Enzymes For Specific Improvements in Tortilla Application

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Tortilla market is rapidly expanding…..

According to Future Market Insights:

Expected global tortilla market 12.3 billion by end of 2028

Source: https://www.prnewswire.com/news-releases/tortilla-market-is-projected-to-be-valued-at-us-12-324-4-mn-by-2028-end-future-market-insights-818122774.html

Tortilla market is rapidly expanding…..

Key driving factors for increasing demand include:

- Innovative flavors
- Health deliverables
- Small portion size of tacos
- Varieties (e.g., corn chips,
- corn tortilla, flour tortilla,
- wraps, etc.)

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Ideal Tortilla Characteristics

- Uniform round shape
- Evenly distributed blisters
- White with opacity or translucence
- Uniform edges with soft texture
- Excellent rollability/flexibility
- Good foldability
- Resistance to cracking/breaking
- No zippering; no sticking
- Optimal shelf-life (NO MOLD)

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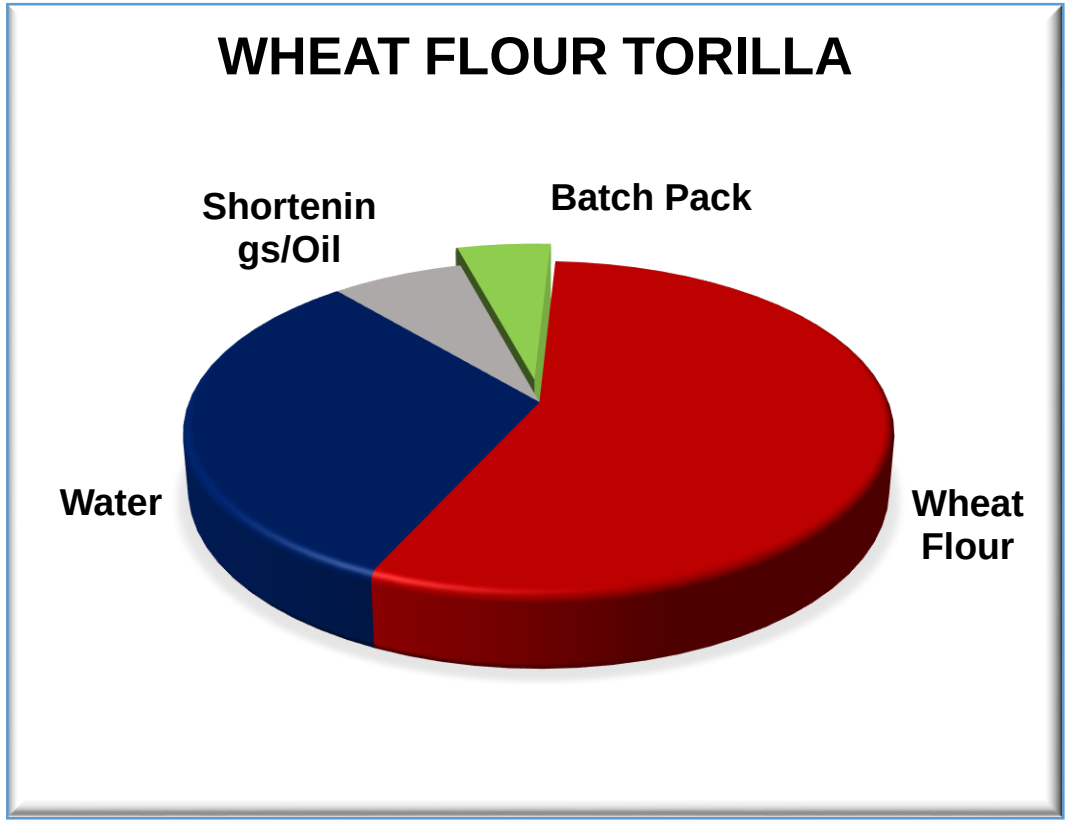
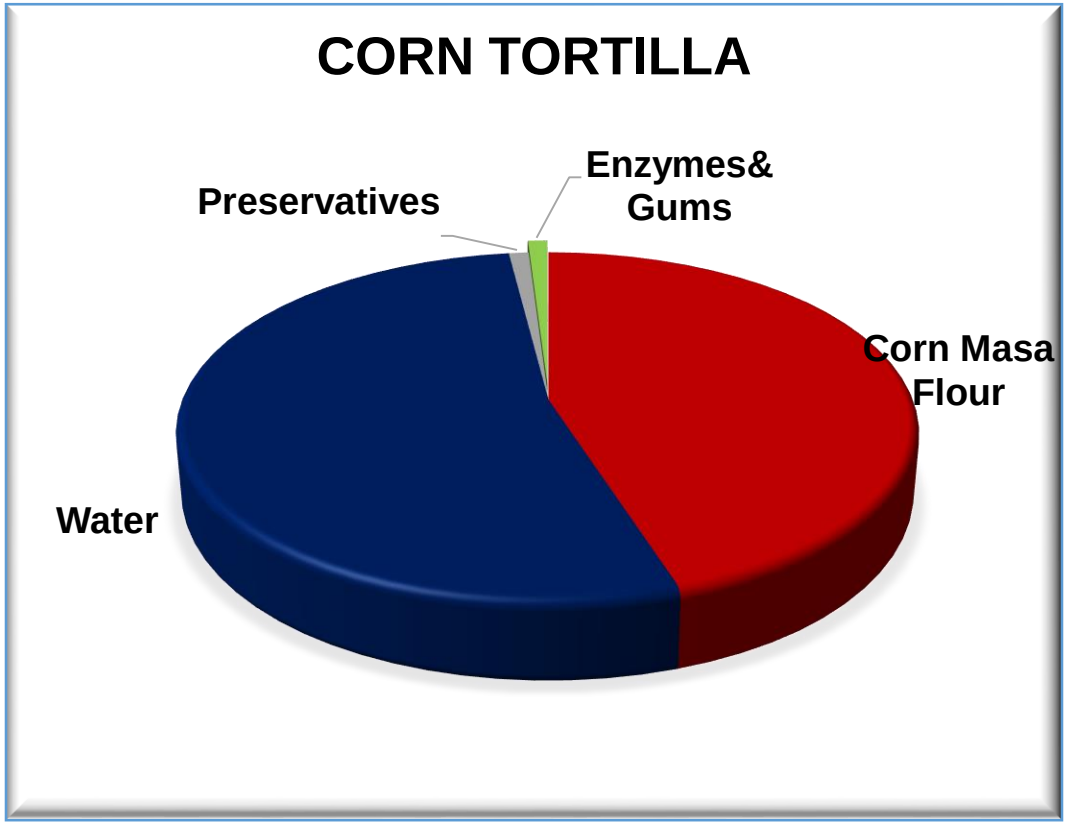
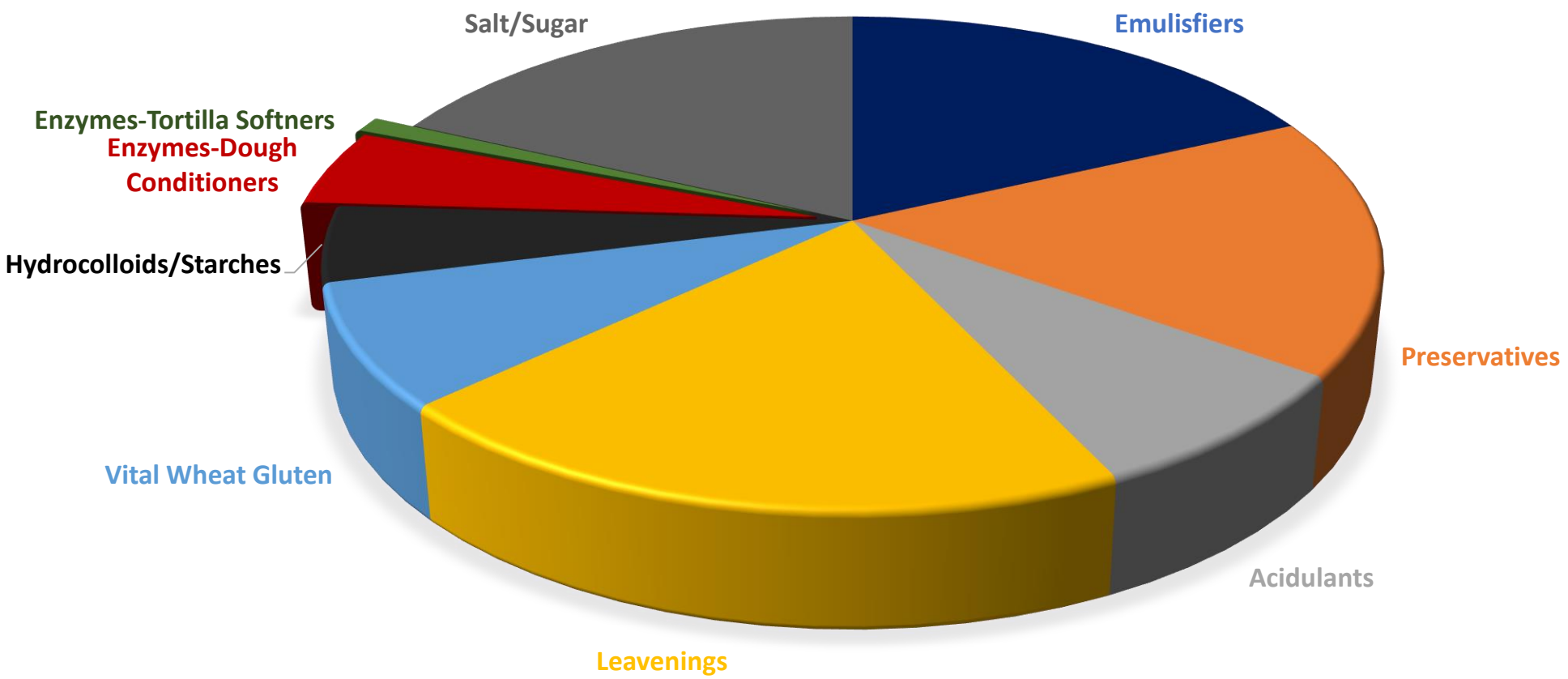
Basic Composition of Tortillas





