Enzyme Functionality and Potential Applications for the Tortilla Industry

Todd Forman
NOVOZYMES

Topics to be covered

- Introduction to Novozymes
- Enzyme Basics
- Enzyme types, functionality and applications
  - Amylases
  - Xylanases
  - Lipases
  - Oxidases
  - Proteases
  - Asparaginase

Novozymes in brief

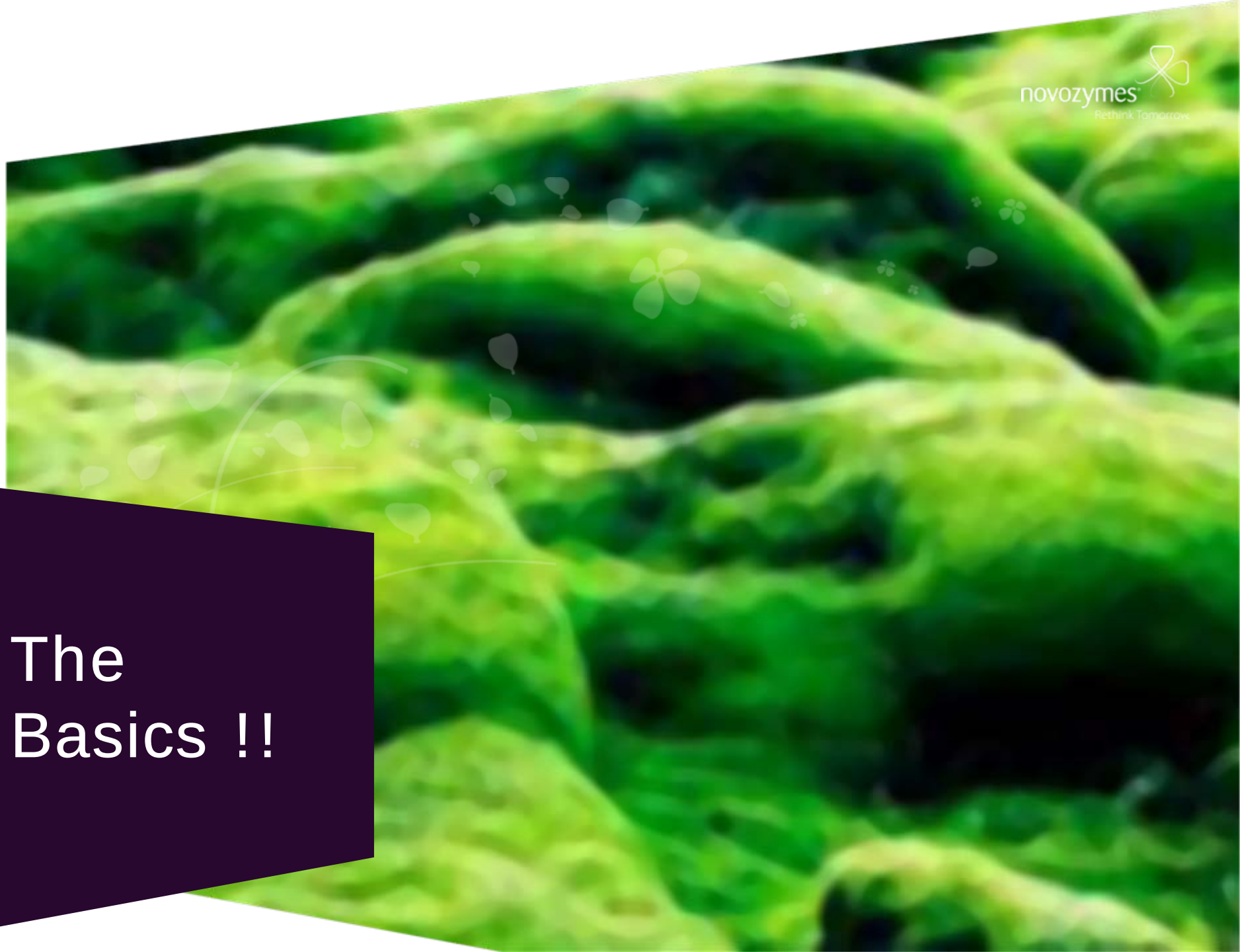
- World leader in Industrial Enzymes & Microorganisms
- Enzymes account for > 90% of turnover
- Market leader in all main industries
- More than 700 products used in 130 countries in > 30 different industries
- More than 6,500 granted patents and pending patent applications
- Main production in USA, China and Denmark
- Sales $US 1.9B (FY2010)
- More than 5,400 employees

Source: Novozymes 2010 estimates

We change the way the world works within

- Household care
- Bioenergy
- Food and Beverage
- Feed, Leather and Textiles
- Microorganisms
- Biopharmaceuticals

