Industrial Enzymes

Enzyme Engineering

Bulk enzymes vs Medical enzymes

Industrial enzymes	Medical/diagnostic proteins
Produced in large quantities	Produced in small quantities
Partially purified at best	Extensively purified
Economic considerations critical	Economic considerations are of secondary importance to functional excellence of product
Function: catalytic	Function: various (hormones, growth factors, cytokines, other regulatory factors, blood factors, vaccines, antibodies, enzymes)
Source: mainly microbial and recombinant	Source: mainly human or animal and recombinant products
Mainly secreted into the extracellular medium by producer strains	May be intracellular or extracellular

Annual Sales of bulk enzymes

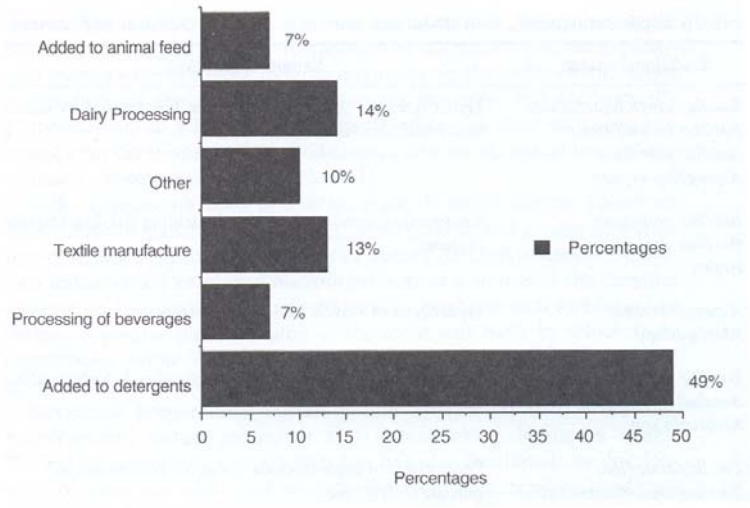
Enzyme	Market value (US$, millions)
Proteases	700
Cellulases	180
α-Amylases	135
Lipases	90
Hemicellulases	75
Glucoamylases	60
Pectinases	60
Lactases	15
Glucose isomerase	15
Pullulanases	15
Others	100

Enzyme	Traditional source	Sample applications
α-Amylase	*Bacillus amyloliquefaciens* *Bacillus licheniformis* *Bacillus subtilis* *Aspergillus oryzae*	Hydrolyzes α 1–4 linkages in starch. Used to liquify starch and reduce its viscosity
β-Amylase	*Bacillus polymyxa* *Bacillus circulans* Barley	Enzymatic degradation of starch yielding the disaccharide maltose
Glucoamylase (Amyloglucosidase)	*Aspergillus niger* *Rhizopus* spp.	Hydrolysis of starch, yielding dextrose
Pullulanase	*Bacillus* spp. *Aerobacter aerogenes* *Klebsiella* spp.	Debranching of starch by hydrolysis of α 1–6 glycosidic linkages
Glucose isomerase	*Bacillus coagulans* *Bacillus stearothermophilus* *Streptomyces* spp. *Arthrobacter*	Production of high-fructose syrup by conversion of glucose to fructose
β-Galactosidase (lactase)	*Bacillus coagulans* *Streptomyces* spp. *Saccharomyces* spp. *Aspergillus* spp.	Hydrolysis of milk lactose yielding glucose and galactose

Invertase (Sucrase)	*Saccharomyces* spp.	Hydrolysis of sucrose, yielding glucose and fructose
Cellulase & hemicellulase	*Trichoderma* spp. *Sporotrichum cellulophilum* *Actinomyces* spp. *Aromonas* spp. *Aspergillus niger*	Enzymatic hydrolysis of cellulose-containing material
Pectinases	*Aspergillus niger* *Fusarium* spp.	Enzymatic hydrolysis of pectin
Proteases	Various bacilli species *Bacillus amyloliquefaciens* *Bacillus subtilis* *Streptomyces* spp. *Aspergillus oryzae* Some animal sources such as calf stomach	Enzymatic hydrolysis of proteins widely used in detergents and in brewing, baking and meat tenderization
Lipases	*Mucor* spp. *Myriococcum* spp. Animal pancreas	Enzymatic hydrolysis of lipids. Used in dairy industry for flavour development in foods and also used in detergents

Bacillus or *Aspergillus* are classified as GRAS (Generally recongnized as safe)

Industries using enzymes



Impact of genetic engineering

- Advantages

1. High expression level
2. High purity
3. Economically attractive
4. GRAS-listed species can be used regardless of the source
5. Allowing alteration of enzyme's characteristics via protein engineering