Basic Composition of Batch Pack



What are Enzymes?

- Enzymes are proteins that function as biocatalysts
- Control vital metabolic processes and exist in all living organisms
- Some of them break down complex food ingredients into simpler ingredients
- Work under relatively mild conditions

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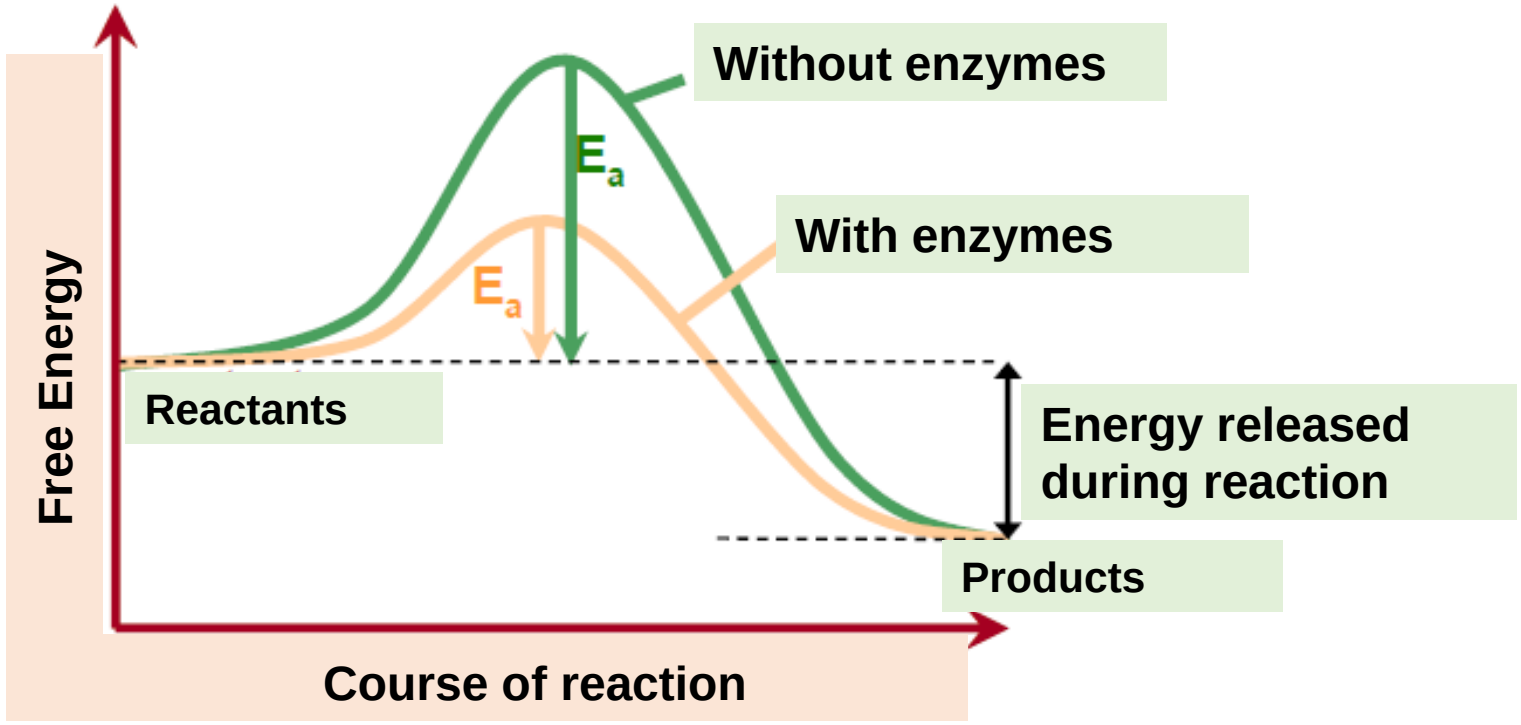
Sources of Commercial Enzymes

	Sources	Enzymes
1	Microbial	Bacterial, Fungal, Yeast
2	Plant	Barley Malt, Papain, Bromelain, Soybean
3	Animal	Trypsin, Rennet

Enzyme Kinetics

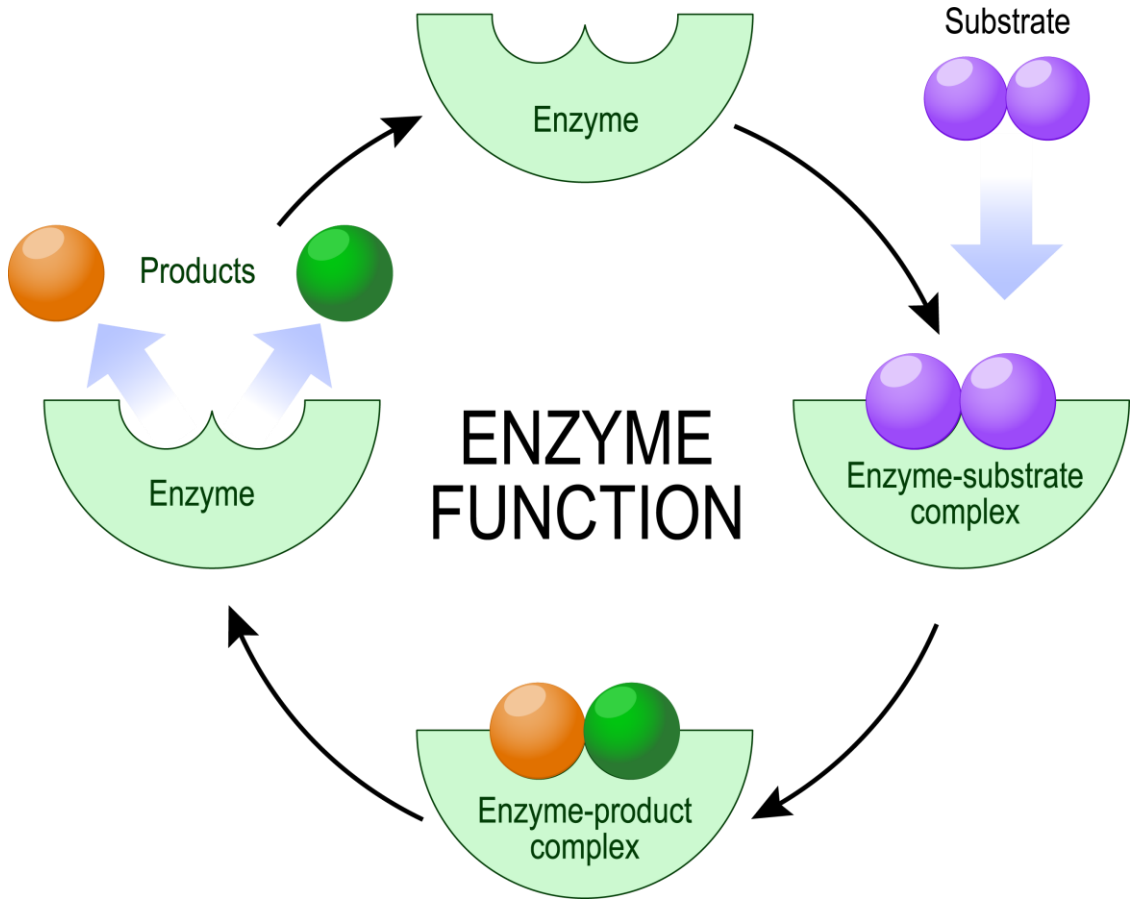
Speed up chemical processes – lower the activation energy needed for reaction to occur



Modified from Oart, M.V. 2010.