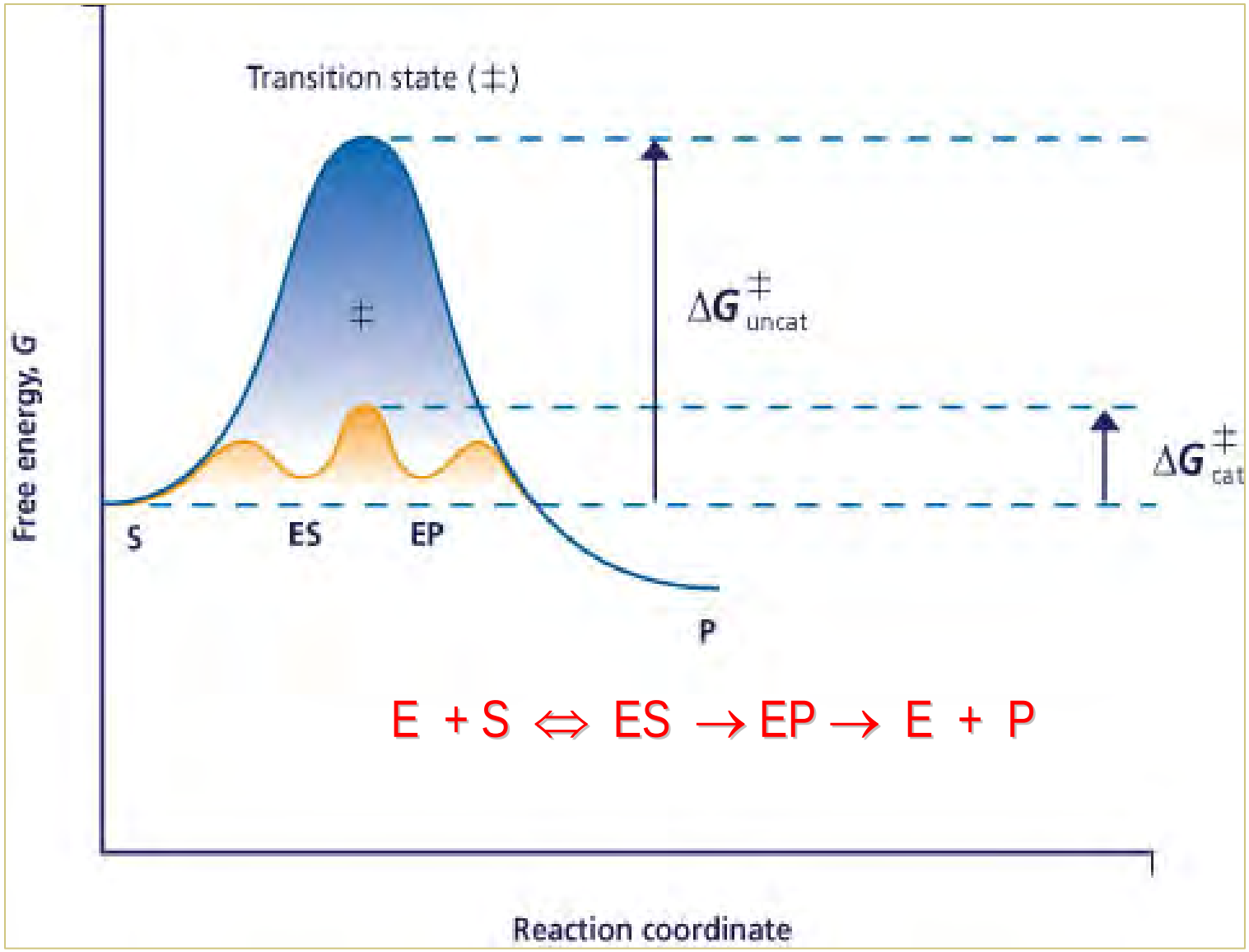
Our vision has never been more relevant



The Basics !!

ENZYMES ARE…

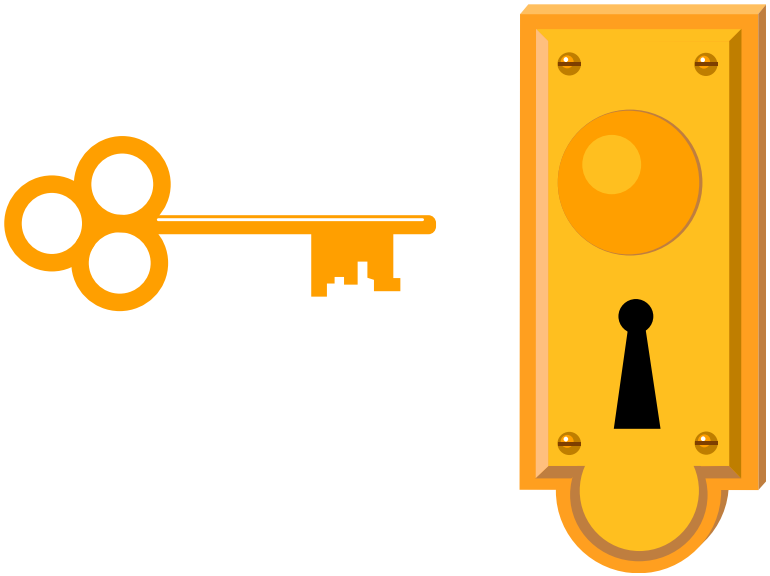
- **NOT ALIVE**
- **PROTEINS**
  - Present in all living cells
  - Control vital metabolic processes
  - Take part in the breakdown of food into simpler materials
  - Work under relatively mild conditions
- **CATALYSTS**
  - Speed up chemical processes – lower the activation energy needed for reaction to occur
  - Are not consumed in the process

