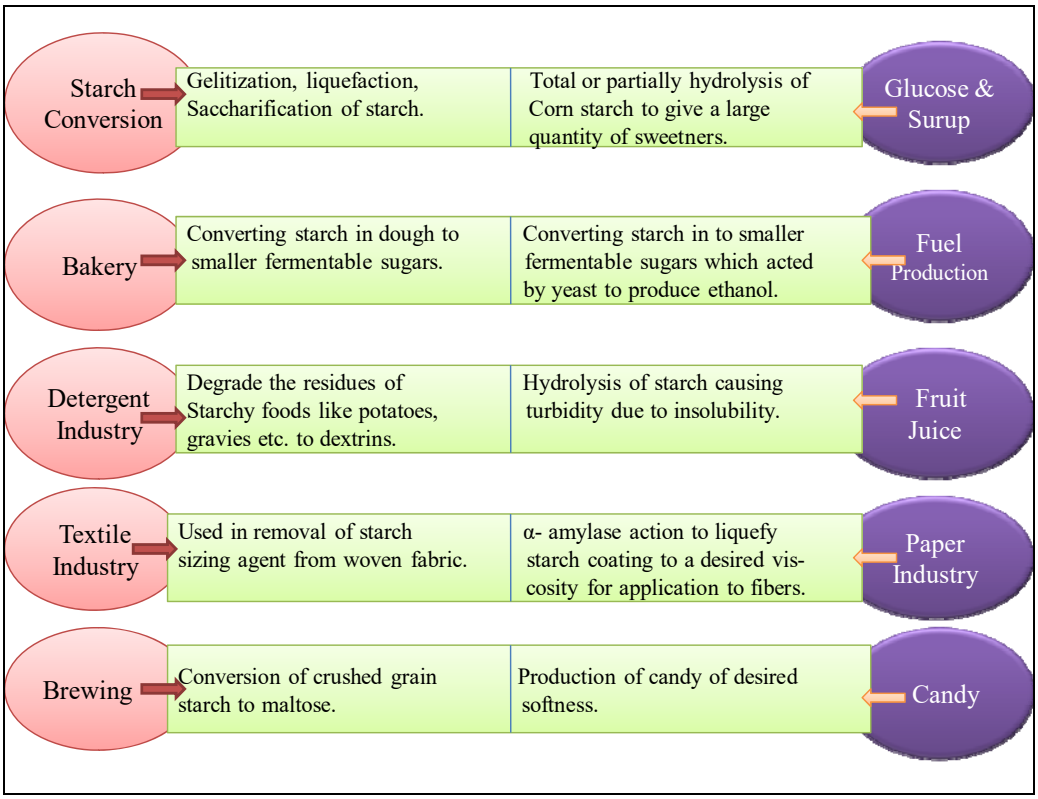


Fig 3: Industrial applications of amylases

5.4 Liquefaction

Liquefaction is a process of dispersion of insoluble starch granules in aqueous solution followed by partial hydrolysis using thermostable amylases. In industrial processes, the starch suspension for liquefaction is generally in excess of 35% (w/v) (Damien *et al*., 2010) [9]. Therefore the viscosity is extremely high following gelatinization (Vander *et al*., 2002) [42]. Thermostable α-amylase is used as a thinning agent, which brings about reduction in viscosity and partial hydrolysis of starch. Retrogradation of starch is thus avoided during subsequent cooling (Sang-Lang *et al*., 2011) [37].

5.5 Bread and chapatti industry

Amylases play important role in bakery products. For decades, enzymes such as malt and fungal alpha-amylases have been used in bread-making. Amylases can degrade starch and produce small dextrins for the yeast to act upon. The alpha-amylases degrade the damaged starch in wheat flour into small dextrins, which allows yeast to work continuously during dough fermentation, proofing and the early stage of baking. The result is improved bread volume and crumb texture. In addition, the small oligosaccharides and sugars such as glucose and maltose produced by these enzymes enhance the Maillard reactions responsible for the browning of the crust and the development of an attractive baked flavour (Lundkvist *et al*., 2007) [17].

5.6 Direct starch fermentation to ethanol

The amylolytic activity rate and amount of starch utilization and ethanol yields increase in several folds in co cultures (Reeta *et al*., 2009) [32]. Moulds amylases are used in alcohol production and brewing industries. The advantages of such systems are uniform enzyme action in mashes, increase rate of saccharification, alcohol yield and yeast growth (Maria *et al*., 2011).

