

# FOOD ENZYMES AND THEIR APPLICATIONS

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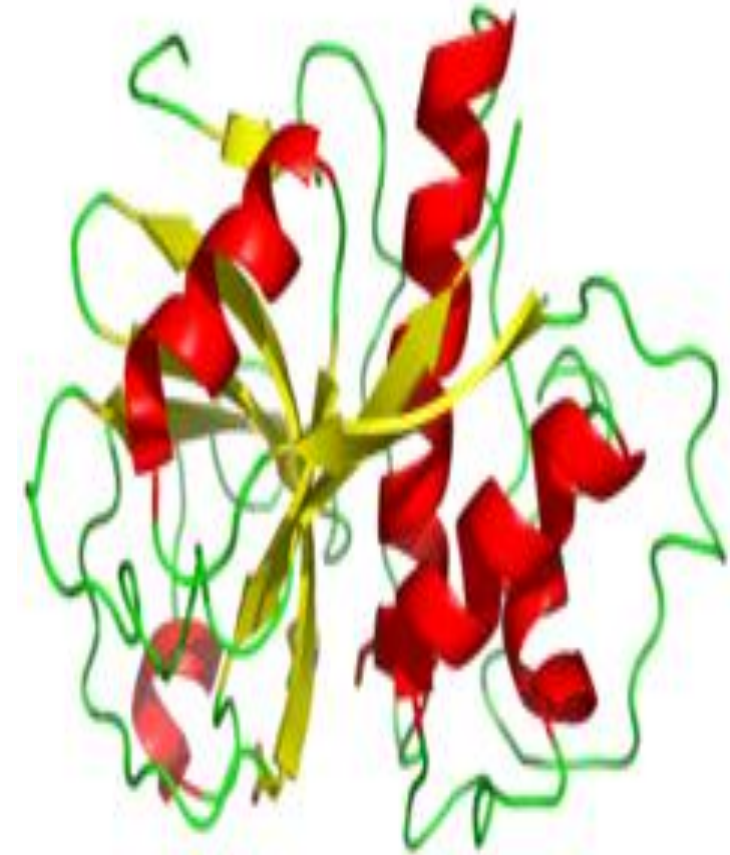
# Application of food enzymes – Glycosyl Hydrolases - Proteases

## III. Proteases

- Enzymes that hydrolyze peptide bonds in proteins

### A) Papain (found in papaya)

- Broad pH (3-11) and temperature stability
- very popular for a variety of food applications
- Broad spectrum protease active over a wide pH and temperature range
- Papain molecule composed of single polypeptide (212 amino acids, a mass of 23,350 Da, pI of 8.9)
- Actually represents four distinct proteases (four genes)
- Papain, chymopapain, caricain, glycyI endopeptidase IV
- Nearly identical size and similar pI
- Activity is enhanced by reducing agents



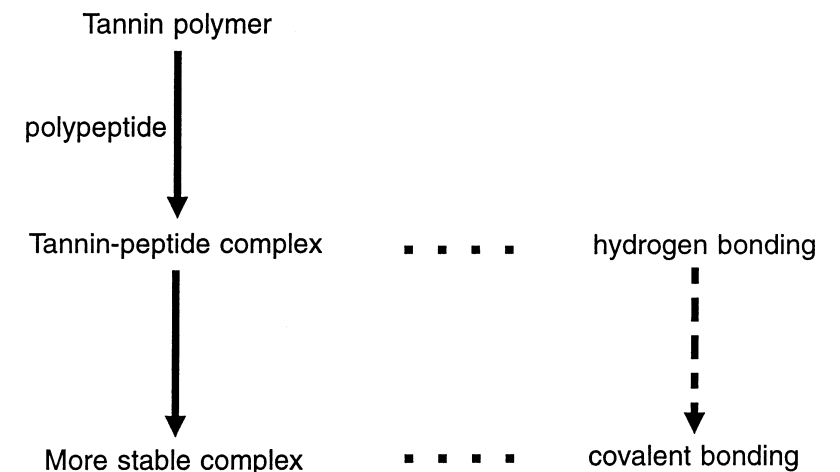
# Applications of Papain

1. Used as a meat tenderizer on inferior meat cuts (can also use slice of pineapple on meat)
  1. The enzyme makes its way into the muscle and hydrolyzes connective tissue proteins and softens muscle
  2. Have to use low amount to prevent liquefaction of muscle
  3. If you mix raw papaya into Jell-O it will not form a gel
2. Dairy products (rennin replacement)
3. Leather industry (bating hides)
4. Textile and laundry (reduce wool felting and stain removal)
5. Beverage industry (beer clarifier)

# Application of food enzymes – Glycosyl Hydrolases - Proteases

5. Papain can be used to clear turbidity (chill haze) in beer
  - When bottled or canned beer at  $<10^{\circ}\text{C}$ , a haze can form
    - Interactions of proteins/polypeptides and tannins in beer
  - This can be prevented using chill-proofing