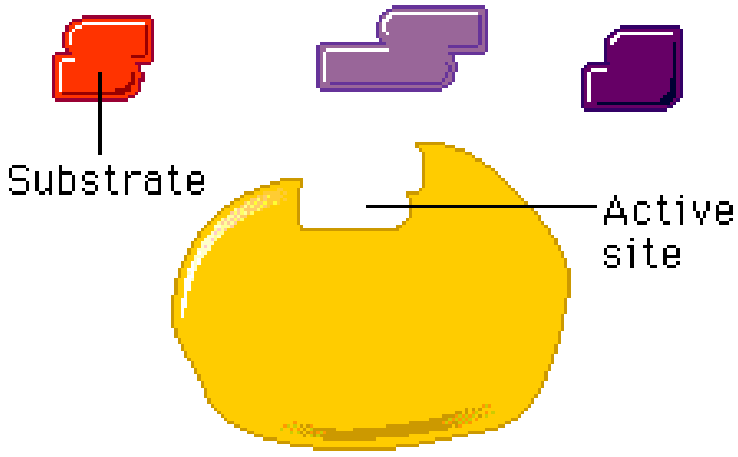


$$E + S \Leftrightarrow ES \rightarrow EP \rightarrow E + P$$

ENZYMES ARE…

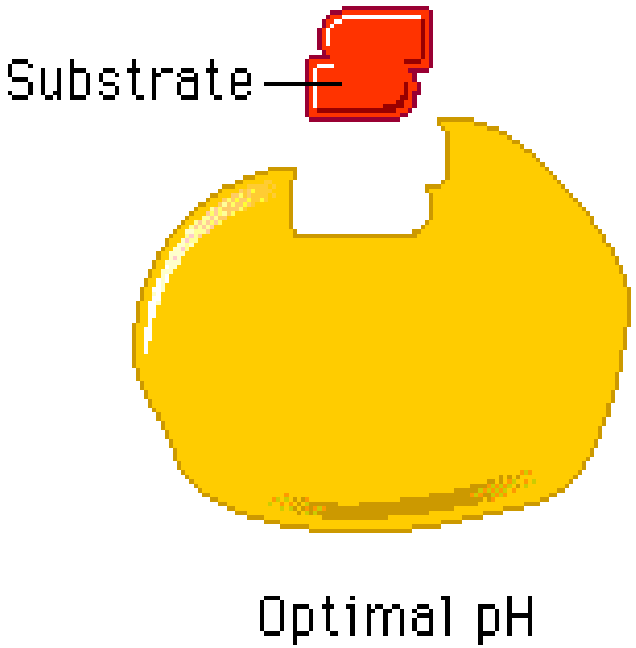
- **SPECIFIC -**
  - An enzyme can breakdown only one particular type of compound. In some cases, the action is limited to specific bonds in the compound
  - Enzymes have a specific shape
  - The enzyme's active site fits onto the substrate
  - Even when different substrate molecules are present, only those that have the specific shape complementary to the active site are able to bind with the enzyme's active site.



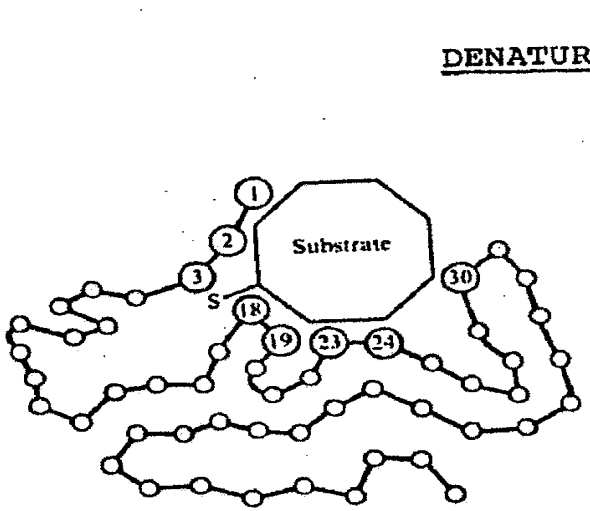


pH and Enzyme Function

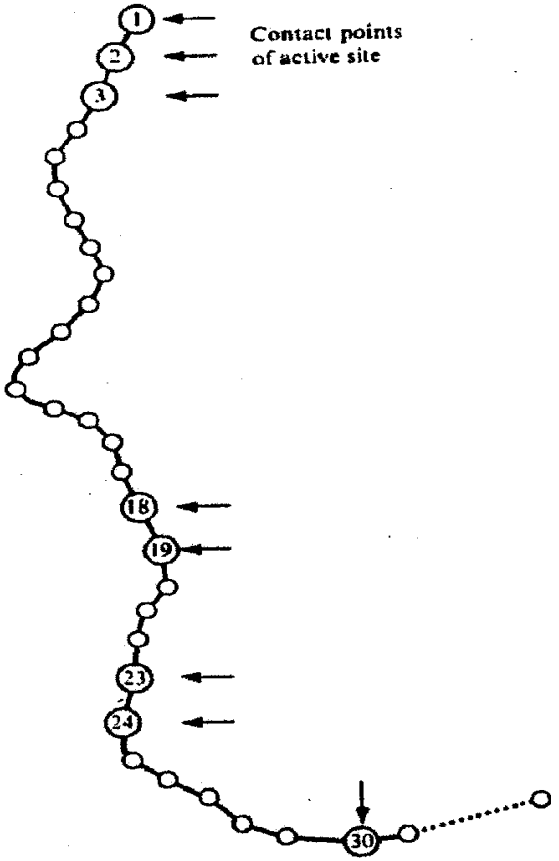
- When the pH changes, the active site progressively distorts.
- In the presence of either excess H^+ or excess OH^- ions, the globular protein's shape is altered.
- The active site is distorted and the enzyme cannot catalyze reactions.



Temperature & Enzyme function

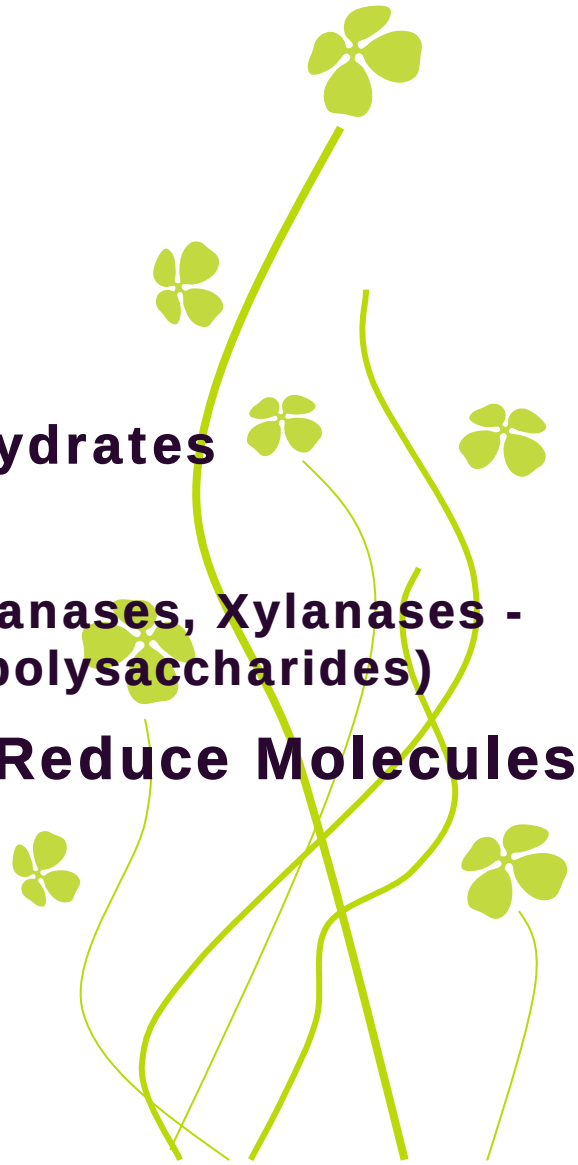


The amino acids are located near one another in the active form but not in the denatured form.