Enzyme Kinetics- Induced Fit Model

- Enzymes are very specific
- Enzymes have one or more active sites
- Active sites continuously reshape itself until substrate is completely bound



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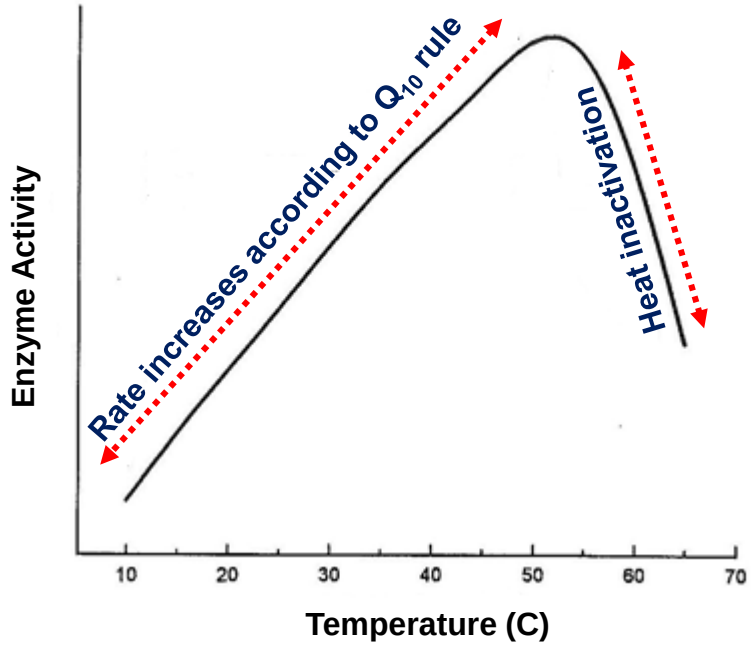
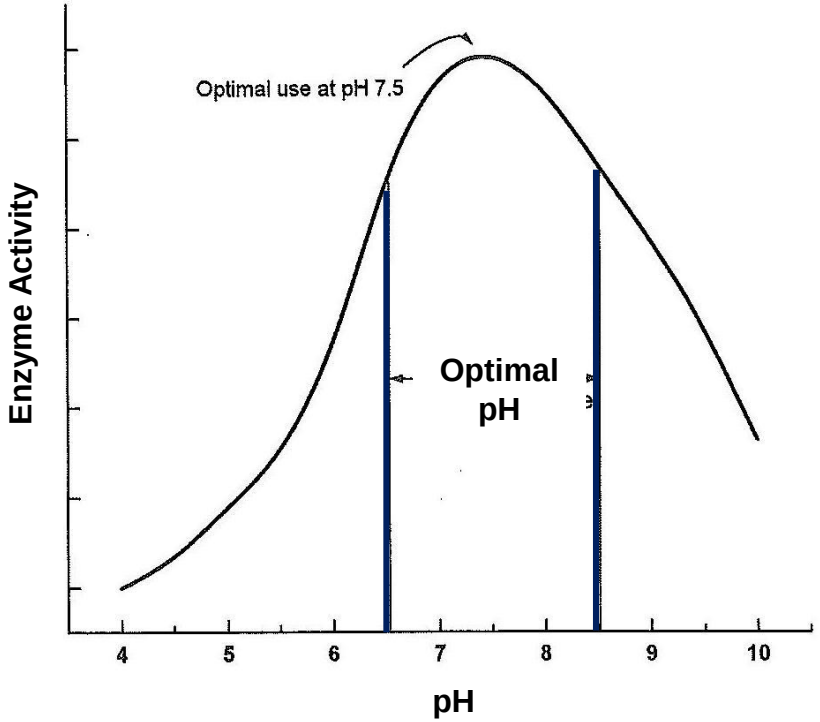
Factors Affecting Enzymes

Temperature



pH



Adapted from Purich, D. L. 2011

Factors Affecting Enzymes

Temperature Characteristics of Starch Degrading Enzymes			
Source	Type	$^{a}T_{Optimum}$ (°C)	$^{b}T_{50}$ (°C)
Sound wheat	α-Amylases	60-66	75
Sound wheat	β-Amylases	48-51	60
Malted wheat	α-Amylases	55-60	65-75
Fungal	α-Amylases	50-60	60-70
Fungal	Glucoamylase	40-45	65-70
Bacterial	α-Amylases	70-80	85-90

$^{a}T_{Optimum:}$ Temperature of optimum activity (pH 5-7);
$^{a}T_{50:}$ Temperature at which 50% of the enzyme is inactivated (pH 5-7);

Hammer, 1992.

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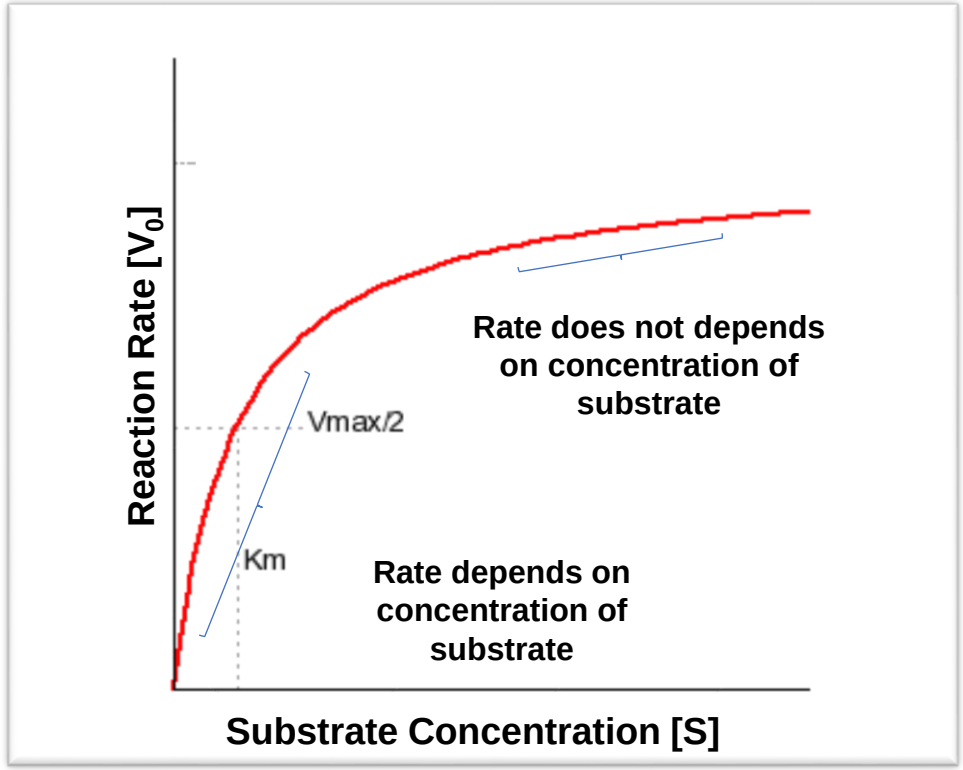
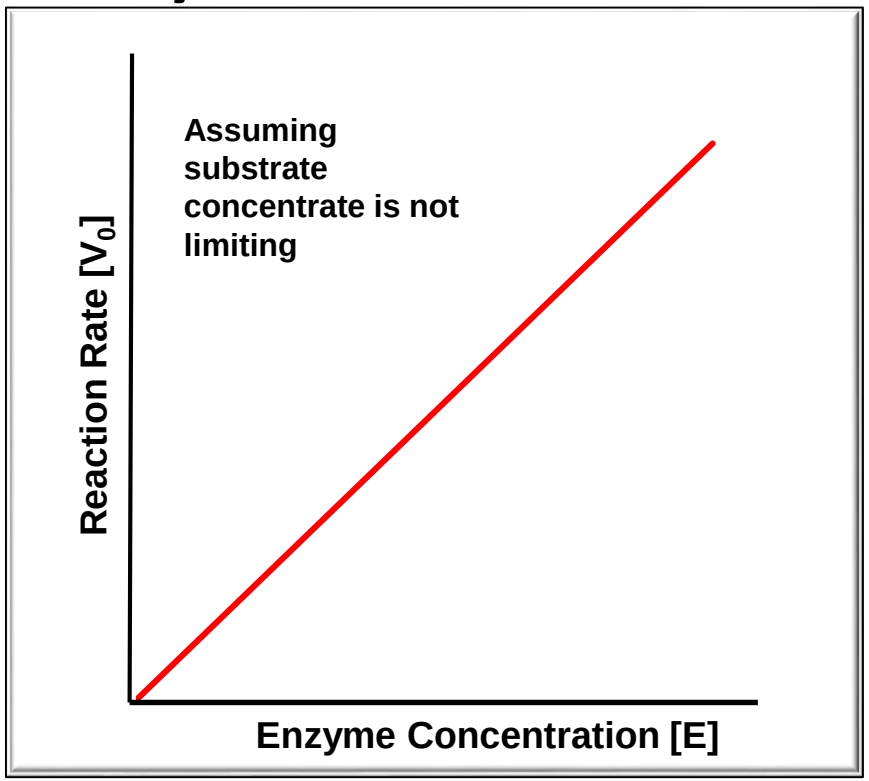
Factors Affecting Enzymes

