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وَ ا حْلُلْ عُقْدَةً مِّنْ لِّسَانِیْ o یَفْقَهُوْا قَوْلِیْ o

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# FST-311. L # 9: ENZYMES USAGE IN FOOD APPLICATION

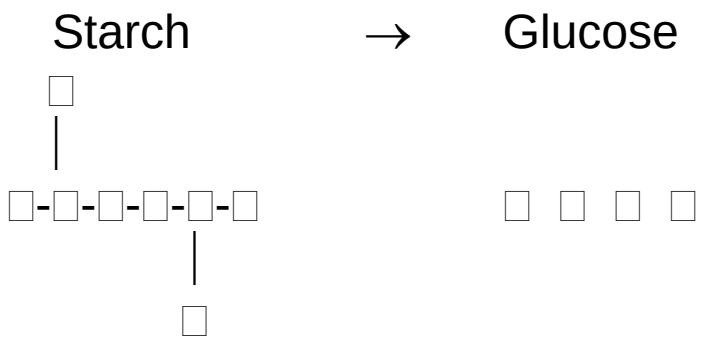
- ENZYMES?
- ENZYMES CATALYZE REACTIONS
- WHAT CONTROLS THE ACTION OF ENZYMES ?
- ENZYMES ADVANTAGES IN THE FOOD INDUSTRY?
- ENZYMES IMPORTANT IN THE FOOD INDUSTRY?
- ENZYME CLASSES

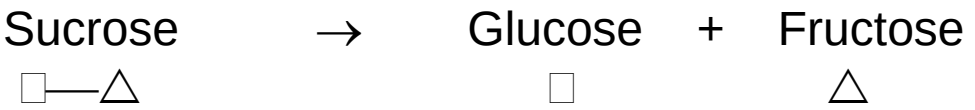
“ENZYME WIDELY SOURCED FROM ANIMALS AND

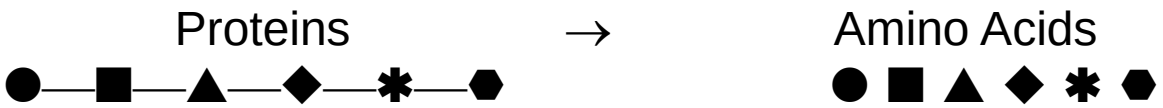
PLANTS USED IN FOOD MANUFACTURING

ENZYME USAGE IN FOOD APPLICATION”

# ENZYMES CATALYZE REACTIONS -



# WHAT CONTROLS THE ACTION OF ENZYMES ?

- **Temperature**
- **Water Content**
- **Concentrations**
- **pH**
- **Chemicals**
- **Alteration of Substrates**
- **Alteration of Products**

# ENZYMES ADVANTAGES IN THE FOOD INDUSTRY?

**Added or used to cause particular reaction with these advantages**

- **Natural & Nontoxic**
- **Catalyze specific reactions**
- **Active under mild conditions**
- **Active at low concentrations**
- **Can control rate of reaction**
- **Can be inactivated**

# ENZYMES IMPORTANT IN THE FOOD INDUSTRY?..

- **Naturally present – may want to inactivate them**
- **Naturally present – may want them to act**
- **Used as indicators of proper processing**
- **Used to measure another compound in the food**

# ENZYME CLASSIFICATION

**Oxidoreductases**

- Catalyze-reactions where oxidation or reduction occurs - cytochrome oxidases and alcohol dehydrogenases

**Transferases**

- Catalyze-transfer of a functional group from one substrate to another - transaminases, aminoacyl transferases

**Hydrolases**

- Catalyze-hydrolytic reactions or hydrolysis of substrates in presence of water, e.g. esterases, proteinases, alkali and acid phosphatases

# ENZYME CLASSIFICATION

**Lyases**

- Catalyze-addition of a group to a double bond or
- Removal of a group from substrate without hydrolysis
- Often leave a compound containing a double bond, e.g., fumerases

**Isomerases**

- Catalyse-Intramolecular rearrangement of substrates, e.g. phosphohexose isomerases, racemases

**Ligases**

- Catalyze-formation of covalent bonds between substrates using energy from ATP, e.g., pyruvate carboxylases

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# FST-311. L # 10:
# ENZYMES FROM NATURAL SOURCES AND FOOD APPLICATIONS