CLASSIFICATION OF ENZYMES

- HYDROLASES
  - PROTEASES - Modify proteins
  - LIPASES - Modify fats
  - CARBOHYDRASES - Modify carbohydrates
    - Amylases - Modify starch
    - Cellulases, Hemicellulases, Pentosanases, Xylanases - Modify fiber or gums (non-starch polysaccharides)
- OXIDO-REDUCTASES - Oxidize or Reduce Molecules
  - Lipoxygenase
  - Glucose Oxidase



SOURCES OF ENZYMES

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PLANT

- Papain, Bromelain
- Barley Malt
- Soybeans

ANIMAL

- Trypsin
- Rennet

MICROBIAL

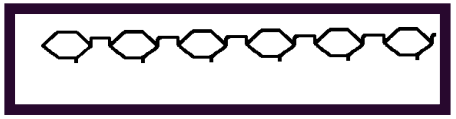
- Fungal
- Yeast
- Bacteria

ENZYME TYPES & FUNCTIONALITY

- **Amylases**
- Xylanases
- Lipases
- Oxidases
- Proteases
- Asparaginase

BASIC STRUCTURE OF STARCH

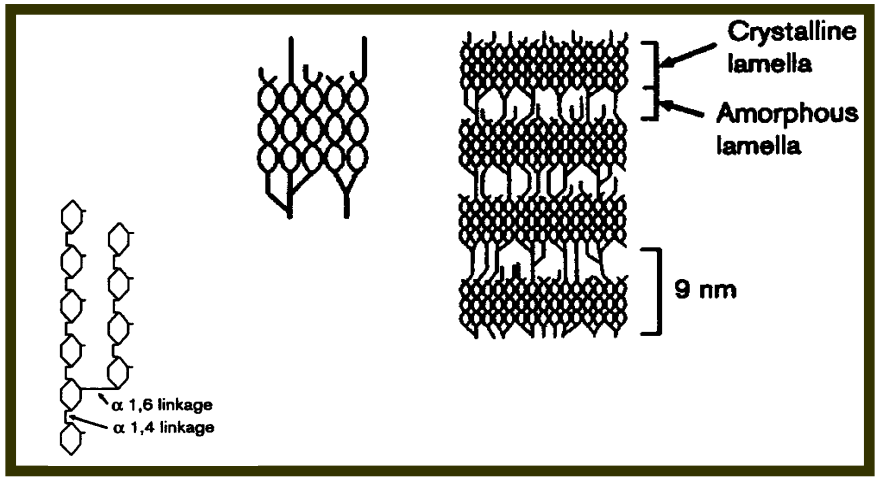
Amylose (1/4 of Starch)



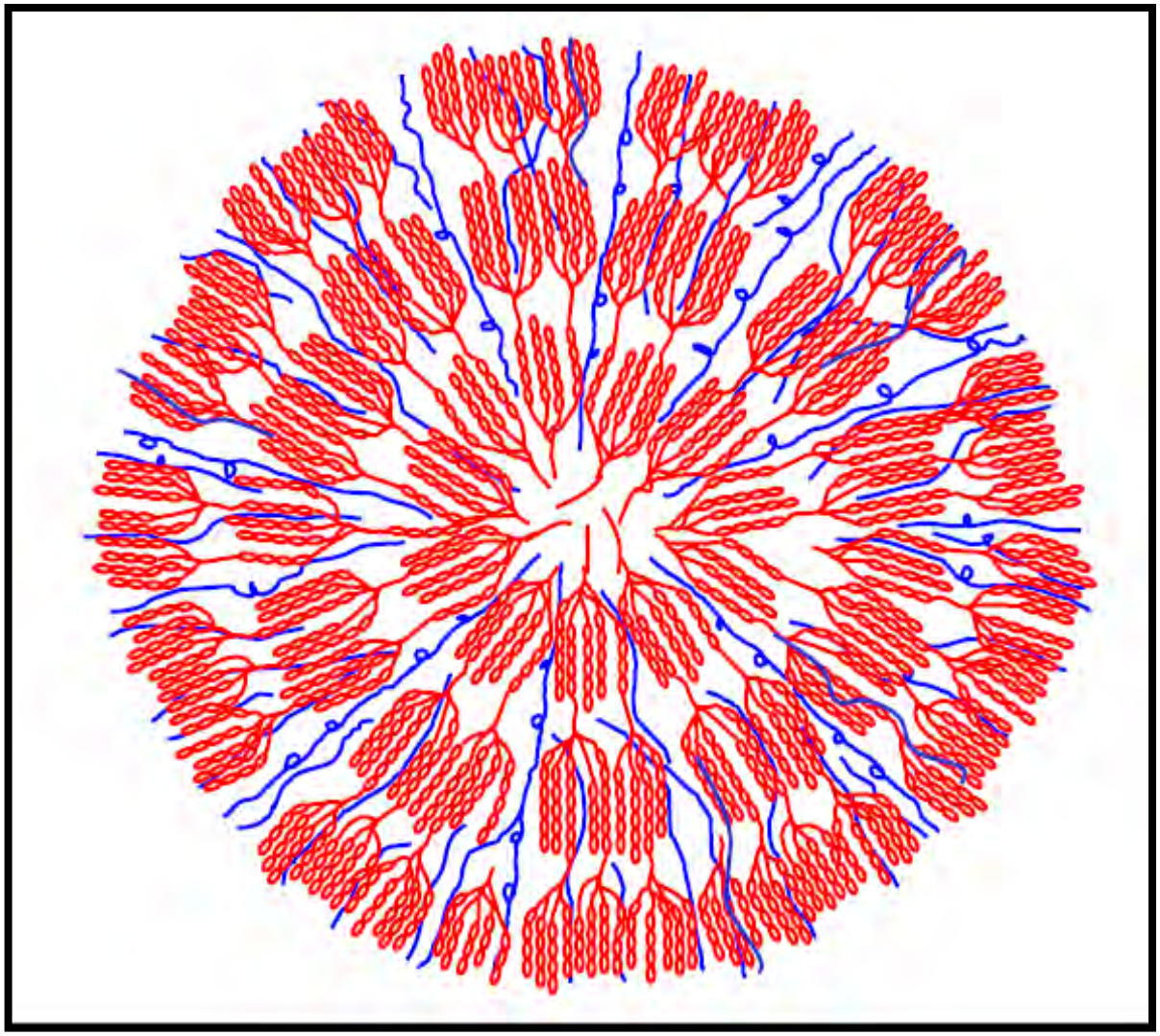
- long linear chain of glucose units
- DP in wheat amylose app. 1300 glucose units