- Protease (papain) added during post-fermentation maturation to hydrolyze the proteins/polypeptides to prevent large aggregates to form on cooling

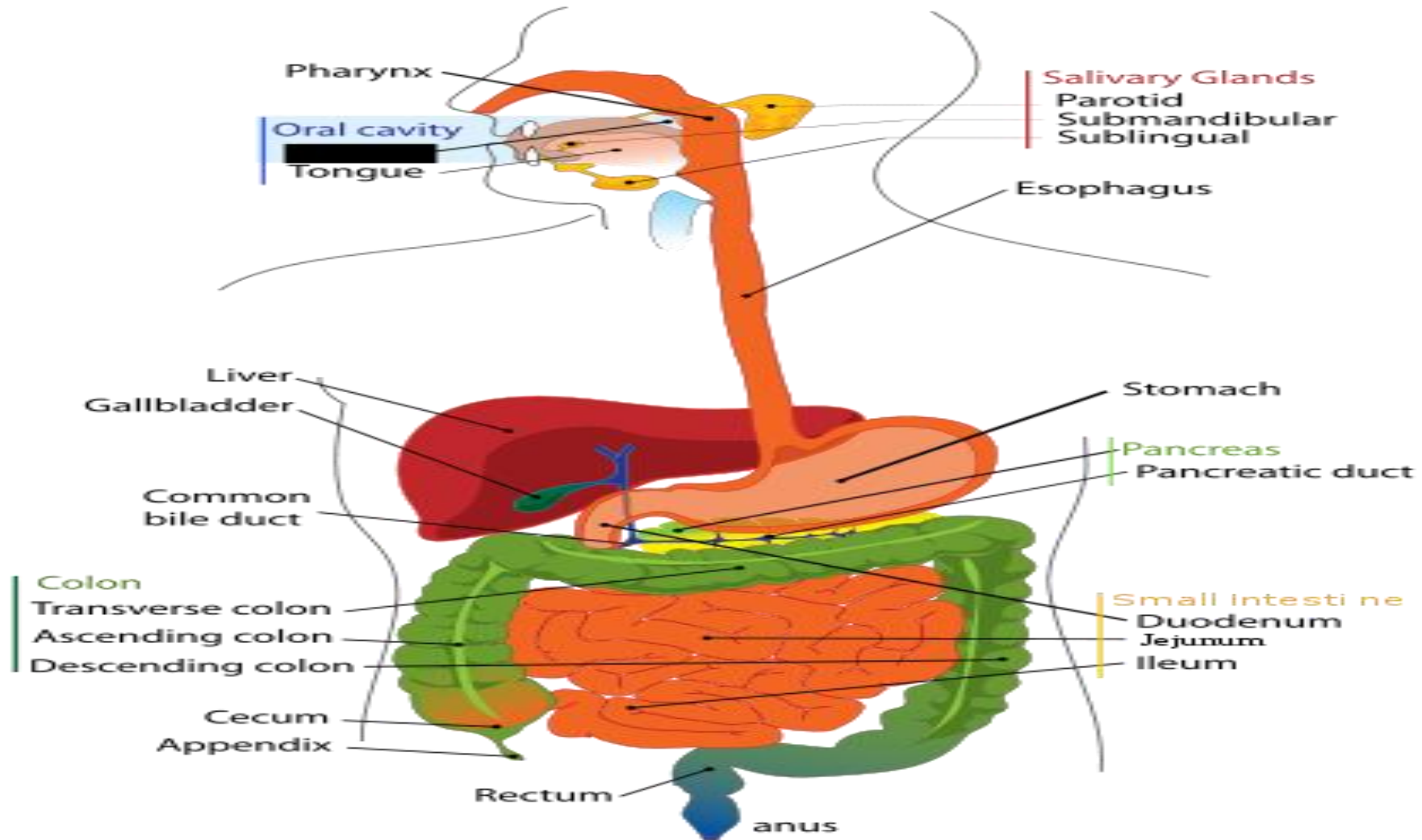


# Application of food enzymes – Glycosyl Hydrolases - Proteases

## B) Digestive proteases

- Trypsin & Chymotrypsin
  - Found in animal pancreas, and active at pH 7 - 9
  - Can cause quality problems in muscle foods if contamination from intestines occurs (e.g. ground products) → over-softening of the meat
  - They are often used to make protein hydrolysates for the food, beverage and the pharmaceutical industry
- Pepsin
  - Very acidic activity pH optima (pH 1.8), limits its use
  - Used in cheese making, chillproofing and also in making protein hydrolysates

Digestive enzymes helps in digestion of food in our body



# Major Digestive enzymes

Enzyme	Produced In	Site of Release	pH Level
<b>Carbohydrate Digestion:</b>			
Salivary amylase	Salivary Glands	Mouth	Neutral
Pancreatic amylase	Pancreas	Small Intestine	Basic
Maltase	Small intestine	Small intestine	Basic
<b>Protein Digestion:</b>			
Pepsin	Gastric glands	Stomach	Acidic
Trypsin	Pancreas	Small intestine	Basic
Peptidases	Small Intestine	Small intestine	Basic
<b>Nucleic Acid Digestion:</b>			
Nuclease	Pancreas	Small intestine	Basic
Nucleosidases	Pancreas	Small intestine	Basic
<b>Fat Digestion:</b>			
Lipase	Pancreas	Small intestine	Basic