Alpha amylases convert starch in to fermentable sugars. Starches such as grain; potatoes etc. are used as a raw material that helps to manufacture ethyl alcohol. In the presence of amylases, the starch is first converted in to fermentable sugars. The use of bacterial enzyme partly replaces malt in brewing industry, thus making the process more economically significant. Alpha amylase can also carries out the reactions of alcoholysis by using methanol as a substrate (Santamaria *et al*., 1999) [38].

5.7 Chocolate industry

Amylases are treated with cocoa slurries to produce chocolate syrup, in which chocolate starch is dextrinizing and thus syrup does not become thick. Cocoa flavored syrups having a high cocoa content and excellent stability and flow properties at room temperature may be produced by using an amylolytic enzyme and a sufficient proportion of Dutch process cocoa to provide a syrup pH of 5.5 to 7.5. The syrup is made by alternate addition of cocoa and sweetener to sufficient water to achieve a solids content of about 58 to 65 weight percent, adding an amylolytic enzyme, heating to a temperature of about 175 -185°F for at least 10 to 15 min, raising the temperature to about 200° F. and cooling. The stabilized cocoa flavored syrups may be added at room temperature to conventional non-acid confection mixes for use in the production of quiescently frozen chocolate flavored confections (Ismail *et al.,* 1992) [13].

5.8 Paper industry

Starch paste when used as a mounting adhesive modified with additives such as protein glue or alum, frequently, causes damage to paper as a result of its embrittlement. Starch digesting enzymes, e.g. alpha amylase, in immersion or as a gel poultice are applied to facilitate its removal. Alpha amylase hydrolyzed the raw starch that is used for sizing and coating the paper instead of expensive chemically modified starches. So, starch is extensively used for some paper size press publications (Okolo *et al*., 1996) [24].

5.9 Detergent, Building product and Feed industries

In detergent industries, the enzyme alpha amylase plays a vital role. It is widely used for improvement of detergency of laundry bleach composition and bleaching without color darkening (Borchet *et al*., 1995; Atsushi and Eiichi, 1998) [7, 6]. The addition of enzyme stabilizes the bleach agent and preserves effectiveness of the bleach in laundry detergent bar composition (Onzales, 1997; Mirasol *et al*., 1997) [25, 21] Modified starch is used in the manufacture of gypsum board for dry wall construction. Enzyme modified the starch for the industry use. Many starches or barely material are present in the feed. So, the nutritional value of the feed can be improved by the addition of alpha amylase.

5.10 Bio fuel production

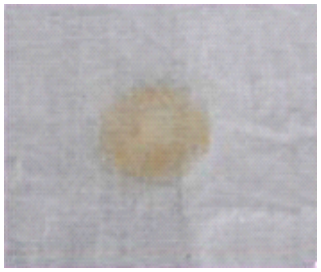
Fossil fuels cause a lot of pollution they do more harm than the benefits. Over the last few decades due the environmental concern and high prices of the fuels, bio fuels have generated so much interest. Bio fuels mainly include ethanol fuel. Ethanol can be derived from renewable resources such as waste generated from the agriculture crops and by products. Enzymes such as alpha amylase and others like glucoamylase and cellulose are important to produce fermentable sugars to produce ethanol (Kirk *et al*., 2002) [16].

5.11 Removal of starchy stains

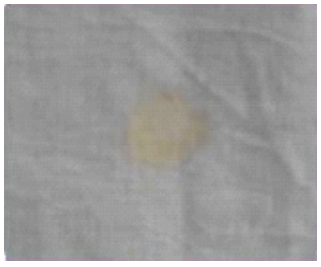
Wash performance (against baby food and chocolate stain) of alkaline α-amylase from Apple in combination with commercial detergent (Tide)'.

- **Cloth stained with chocolate**

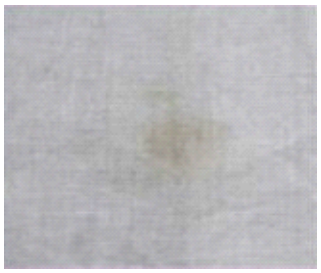
- **Stained cloth washed with water only**



- **Stained cloth washed with enzyme only**



- **Stained cloth washed with detergent only**



- **Stained cloth washed with detergent and enzyme**

Fig 4: Removal of starchy stains by apple amylase

Amylase from apple was tested for its application in for the removal of starchy stains from the fabrics and has shown potential results in removal of stains in combination with detergent (Saini *et al*., 2016) [34].

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