Amylopectin (3/4 of starch)

- branched “tree” of glucose units
- DP app. 10.000 glucose units



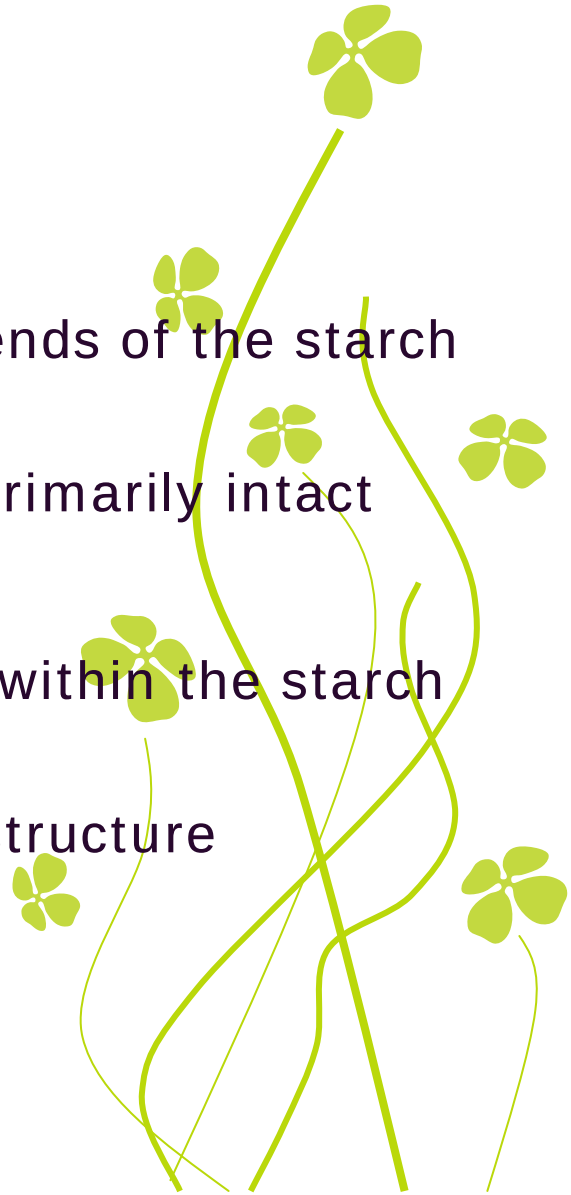
Cross Section of Starch Granule



Dr. Jay-lin Jane
Iowa State University

Classification of Amylases

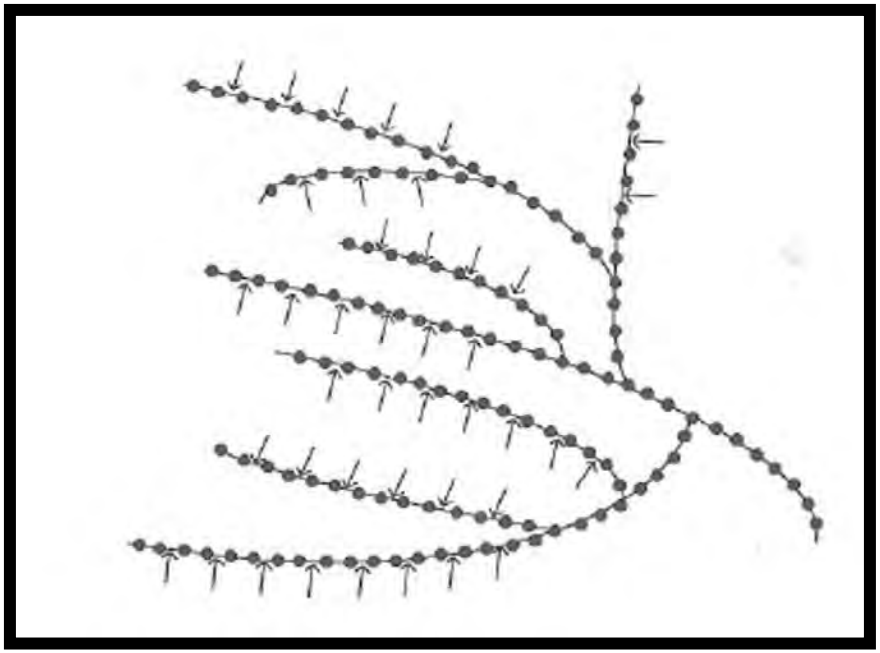
- Action pattern on amylopectin
  - *Exo*-acting
    - Break 1,4 starch bonds from the ends of the starch molecule
    - Leave the amylopectin structure primarily intact
  - *Endo*-acting
    - Break 1,4 starch bonds randomly within the starch molecule and from the ends
    - Essentially destroys amylopectin structure