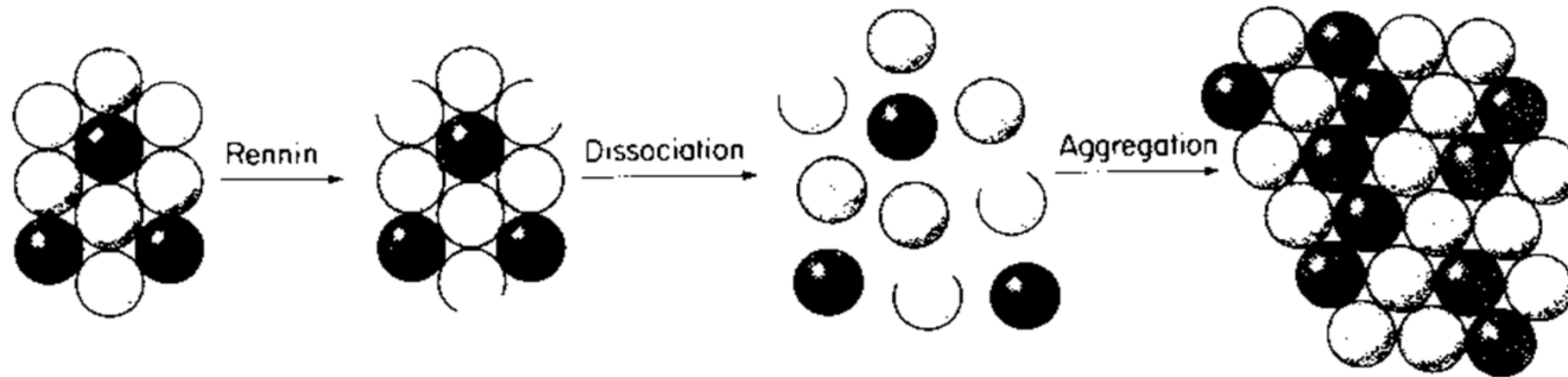
# Application of food enzymes –Glycosyl Hydrolases - Proteases

- Chymosin (rennin)
  - Essential for the manufacture of good quality cheeses
  - Found in the fourth stomach of suckling calf's
  - Very expensive and “inhumane” to process now
  - So engineered into bacteria for mass production
  - Has a very specific activity
    - Hydrolyzes only one bond in  $\kappa$ -casein, one of the many proteins that make up the milk casein protein complex ( $\kappa$ -,  $\alpha$ -,  $\beta$ -casein)
    - This breaks up the casein complex (micelle) and it aggregates leading to a clot, the first step in cheese production



# Application of food enzymes – Glycosyl Hydrolases - Proteases

- Most other proteases can initiate a milk clot like chymosin
- However they would continue the casein hydrolysis producing bitter peptides and eventually breaking the clot itself



**Figure 10** Schematic representation of events in clotting of milk. The  $\alpha_s$ -,  $\beta$ -, and  $\kappa$ -caseins are shown by striped, stippled, and white balls, respectively. The involvement of  $\text{Ca}^{2+}$ , attached to phosphate groups of the caseins, is not shown.

# Coagulation of Milk by Rennet: Experiment

- Pipette 10 ml of milk into each of 3 test tubes.
- To two tubes, add ~1.5 ml of a 1% rennet solution, mix
- The 3rd tube will serve as a control. It contains no rennet
- Place one of the two tubes with rennet into water bath at ~37°C
- Observe the coagulation



Control

# Coagulation of Milk by Rennet: Experiment

- 1% Rennet Solution, No Heat



5 min



10 min



- 1% Rennet Solution, at 37°C

# Coagulation of Milk by Rennet: Enzyme Action

- An enzyme obtained from fourth stomach of ruminant animals, and from some microorganisms
- Cleaves particular bond in K-casein of milk to initiate milk coagulation
- Coagulates milk protein in cheese making
- Aids in development of flavor and texture in ripened cheese.
- Mild heat speeds up the enzyme reaction

# Application of food enzymes – Microbial Proteases

## C) Microbial proteases

- Fungal & bacterial proteases are used in the food industry
- Fungal proteases
  - Some have equal ability to form cheese, like chymosin
  - A protease cocktail from *Aspergillus oryzae* is used to partially break down bread proteins (glutens) to reduce mixing time and making them more extensible
  - Some are added to help with flavor and texture development and speeding up fermentation in fermented dairy products
  - Fungal proteases are also used to tenderize meat
- Bacterial proteases
  - Subtilisin from *Bacillus subtilis* is popular and is used in combination with papain in beer chill proofing