Substrate Concentration



Enzyme Concentration



Adapted from Purich, D. L. 2011

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Enzymes in Bakery

Enzyme Type	Substrate	Function in Tortilla
Amylases	Starches	Modification of gelatinized starch
Pentonases	Hemicellulose, Xylan	Modification of Pentosan
Proteases	Proteins	Prevent strong gluten network;
Oxidases	Proteins	Improves gluten strength
Transglutaminase	Proteins	Improves gluten strength
Lipases/phospholipases	Lipids	Help to generate emulsifier like structure
Phytase	Phytic acid	It breaks down phytic acid present in bran
Asparaginases	Asparagine	Removes precursor of acrylamide

Austin, 2016

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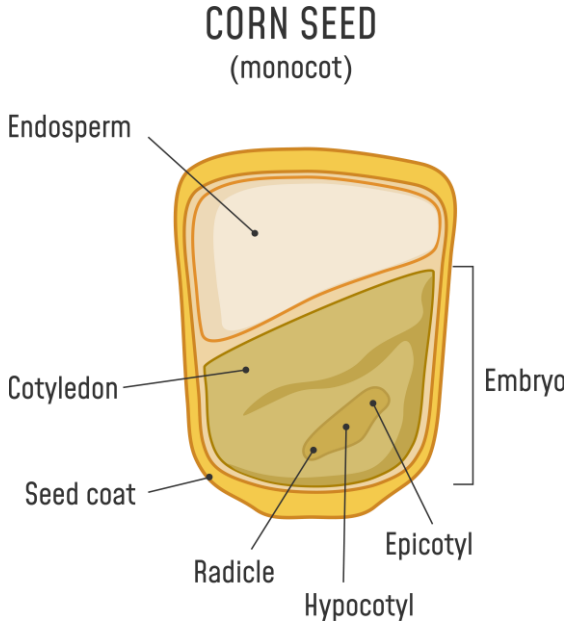
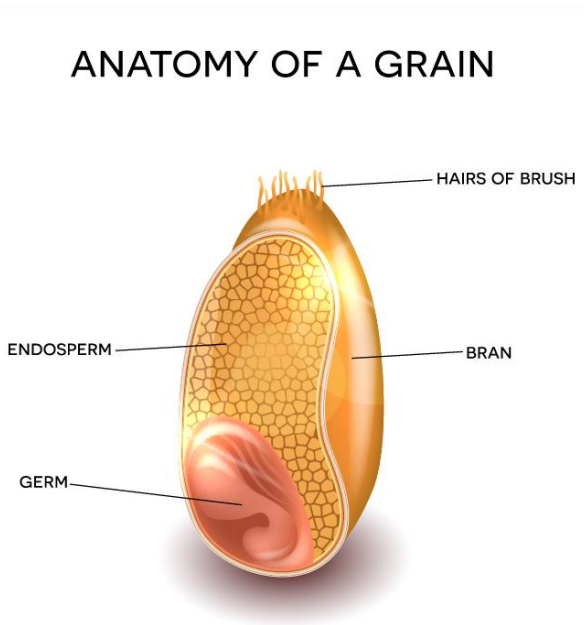
Major Ingredients: Wheat & Corn Flour





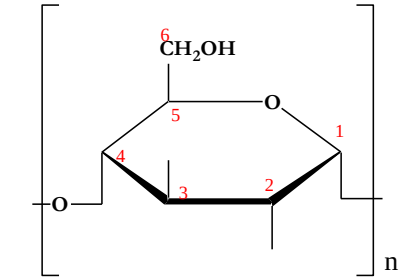
	Endosperm	Starch	Protein	Quality of Protein
Corn	83%	78-85%	9-18%	47% is zein protein; insoluble in water
Wheat	82%	65-75%	9-15%	80% is Gluten; known for water absorption

Plant Foods Hum Nutr (2000) 55: 15

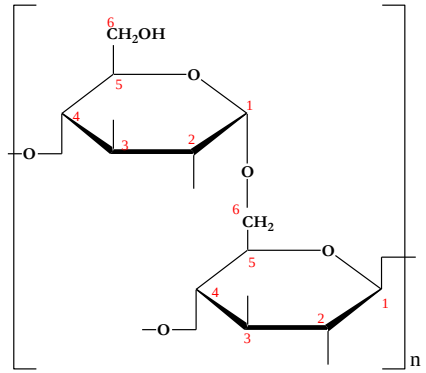
Starch

Starch has two components:

- **Amylose** Linear glucose polymer **(20-25%)**:
- α-linked glucose units (Glucan) α, 1→4 linkage



- **Amylopectin** Branched glucose polymer **(75-80%)**:
- α, 1→4 and α, 1→6 linked glucose units (Glucan)



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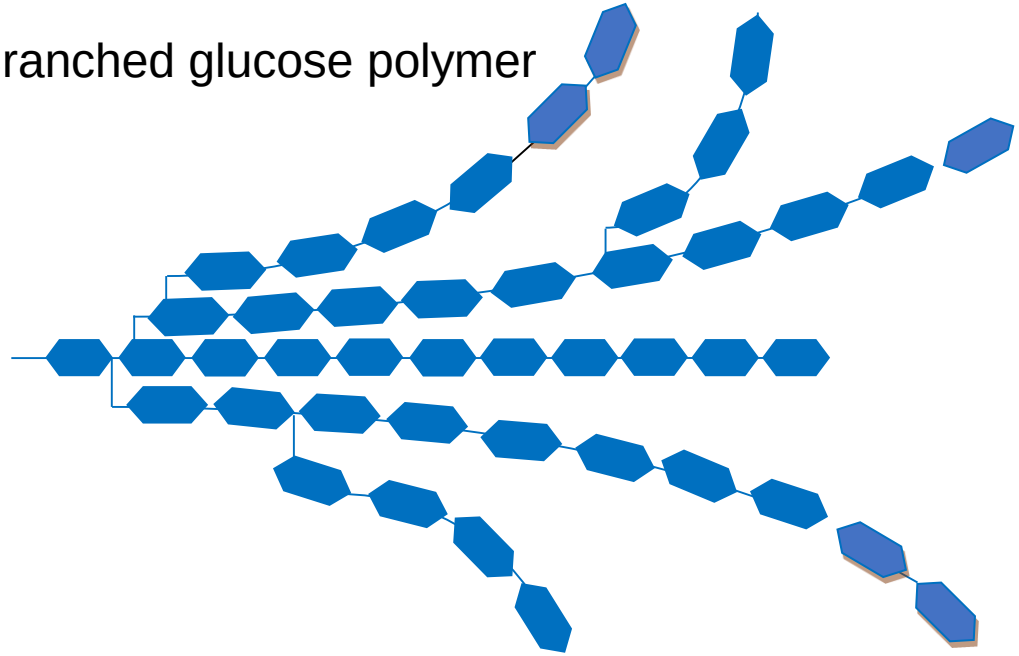
Starch