- **Enzyme**
- **Sources**
- **Actions in Food**
- **Food applications**

| Enzyme | Source | Action in Food | Food application |
| --- | --- | --- | --- |

| Enzyme | Source | Action in Food | Food application |
|---|---|---|---|
| α-Amylase | Cereals<br>Wheat, Barley | **Starch Hydrolysis** to **Oligosaccharides** | **Bread** Making<br>**Brewing** (Malting) |
| β-Amylase | Sweet Potato | **Starch Hydrolysis to Maltose** | Production of High **Malt Syrups** |
| Papain | Latex of unripe Papaya fruit | Food and Beverage **Protein Hydrolysis** | Meat Tenderization, Chill Haze Prevention in Beer |
| Bromelain | Pineapple<br>Juice and Stem | **Muscle** & **Connective** Tissue **Protein Hydrolysis** | **Meat Tenderization** |
| Ficin | Fig Fruit Latex<br>**(milky white fluid)** | **Muscle** & **Connective** Tissue **Protein Hydrolysis** | **Meat Tenderization**<br>(not widely used due to cost) |

| Enzyme | Source | Action in Food | Food Application |
|---|---|---|---|
| Lipoxygenase | Soya Bean | Oxidation of Unsaturated Fatty acid in Flour | Bread Dough Improvement |
| Lysozyme | Hen Egg White | Hydrolysis of Bacterial Cell Wall Polysaccharides | Prevention of Late Blowing defects in Cheese by Spore forming Bacteria |
| Lactoperoxidase | Cheese Whey<br>Bovine Colostrum | Oxidation of Thiocyanate ion to Bactericidal Hypothiocyanate | Cold Sterilization of Milk |

| ENZYME | SOURCE | ACTION IN FOOD | FOOD APPLICATION |
|---|---|---|---|
| **Trypsin** | Bovine (Cow) Pancreas<br>Porcine (Pig) Pancreas | Food **Protein Hydrolysis** | Production of **Hydrolyzates** for **Food Flavoring** (mostly replaced by microbial Proteinase) |
| **Chymosin** | Calf Abomasum ($4^{th}$ Compartment of Ruminant Stomach) | **K-Casein Hydrolysis** | **Coagulation** of **Milk** in **Cheese** Making |
| **Pepsin** | Bovine Abomasum | **Chymosin + Casein Hydrolysis** | Usually present with **Chymosin** as part of **Rennet** |
| **Lipase / Esterase** | Gullet (tube from mouth to stomach) of Goat & Lamb<br>Calf Abomasum<br>Pig Pancreas | **Triglyceride (Fat) Hydrolysis** | **Flavor** Enhancement in **Cheese product**<br>**Fat** function modification by inter **Esterification** |

# CLASS TASK

- **Acetoin**
- **Beer**
- **Wine**
- **Whisky**
- **Brandy**
- **Saccharification**
- **K-Casein (Kappa-Casein)**
- **Rennet**
- Late Blowing defects in Cheese

## Acetoin

- Acetoin is a colorless or pale yellow to green yellow liquid with a pleasant, buttery odor
- Acetoin, along with diacetyl, is one of the compounds giving butter its characteristic flavor

## Beer (Ethanol: 2-12 % ; usually: 4-6%)

- Beer is an Alcoholic beverage produced by the Saccharification of Starch and Fermentation of the resulting sugar
- Beer is the World's most widely consumed alcoholic beverage and the Third-most popular drink overall, after Water and Tea

## Wine (Ethanol: 9-16 % ; most often: 12.5-14.5%)

- Wine is an alcoholic beverage made from Fermented Grapes or other fruits

## Whisky (Ethanol: 40-68 % ; usually: 40-46%)

- Whisky or whiskey is a type of Distilled Alcoholic beverage made from Fermented Grain mash

## Brandy (Ethanol: 35-60 % ; usually 40%)

- Brandy is a Spirit produced by Distilling Wine

## Saccharification

- The **Hydrolysis** of **Polysaccharides** to **Soluble Sugars**
- **MALT** made from **Barley** is used as a source of **β-amylase** to break down **Starch** into the Disaccharide **(Maltose)**, which can be used by **Yeast** to produce **Beer**
- Other **Amylase** enzymes may convert **Starch** to **Glucose** or to Oligosaccharides

## K-Casein (Kappa-Casein)

- **A mammalian milk protein involved in a number of important physiological processes**
- **Chymosin (EC 3.4.23.4) is an aspartic protease that specifically hydrolyzes the peptide bond in Phe105-Met106 of κ-Casein and is considered to be the most efficient protease for the CHEESE making industry**

## Rennet

- A complex set of enzymes produced in the stomachs of ruminant mammals
- Chymosin, its key component, is a protease enzyme that curdles the casein in milk.
- In addition to chymosin, rennet contains other enzymes, such as pepsin and a lipase
- Rennet is an enzyme used to coagulate milk, in order to form a thick curd.
- Rennet begins working at temperatures between 85-105F, even at higher temperatures it will not be deactivated until it reaches 140F

## Cheese

- A dairy product, derived from milk and produced in wide ranges of flavors, textures and forms by coagulation of the milk protein casein.
- It comprises proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep.