Classification of Amylases (cont'd)

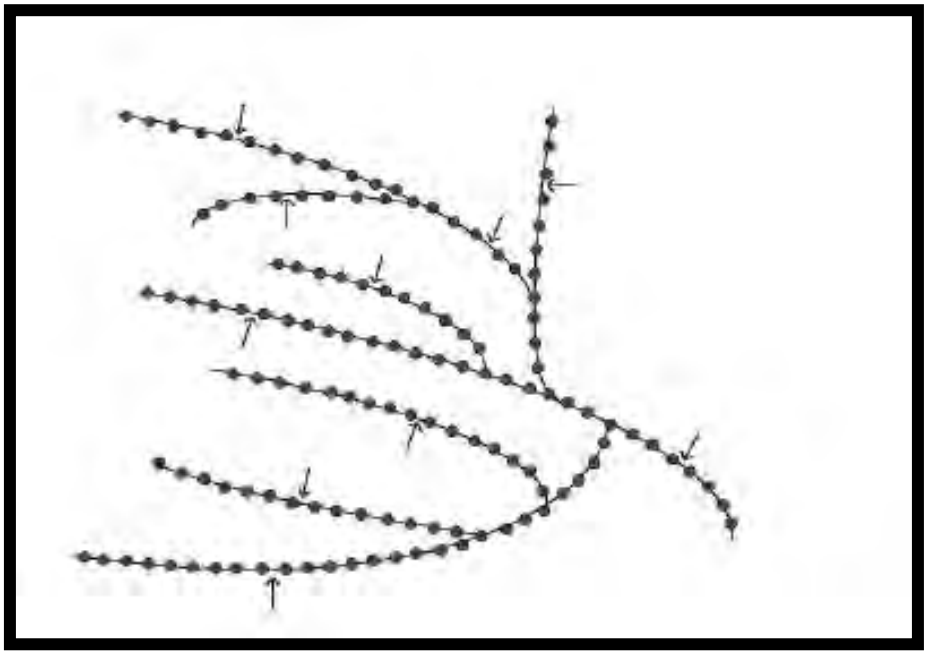
- Thermostability
  - Low thermostability
    - Are inactivated before starch gelatinization temperature
    - Work mainly on damaged starch
  - Medium thermostability
    - Active above starch gelatinization temperatures but are inactivated at end of baking process
  - High thermostability
    - May still be active after the baking process

Amylase Action Pattern



Beta Amylase

(An exo-amylase)



Alpha Amylase

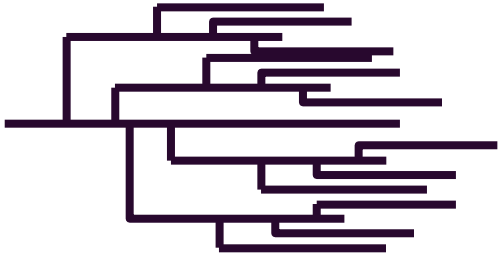
(An endo amylase)

Comparing Traditional Amylase to Maltogenic Amylase

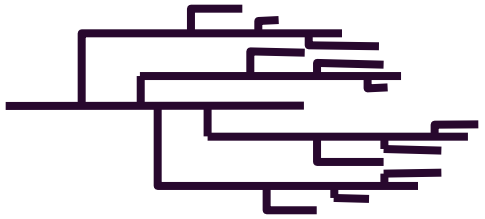
AMYLOPECTIN

Thermostable bacterial amylases

Soft, but gummy and inelastic bread crumb



Maltogenic Amylase



+ small sugars

Soft, elastic bread crumb

Theories of Staling

▪ STARCH THEORY

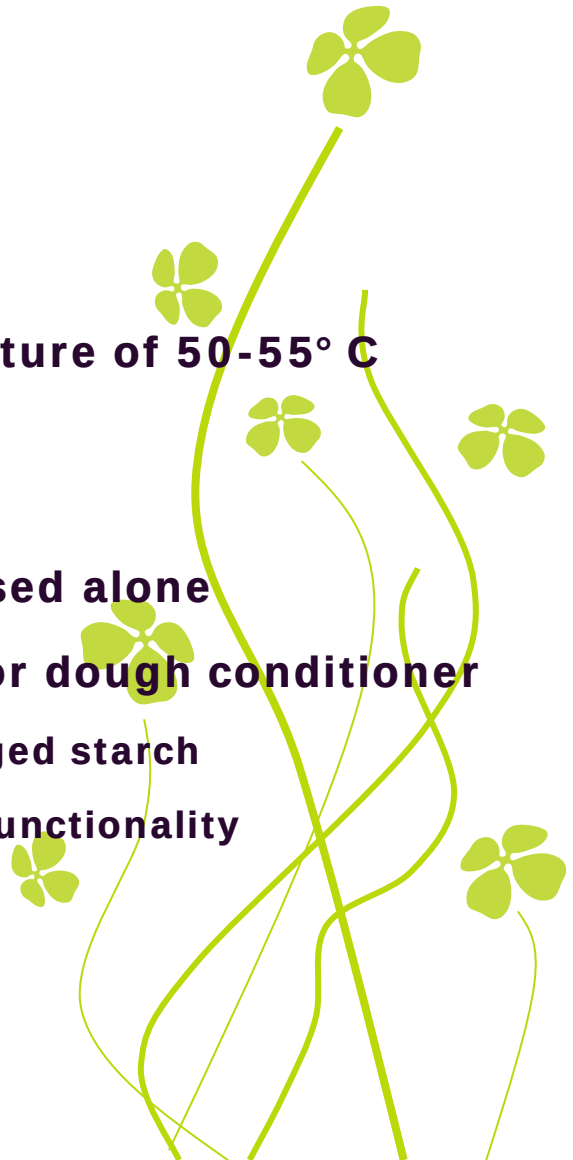
- ➢ During cooling, amylose reverts fast towards more stable state
- ➢ The amylose aggregation “locks” the structure of the crumb
- ➢ The dextrins made by amylase hydrolytic action contribute to a more moist crumb by binding water
- ➢ The gelatinised amylopectin makes nice, soft granules, but as they slowly start to change back to thermodynamically crystalline structure, the granules become harder, making the crumb more firm
- ➢ Textural contributors: amylose and amylopectin dramatically influence the changes in elasticity & softness over the lifetime of the bread