Isolation step	Baker's yeast	Recombinant yeast
Purification steps	10	4
Biomass (tonnes)	236	10
Specific activity (kU/g)	0.4	11
Yeast cell debris (tonnes)	440	12
Ammonium sulfate (tonnes)	1100	25
Potassium phosphate (tonnes)	25	0.5
Alumina absorbent (tonnes)	90	0
Filtration aids (tonnes)	133	5
Water (m^3)	3700	50
Electricity (kWh)	45 000	9000

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Some protein engineered commercial enzymes

Enzyme (Tradename)	Manufacturer	Description
Purafect 0x Am	Genencor Int.	Oxidation resistant α-amylase
Duramyl	Novo Nordisk	Oxidation resistant α-amylase
Lipomax	Gist Brocades	Oxidation resistant lipase
Lipolase ultra	Novo Nordisk	Oxidation resistant lipase
Maxapem	Gist Brocades	Oxidation resistant protease
Everlase	Novo Nordisk	Oxidation resistant protease

1. Proteases

- Four kinds of proteases

Protease class	Examples
Serine proteases	Trypsin, chymotrypsin, elastase, subtilisins, proteinase K
Aspartic proteases	Pepsin, rennin (chymosin), microbial aspartic proteases
Cysteine proteases	Papain, ficin, bromelain
Metalloproteases	Collagenase, elastase, thermolysin

1. Proteases

Industry	Use
Beverage	Solubilization of grain proteins, stabilization of beer
Detergent	To catalytically degrade protein-based stains on clothing
Bread/confectionery	To modify gluten elasticity
Cheese production	To coagulate casein, forming curds. To ripen cheese
Leather processing	To dehair hides; to bate (soften) leather
Meat	To tenderize meat

1. Proteases in detergent

- Biological “dirt” includes protein-, lipid-, and carbohydratre-based stains
- Protein (blood, egg, etc..) are denatured and aggregated by washing process
- Proteolytic enzyme must be stable in alkaline condition and at relatively high temp.→ serine protease
- Produced from *Bacillus licheniformis*, *B. lentus*, *B. amyloliquefacience*, etc.
- Subtilisin Carsberg has Michelis-Menten kinetics and is active at pH 8-10 and 50 C
- Oxidation resistance is needed due to the increasing use of bleach → Changing methionine to other amino acids

Proteases in cheese production

- The first step of cheeses making is coagulation of milk
- Rennin catalyzes milk κ-casein and promotes coagulation
- Produced as a form of prorennin
- Rennin is obtained from fourth stomach of calves : Supply fluctuated → Recombinant rennin from *E. coli* was first approved in 1990 (The first food ingredient made by recombinant DNA technology)

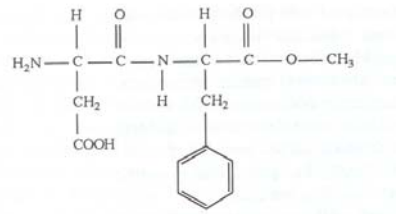
1. Proteases in Meat tenderization and Leather processing

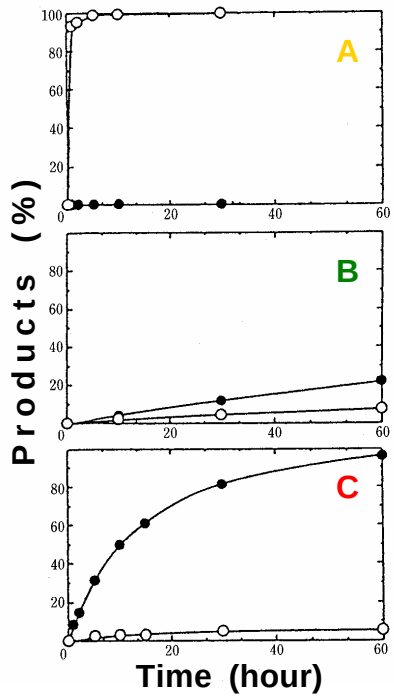
- Meat from old animals have a high degree of cross-linked collagen, needing tenderization
- Storing cold room for a few days or adding papain (a cytein protease) can be used
- Papain can be injected to animal before slaughter

- Leather treatment involves removing lipid, water, and some of the surface protein (mainly collagen)
- Pancreatic or microbial proteases are used to smoothening the animal skin
- Enzymatic dehairing or dewooling process reduce the environmental problem

1. Proteases for aspartam synthesis

- Aspartame is 200 times sweeter than sugar
- Its taste depends on L-configuration, making chemical synthesis difficult
- Synthesized by neutral metalloprotease thermolysin from *Bacillus thermoproteolyticus*
- By controlling reaction conditions, protease can be used to synthesize peptide bonds