## Late Blowing defects in Cheese

- Butyric acid Fermentation, also known as **Late Blowing Defect (LBD),** is a major cause of spoilage in semi-hard and hard cheeses. It results in the appearance of texture and flavor defects that generate severe economic losses at the cheese industry.

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# FST-311. L # 11:
# ENZYMES FROM MICROBIAL SOURCES AND FOOD APPLICATIONS

- **Enzyme**
- **Sources**
- **Actions in Food**
- **Food applications**

| ENZYME | SOURCE | ACTION IN FOOD | APPLICATION IN FOOD |
|---|---|---|---|
| **α-Amylase** | *Aspergillus* spp.<br>*Bacillus* spp.<br>*Microbacterium imperiale* | Wheat **Starch** Hydrolysis | Amylase **Dough Softening**<br>Increased **Bread Volume**<br>Production of **Sugars** for **Yeast Fermentation** |
| **α-Acetolactate** | *Bacillus subtilis* | Converts **Acetolactate** to **Acetoin** | Reduction of **Wine Maturation Time** by circumventing need of **Decarboxylase** for **secondary Fermentation** of **Diacetyl**e to **Acetoin** |
| **Amyloglucosidase** | *Aspergillus niger*<br>*Rhizopus spp.* | Hydrolyzes **Starch Dextrin** to **Glucose** (Saccharification) | One stage of **High Fructose Corn s**yrup production.<br>Production of **Lite Beers** |
| **Aminopeptidase** | *Lactococcus lactis*<br>*Aspergillus spp.*<br>*Rhizopus oryzae* | Release free **Amino acids** from N-terminus of **Proteins** and **Peptides** | **Debittering Protein Hydrolyzates** accelerating **Cheese** Maturation |

| ENZYME | SOURCE | ACTION IN FOOD | APPLICATION IN FOOD |
|---|---|---|---|
| **Catalase** | *Aspergillus niger*<br>*Micrococcus luteus* | Break down $H_2O_2$ to $H_2O$ & $O_2$ | $O_2$ Removal Technology combined with **Glucose oxidase** |
| **Cellulase** | *Aspergillus niger*<br>*Trichoderma spp.* | Hydrolyze **Cellulose** | **Fruit Liquefaction** in Juice Production |
| **Chymosin** | *Aspergillus awamori*<br>*Kluyveromyces lactis* | Hydrolyzes **K- Casein** | **Coagulation** of **Milk** for **Cheese** making |
| **Cyclodextrin glucanotransferase** | *Bacillus spp.* | Synthesize **Cyclodextrins** from liquefied **Starch** | **Cyclodextrins** are **Food grade** microencapsulant for **Color** **Flavors** and **Vitamins** |
| **β-Galactosidase (lactase)** | *Aspergillus spp.*<br>*Kluyveromyces spp.* | Hydrolyzes **Milk** **Lactose** to **Glucose** and **Galactose** | **Sweetening Milk** and **Whey** products for **Lactose Intolerant** individuals<br>Reduction of crystallization in **Ice Cream** containing whey |

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# FST-311. L # 12:
# ENZYMES FROM MICROBIAL SOURCES AND FOOD APPLICATIONS

- **Enzyme**
- **Sources**
- **Actions in Food**
- **Food applications**

| ENZYME | SOURCE | ACTION IN FOOD | APPLICATION IN FOOD |
|---|---|---|---|
| **β-Glucanase** | *Aspergillus ssp.*<br>*Bacillus subtilis* | Hydrolyzes **β-glucans** in **Beer** mashes | **Filtration aids**<br>**Haze** prevention in **Beer** production |
| **Glucose isomerase** | *Actinoplanes missouriensis*<br>*Bacillus coagulans*<br>*Streptomyces lividans*<br>*Streptomyces rubiginosus* | Converts **Glucose** to **Fructose** | Production of **High Fructose Corn** Syrup (Beverage sweetener) |
| **Glucose oxidase** | *Aspergillus niger*<br>*Penicillum chrysogenum* | Oxidizes **Glucose** to **Gluconic acid** | **$O_2$**removal from **packagin**g<br>**Removal** of **Glucose** from **Egg White** to prevent **Browning** |
| **Hemicellulase & Xylanase** | *Bacillus spp.* | Synthesize **Cyclodextrins** from liquefied **Starch** | **Cyclodextrins** are **Food grade** microencapsulate for **Color Flavors** and **Vitamins** |
| **B-Galactosidase (lactase)** | *Aspergillus spp.*<br>*Bacillus subtilis*<br>*Trichoderma reesei* | Hydrolyzes **Hemicellulose** (insoluble non-starch polysaccharide in flour) | **Bread** improvement through **improved crumb structure** |