• STARCH-GLUTEN INTERACTION

- ➢During ageing more cross links are formed between gluten and starch
- ➢This may cause more rigidity

AMYLASES FOR FRESH-KEEPING

- **FUNGAL ALPHA AMYLASE**
  - **Endo attack - Creates dextrins**
  - **Low thermostability - optimum temperature of 50-55° C (120-130° F)**
  - **Acts on damaged starch**
  - **Limited effect on fresh-keeping when used alone**
  - **Traditionally used as flour supplement or dough conditioner**
    - **Reduces water holding capacity of damaged starch**
    - **Allows for greater gluten hydration and functionality**

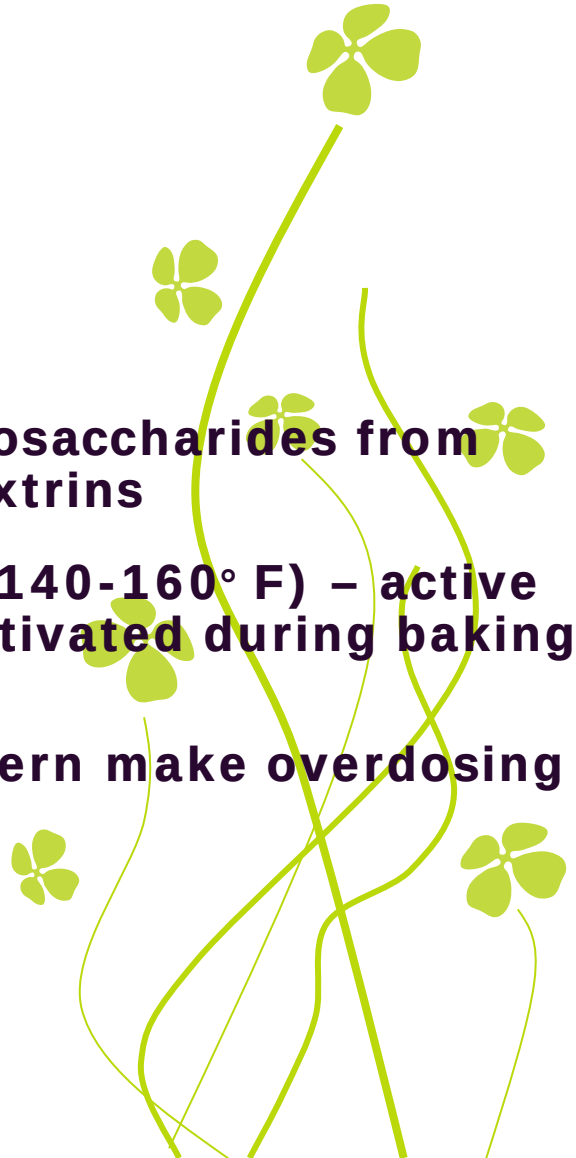


AMYLASES FOR FRESH-KEEPING

- **BACTERIAL ALPHA AMYLASE**
  - Endo attack - creates large dextrins
  - Thermostable-Optimum temperature of 80-90°C (175 -195°F)
  - Not completely inactivated during baking process
  - Quite effective but difficult to control
  - Action pattern + thermostability can lead to unacceptable product if even slightly overdosed

AMYLASES FOR FRESH-KEEPING

- **MALTOGENIC ALPHA AMYLASE**
  - **Medium thermostability**
  - **Unique action pattern - creates oligosaccharides from amylopectin, not large branched dextrins**
  - **Temperature optimum of 60-70° C (140-160° F) – active above starch gelatinization but inactivated during baking process**
  - **Temperature profile and action pattern make overdosing very difficult**



CORN AND FLOUR TORTILLAS REQUIRE AMYLASES WITH DIFFERENT CHARACTERISTICS FOR OPTIMAL FRESHKEEPING

Flour Tortillas

- Starch is gelatinized during baking process
  - High fat content raises gelatinization temperature
  - Extremely limited opportunity for enzyme to work
- An amylase with higher *endo* activity and higher thermostability may be the best option
  - Aggressive action pattern can modify amylopectin rapidly
  - Dosage must be well controlled to avoid tortillas from sticking together in package
- Amylase – emulsifier blends are often used to maintain quality and to prevent stickiness

Corn Tortillas (made from masa flour)

- Starch is “opened up” during nixtamalization and drying process
  - Accessible for enzyme modification during dough mixing and resting
- An amylase with high *endo* activity will create sticky dough and tortillas with very fragile integrity
- Maltogenic amylase can be used to extend freshness of corn tortillas
  - Maintains integrity of amylopectin

Corn Tortillas (cont'd)

- From testing done at University of Nebraska, Lincoln
  - "Adding maltogenic amylase in a corn tortilla formulation containing CMC:
    - improved corn tortilla processing/handling/sheeting properties
    - enhances tortilla rollability and flexibility
    - significantly reduced DSC measured starch retrogradation.
  - The shelf life of corn tortillas with this enzyme product can be significantly extended"