- **Amylose** Linear glucose polymer **(20-25%)**:

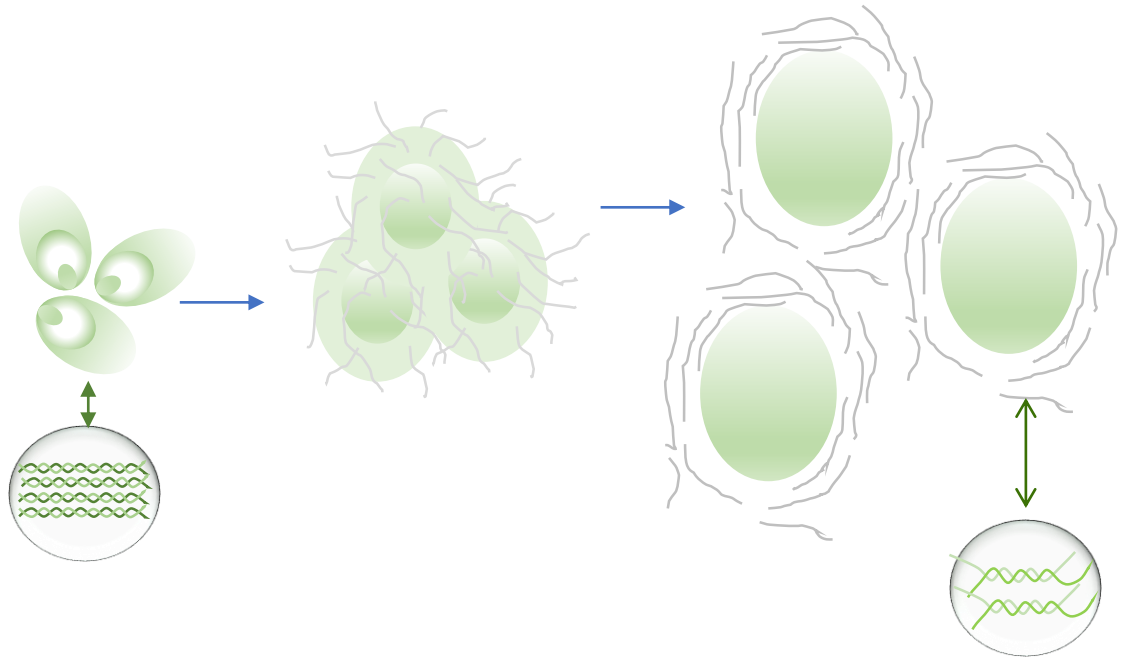
- **Amylopectin** Branched glucose polymer



Retrogradation of Starch Molecules

Starch Water Interaction

- Gelatinization: In the presence of water and heat starch absorb water and swells up; loses its crystalline structure, viscosity increases
- Retrogradation: After gelatinization starch tend to regain its crystalline structure



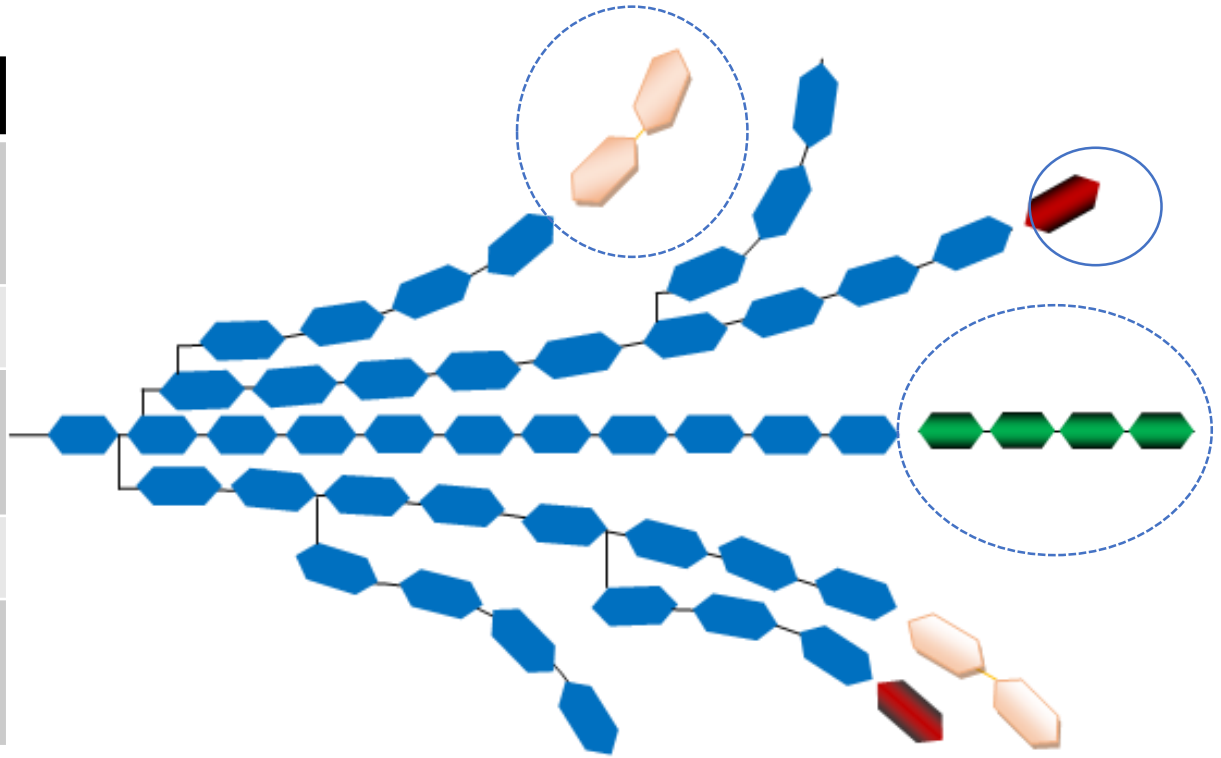
Starch Granules **Heating→** **Cooling/Storage**

Delcour et al., 2005

Enzymes for Starches

Activity of various amylase

Enzyme Type	Products Produced
α-amylase	Maltose/ Oligosaccharides
β-amylase	Maltose
Amylo-glucosidase	Glucose
G4 Amylase	Malto-oligosaccarides
Maltogenic amylase	Maltose and Maltodextrins



Oart,2010

Impact of Retrogradation on Tortillas

Retrogradation (staling) is a major concern for corn and flour tortillas resulting in…

- Loss of freshness/softness
- Hardness
- Loss of foldability

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Impact of Retrogradation on Tortillas

Retrogradation (staling) is a major concern for corn and flour tortillas resulting in…