| ENZYME | SOURCE | ACTION IN FOOD | APPLICATION IN FOOD |
|---|---|---|---|
| **Lipase / Estrase** | *Aspergillus spp.* *Candida spp.* | Hydrolyzes **Triglycerides** to **Fatty acids** and **Glycerol** | **Flavor** enhancement in **Cheese** products |
| **Pectinase** | *Aspergillus spp.* *Penicillum funiculosum* | Hydrolyze **Pectin** | Clarification of **Fruit Juice** by **De-Pectinization** |
| **Pectinesterase** | *Aspergillus spp.* | Removal **Methyl** groups from **Galactose** units in **Pectin** | With **Pectinase** in **De-Pectinization** technology |
| **Puliulanase** | *Bacillus spp.* *Klebsiella spp.* | Hydrolyzes **1-6 bonds** that form branches in **Starch** structure | Starch **Saccharification** (improves efficiency) |
| **Protease / Proteinase** | *Aspergillus spp.* *Bacillus spp.* *Penicillium citrinum* | Hydrolyzes of **K-Casein** Hydrolysis of **Wheat Glutens** | **Milk Coagulation** for **Cheese** making **Hydrolyzates** production for **Soups** and **Savory** foods **Bread Dough** improvement |

# CLASS TASK

- ***Aspergillus***
- ***Bacillus***
- ***Microbacterium***
- ***Rhizopus***
- ***Saprophytes***
- ***Lactococcus lactis***
- ***Buttermilk* (LASSI)**
- ***Kluyveromyces***
- ***Trichoderma***
- ***Candida***
- ***Actinoplanes***
- **Butyric acid fermentation**
- **Wine aging**
- **Malt**

- ***Aspergillus*** is a genus consisting of a few hundred **mold** species found in various climates worldwide
- ***Bacillus*** is a genus of **Gram-positive**, **Rod-shaped** (bacillus) bacteria; can be **Obligate aerobes** ($O_2$ reliant), or **Facultative anaerobes** ($O_2$ reliant or **NOT**)
- ***Microbacterium*** is a genus of **bacteria** in the family **Microbacteriaceae;** consists of **63** species
- ***Rhizopus*** is a **genus** of common **Saprophytic Fungi** on plants and specialized parasites on animals
- ***Saprophytes*** are plants, **fungi**, or micro-organisms more accurately called myco-heterotrophs because they actually parasitize fungi, rather than dead organic matter directly. **They live on dead or decomposing matter**.

- ***Lactococcus lactis*** is a **Gram-positive** bacterium used extensively in the production of **Buttermilk** and **Cheese**
- ***Buttermilk*** **(LASSI)** refers to a number of **dairy drinks** (**liquid left** behind **after churning butter)**
- ***Kluyveromyces*** is a genus of Yeasts in the Ascomycetes family of Saccharomycetacea
- ***Trichoderma*** is a genus of **Fungi** that is present in all soils, where they are the most prevalent culturable fungi
- ***Candida*** is a genus of **yeasts** and is the **most** common **cause** of fungal **infections** worldwide
- ***Actinoplanes*** species are Gram-positive, soil-inhabiting, filamentous bacteria

- Wine aging refers to a **group of reactions** that tend to improve the taste and flavor of a wine over time. The term wine 'maturation' refers to changes in wine after fermentation and before bottling.

- Malt is **germinated** cereal grain that has been dried in a process known as "malting". The grain is made to germinate by soaking in water and is then halted from germinating further by drying with **hot air**.
- Fruit Liquefaction in Juice: As an alternative to press systems, some processors have gone to total enzymatic liquefaction of the fruit mash. **Cellulase** and **pectinase** enzymes are added, and the mash is **heated** in order to accelerate the enzyme's performance.

# HIGH-FRUCTOSE CORN SYRUP (HFCS)

- Also known as **glucose-fructose**, **isoglucose** and **glucose-fructose** syrup, is a **sweetener** made from **corn starch**
- As in the production of conventional corn syrup, the **starch** is broken down into **glucose** by enzymes
- To make HFCS, the corn syrup is further processed by **glucose isomerase** to convert some of its glucose into fructose
- "**HFCS 42**" and "**HFCS 55**" refer to **42** % and **55** % **fructose** composition respectively, the rest being **glucose** and **water**