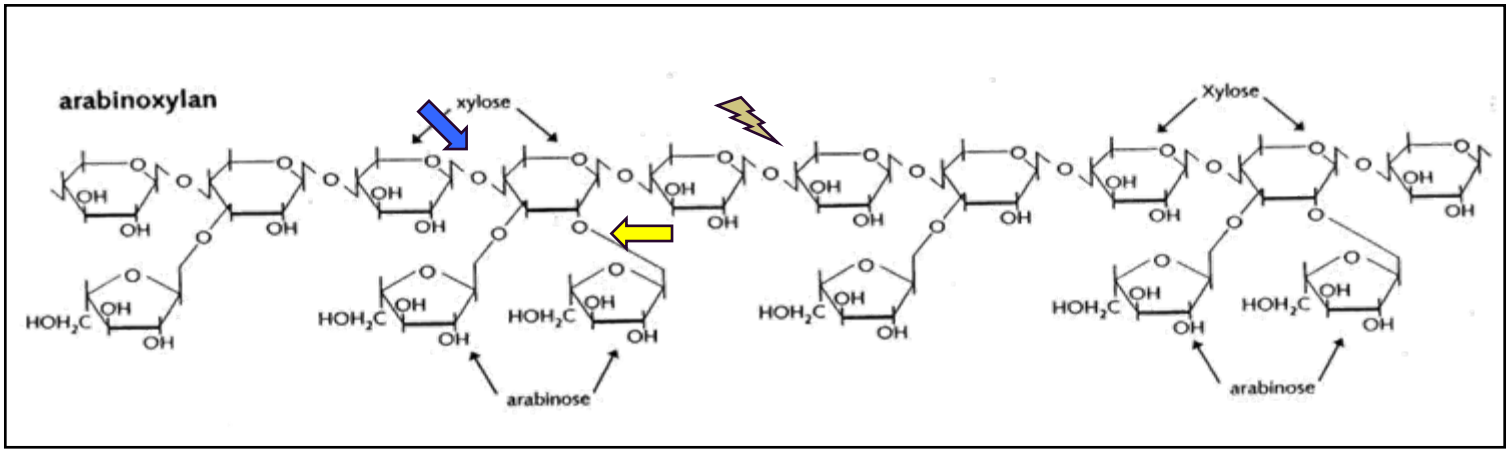
ENZYME TYPES & FUNCTIONALITY

- Amylases
- **Xylanases**
- Lipases
- Oxidases
- Proteases
- Asparaginase

Non-Starch Polysaccharides

- Found at a level of 1-3% in white flour
- Both water soluble (extractable) and insoluble (unextractable)
- Hold many times their weight in water
- Level can vary between wheat extraction, variety and growing season
- Sometimes referred to as flour gums, hemicellulose, pentosans
- Arabinoxylans are most important in terms of baking quality

ARABINOXYLAN

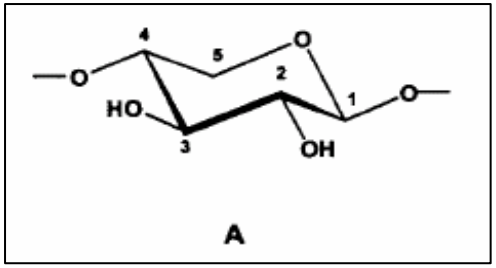


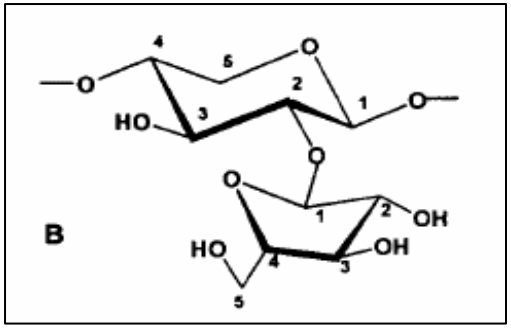
endo-1,4-β-xylanase (EC 3.2.1.8)

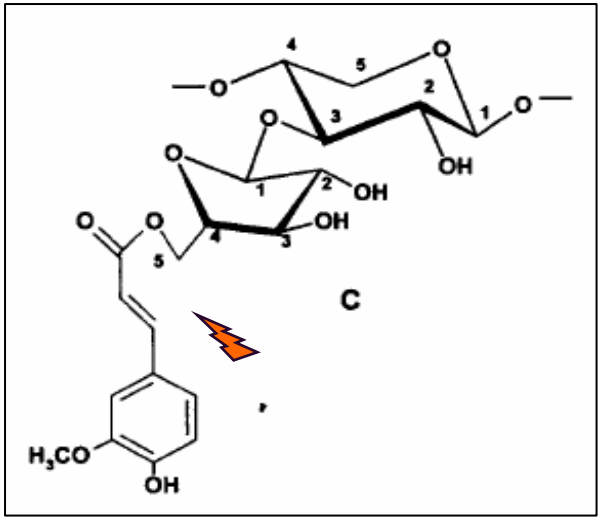
β-xylosidase (EC 3.2.1.37)

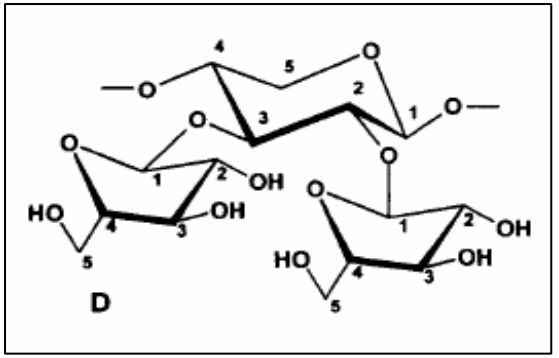
feruloyl (p-coumanroyl) esterase (EC 3.2.1.55)

α-L-arabino-furanosidase (EC 3.2.1.55)









A. Xylose element

B. Xylose with substituted Arabinose

C. B with link to ferulic acid

D. Xylose with two substituted Arabinose

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RECAP

ENZYMES…

- …are catalytic proteins that work under mild conditions
- …are highly specific
- …are influenced by substrate concentration and accessibility, temperature, ph
- …improve product quality and processing and reduce waste
- …can improve the healthfulness of food products by preventing the formation of potentially harmful compounds

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Thank You!
Gracias!