Corn Tortilla without enzymes

Corn Tortilla with enzymes

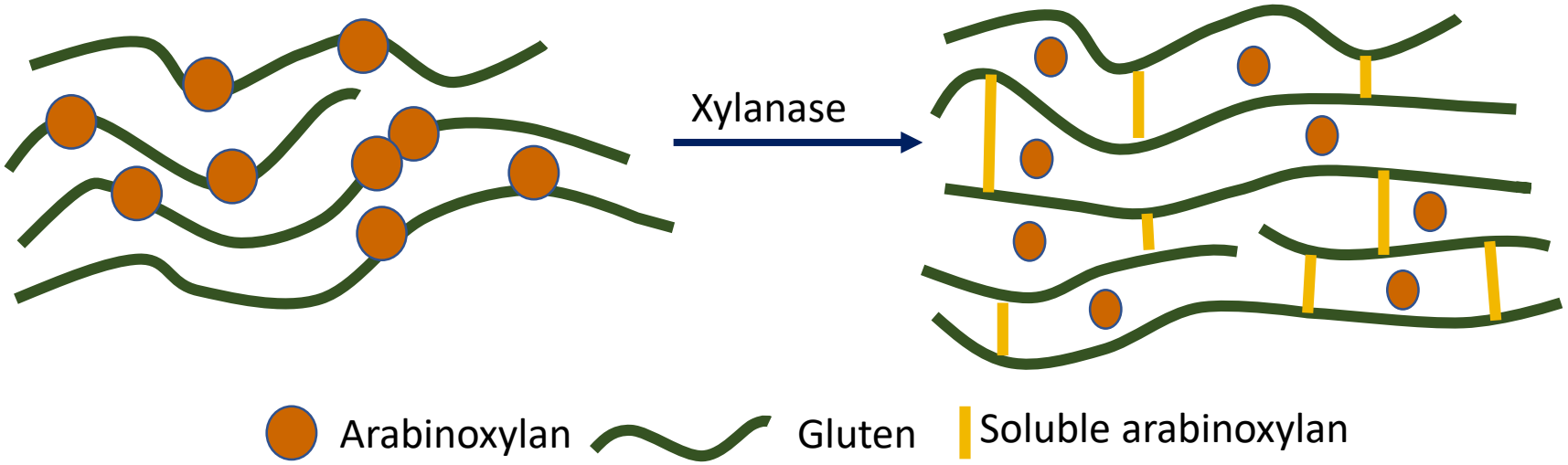
Pentosans (e.g., arabinoxylans)

- Mostly insoluble
- Interfere with gluten development due to their strong water absorption

Composition	%	Distribution of Water in Dough
Starch	58	26.4
Damaged Starch	9.2	19.1
Gluten	14	31.2
Pentosan	1.5	23.4

Li et al., 2012

Enzymes - Xylanases



- Xylanases hydrolyze the xylan
- Xylan breakdown releases water for distribution to starch and gluten
- Dough becomes softer and easier to process
- Reduces mixing time

Anderson, C. and Simsek, S. (1991)

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Enzymes – Protein (Gluten)

- Gluten is major protein present in wheat flour
- Gluten is subdivided into :
    1. Gliadins act as plasticizer and contribute to dough viscosity and extensibility
    2. Glutenin contribute to the dough strength, elasticity and resistance to extension
- Gluten network gives viscoelastic properties (extensibility and elasticity) to dough properties
- Strong gluten matrix is created by disulfide linkages between the amino acids of polypeptide chain

Wieser, 2007; Delcour, 2009

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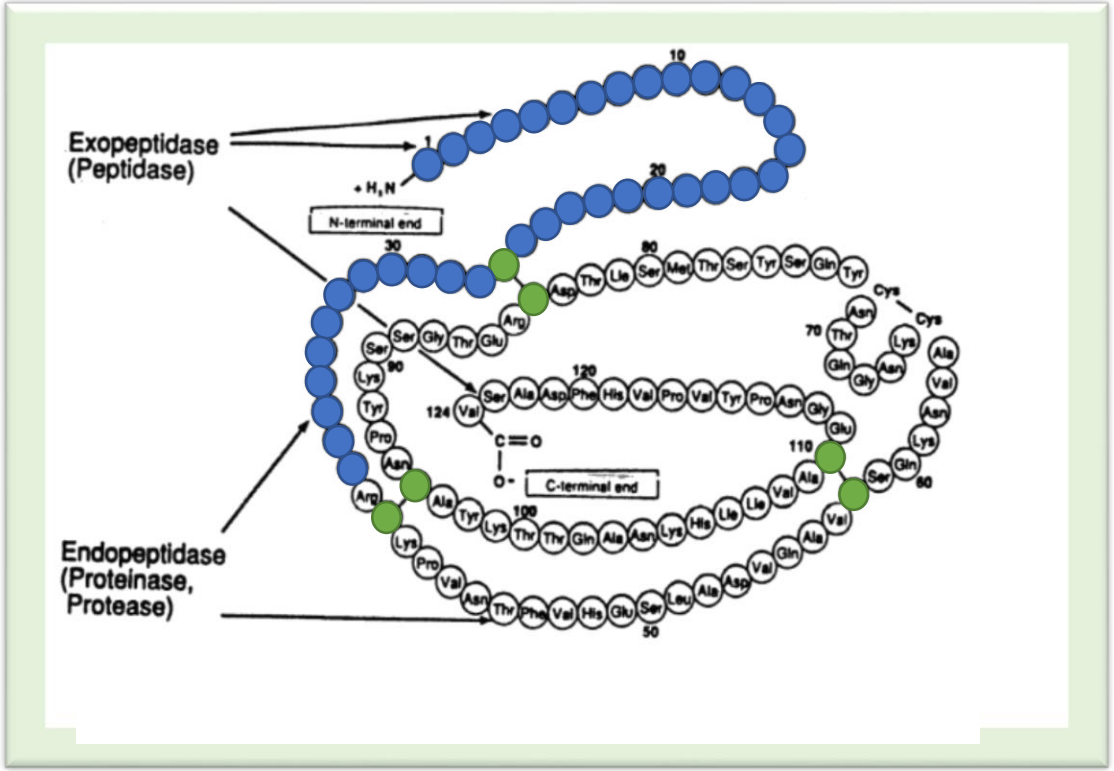
Enzymes – Protease

- Protease is proteolytic enzyme which can be subdivided into exopeptidase and endopeptidase
- Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate
- Endopeptidases cleave peptide bonds distant from the termini of the substrate



Adapted from Anon, 1988

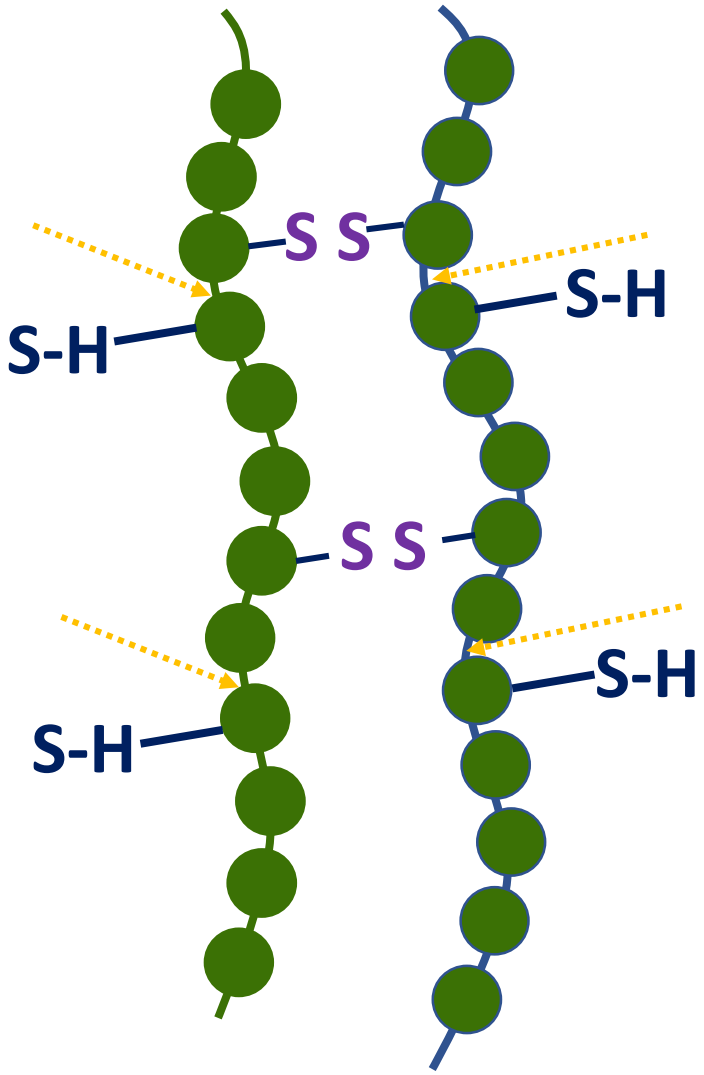
Enzymes – Protease

Gluten quality is determined by:

- Glutenin polymer structure
- Size distribution and subunit composition
- Gliadin/glutenin ratio

Protease can help:

- Reduces the size of glutenin polymers and redistribute SH/SS ratios
- Dough softening, reduced mixing time and improved dough machinability



Adapted from Anon, 1988

Enzymes – Protease

- Protease improve softness, dough machinability and handling
- Reduces mixing time and improves water absorption
- “Cleaner” replacement for L-cysteine or sodium metabisulfite

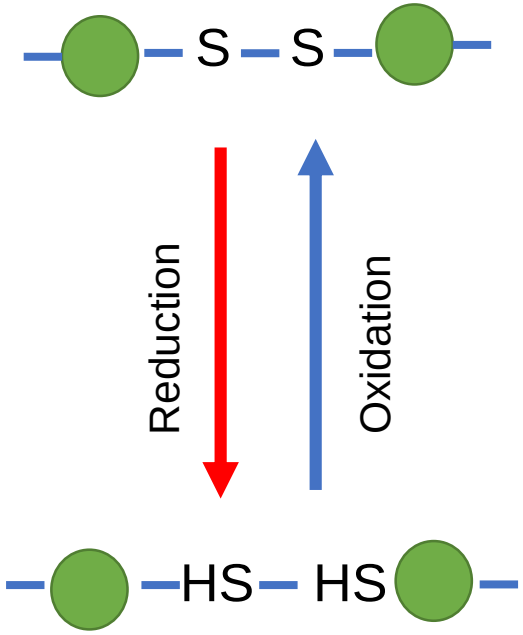
Enzymes – Glucose oxidase

Glucose oxidase – Oxidizing agent

1. Glucose oxidase indirectly oxidize SH into SS by oxidizing glucose and generating hydrogen peroxide and promote disulfide linkages

2. Gliadin–glutenin crosslinking during baking by decreasing the level of free SH groups

3. Minimize SH/SS interchange reactions



Delcour, et al, 2012

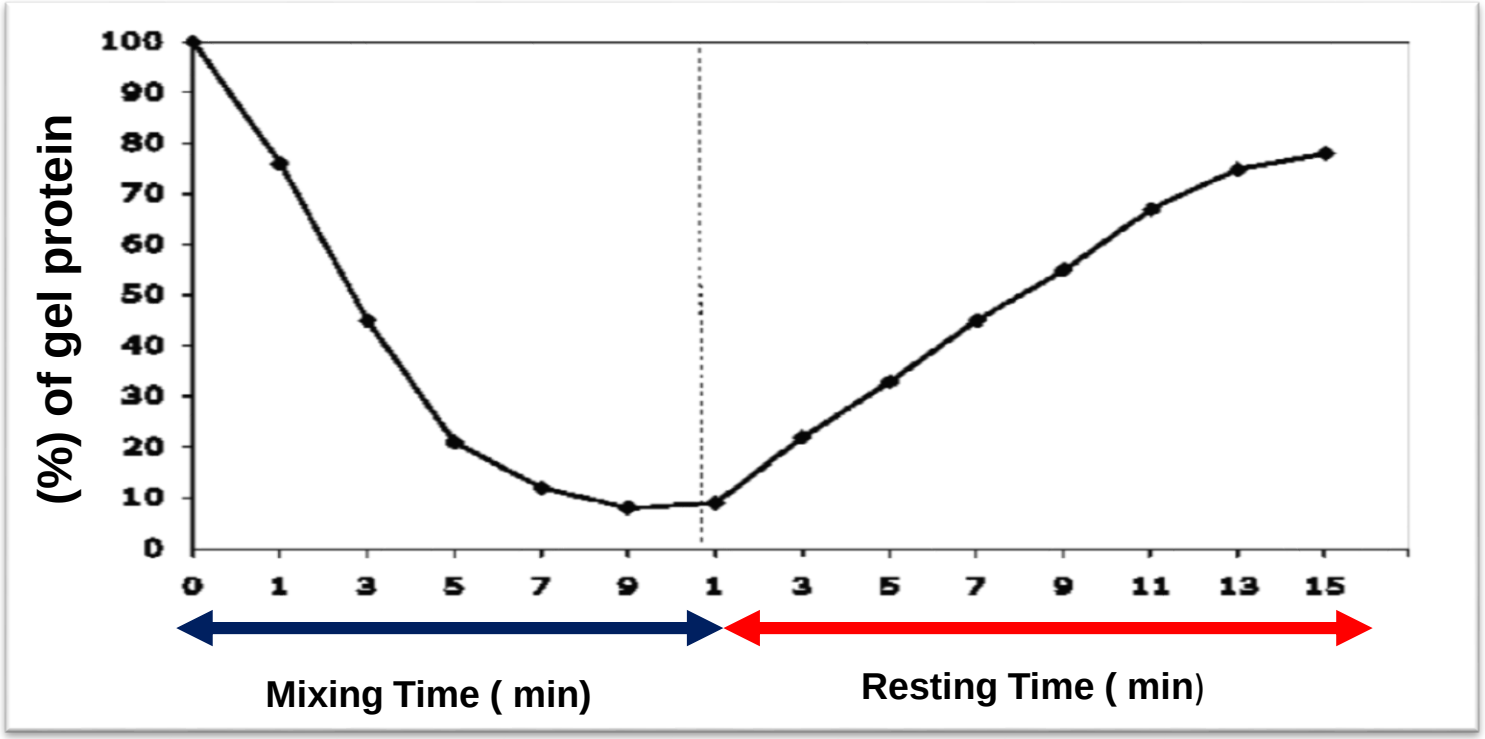
Enzymes – Glucose oxidase

Glucose oxidase: Oxidation of flour thiol with oxidases

	Reactive SH-Groups (µmol/g)
Control Flour	1.925
Glucose oxidase (1000 U/Kg) Supplement flour	0.379
Glucose oxidase (1000 U/Kg)+ Sulfhydryl oxidase (77 U/Kg) Supplement flour	0.363

Haarasilta et al, 1991

Enzymes – Dough Structure



Breakdown and rebuilding of gel protein during dough mixing and dough resting

Schofield et al, 1991

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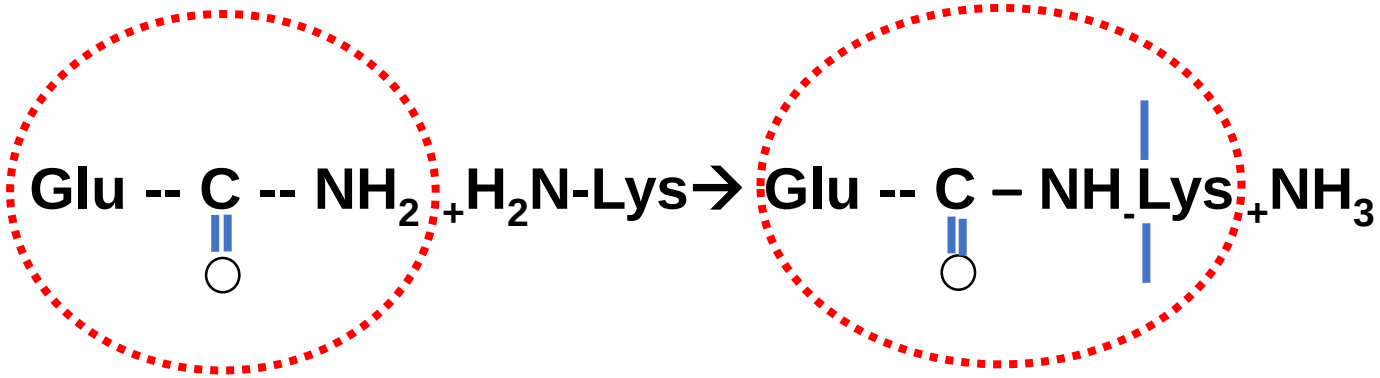
Enzymes – Transglutaminase

Catalyzes formation of cross links between the γ-carboxyamide group of peptide bound glutamine residues and various amines