Or by making mutants

A : Native subtilisin

B : A의 Ser(221) → Cys(221)

C : A의 Ser(221) → Cys(221)
Pro(225) → Ala(225)

○ : Proteolysis
● : Synthesis

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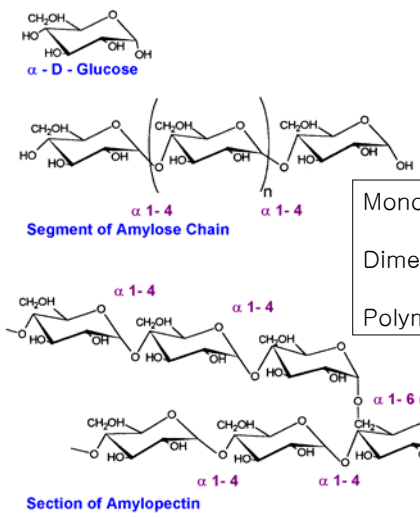
2. Carbohydrases

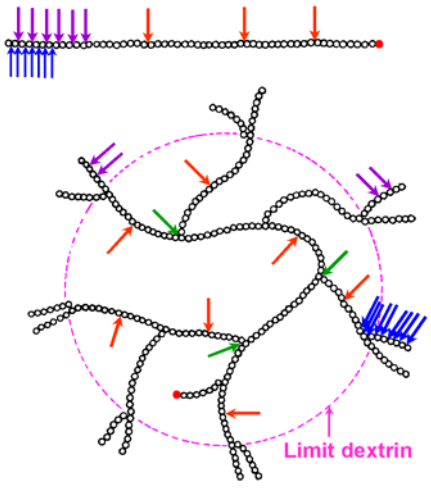


Monomer : Glucose, Fructose, Galactose

Dimer : Sucrose, Maltose, Lactose

Polymer : Starch, Cellulose, Pectin

2. Carbohydrases

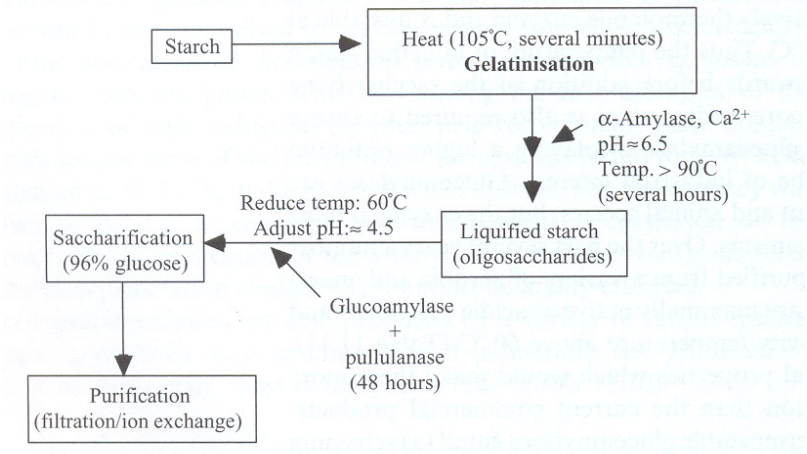


2. Carbohydrases

Industry/process	Amylolytic enzymes employed
Production of glucose/ maltose syrups	α-Amylases, β-amylase, debranching enzymes
Brewing/alcohol production	α-Amylase, β-amylase, amyloglucosidase
Animal feed additive	α-Amylase
Baking industry	α-Amylase, β-amylase, amyloglucosidase, Debranching enzymes
Laundry detergent additive	α-Amylase
Production of dextrins	α-Amylase
Fruit juice processing	α-Amylase, amyloglucosidase
Textile deisizing	α-Amylase