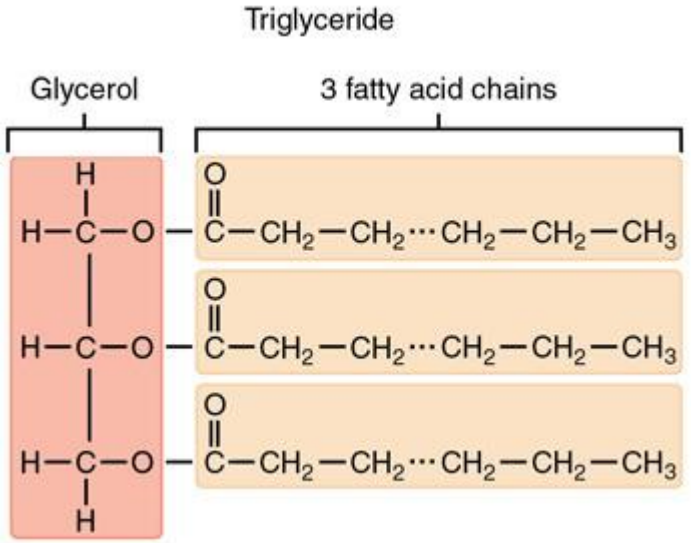
- **Improves the protein network**
- **Increases water holding capacity**
- **Increases water absorption**
- **Viscoelastic and thermal properties**



Song et al, 2010

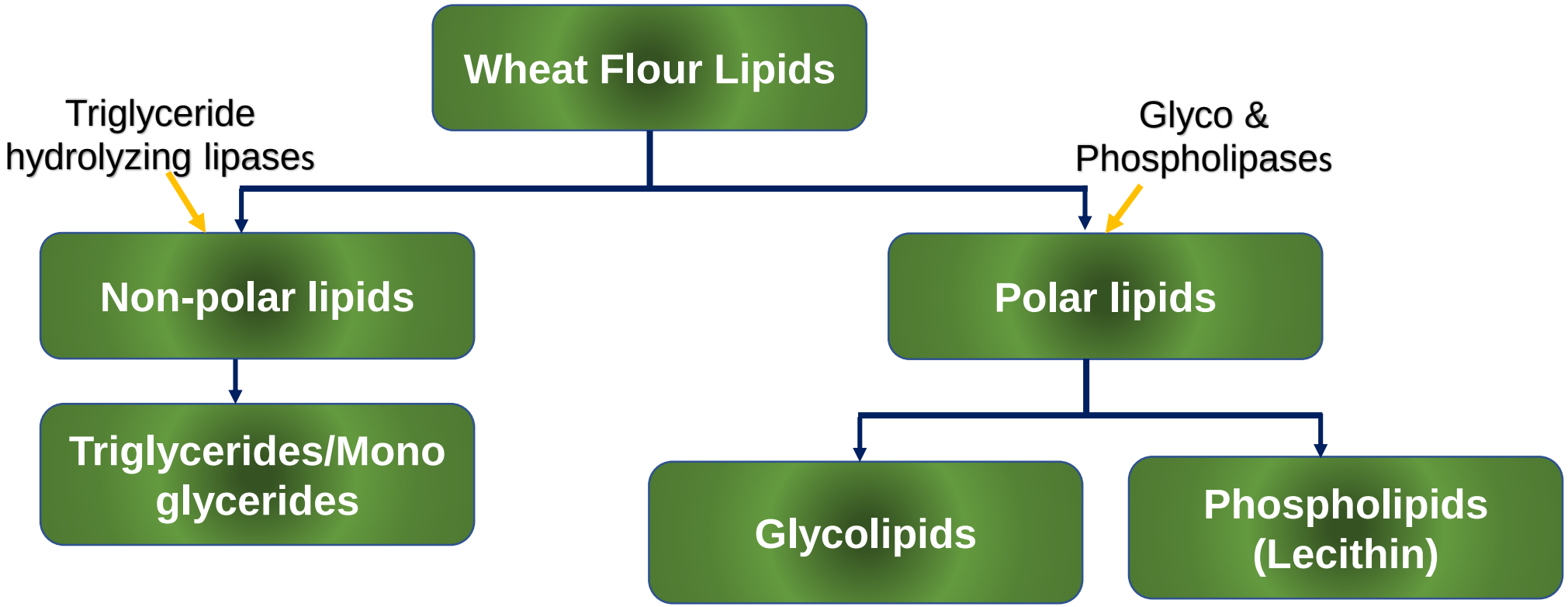
Enzymes - Lipase

- Hydrolyze triacylglycerol (TAG) and produce monoacylglycerols (MAG) , diacylglycerol (DAG), Glycerol and free fatty acid
- Lipases can be 1,3 specific, removing fatty acids from position 1 and 3 and produce mono and –diglycerides
- Mono and – diglycerides improve dough rheology, machinability, increase dough strength and stability
- Also helps in reducing stickiness of tortilla



Basri et al, 2006, Stauffer & Kamel, 2006

Lipases, Glycol & Phospholipases



Pomeranz, 1985

Lipase can work on both polar and non polar lipids

Additional Enzymes

Phytase (Nutritional Improvement)

- Phytic acid is present in bran
- Phytic acid adversely affects bioavailability of minerals (e.g., Zn^{2+} , Ca^{2+} , Mg^{2+})
- Phytase breaks down phytic acid into inositol and phosphoric acid

Asparaginase (Health Concern)

- Acrylamide is classified as probable human carcinogen
- Formed via Maillard reaction between asparagine and a carbonyl source
- Asparaginase catalyzes hydrolysis of asparagine into aspartic acid and ammonia

Gisela Maria Dellamora-Ortiz et al., 2013

Benedito et al., 2001

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Enzyme Blending (Premixes)

- Enzymes are mostly used in combinations to give synergistic effects
- Added at very low concentration
- Diluted to very low concentrations
- Storage in a controlled atmosphere is critical
  - Enzymes are affected by temperature and moisture

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Enzyme Formulation

Kemin has rigorous screening and testing procedures

- Enzyme efficacy & performance evaluation is used to screen and select

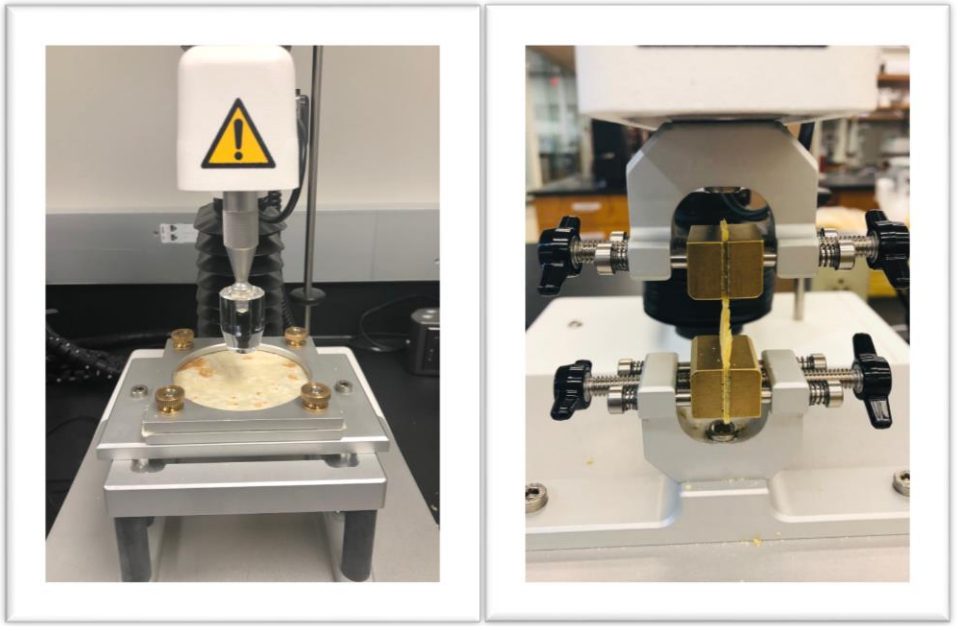
- Enzyme performance testing
  - Lab scale
  - Mimic commercial trials
  - Shelf-life studies

Enzyme Formulation – Process Qualification

Enzyme blending to scale-up level is critical

- Process qualification criteria is important to confirm the blending operation

- Every batch is evaluated to ensure consistency
  - Enzyme activity
  - Performance test



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Kemin Products

- **TillaPack™ FSS:** Batch pack with enzyme blend for Burrito Style Tortilla
- **TillaPack™ GS:** Batch pack with enzyme blend for Gordita Style Tortilla
- **TillaSoft™:** Dough conditioner with enzymes & reducing agents
- **TillaZyme™:** Enzyme, gum blends

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THANK YOU

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Questions

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