2. Carbohydrases

- Hydrolysis of starch



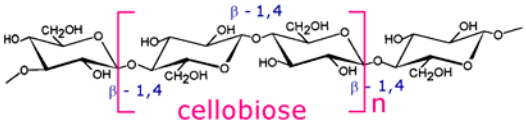
2. Carbohydrases

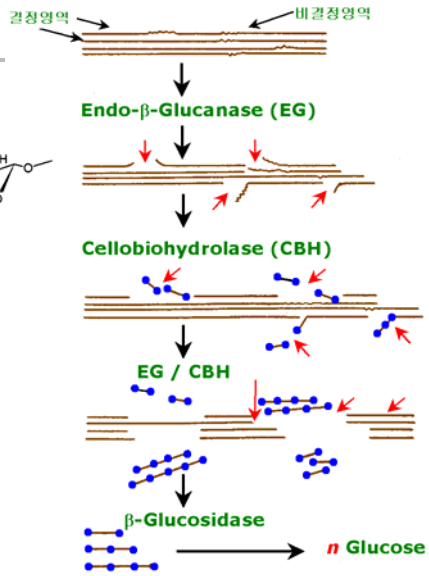
- α-Amylase : The gelatinization process achieved at 100 C for several minutes → Heat stable
  - *Bacillus amyloliquefaciens* or *B. lichemiformis*
  - GMO Tobacco with *B. lichemiformis* α-Amylase (highly glycosylated) improved the process
- Glucoamylase : Producing glucose (Monosaccharide)
  - *Aspergillus niger*
- β-Amylase : Producing maltose (Disaccharide)
  - Produced from plant, *Bacillus*, or *Clostridium*
- Pullulanase or Isoamylase (α1→6 Glucosidase)
  - Linear α1→6 Glucosidase (Pullulanase) or branched (Isoamylase)

2. Carbohydrases

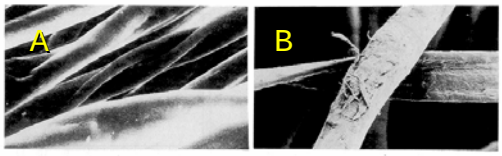
- Glucose isomerase
  - Conversion from glucose to sweeter monosaccharide fructose (과당)
  - Intracellular enzymes (production is more demanding)
  - Used in immobilized state
- α-Amylase has wider applications in detergent applications or textile desizing

3. Cellulases





3. Cellulases

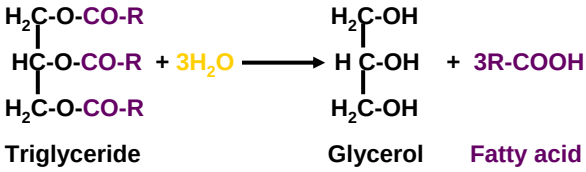


Rmoving microfibril by Cellulase

A: New Textile B: Old Textile
C: Old textile after treated with Cellulase

- Detergent application
- Textile industry (Stonewashing of denim or biopolishig)
- Deinking of newspaper

4. Lipases



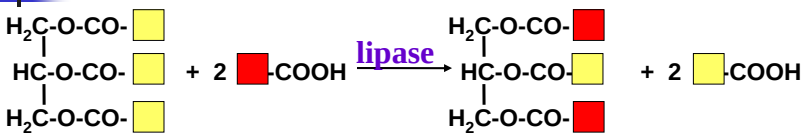
- Detergent application
- Food industry
- Organic synthesis
- Pulp processing

4. Lipases

- Lipid is the most difficult to remove
- Lipases was added in detergent from 1970's
- Multi-cycle effect : Lipids are removed after second cycle of washing because lipases are active when water content of fabric is low

Enzyme brand name	Manufacturer	Lipase gene sourced from	Lipase gene expressed in
Lipolase	Novo Nordisk	*Humicola lanuginosa*	*Aspergillus oryzae*
Lipomax	Gist Brocades	*Pseudomonas alcaligenes*	*Pseudomonas alcaligenes*
Lumafast	Genencor	*Pseudomonas mendocina*	*Bacillus* sp.
Lipolase Ultra	Novo Nordisk	Protein engineered variant of lipolase	
Lipo Prime	Novo Nordisk	Protein engineered variant of lipolase	

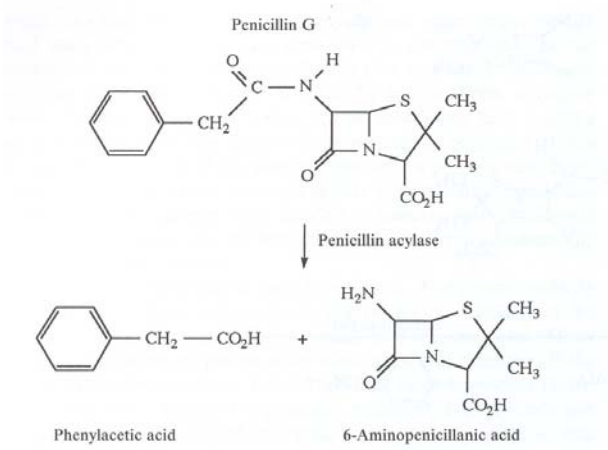
4. Lipases



Olive oil **Saturated FA** **cacao butter** **Unsaturated FA**

- Synthesis of cocoa butter
- Lipases are used in degradation, synthesis, or purification of stereospecific esters : Important in pharmaceutical industry

5. Penicillin acylase



6. Cyclodextrin glycosyltransferase (CGTase)