

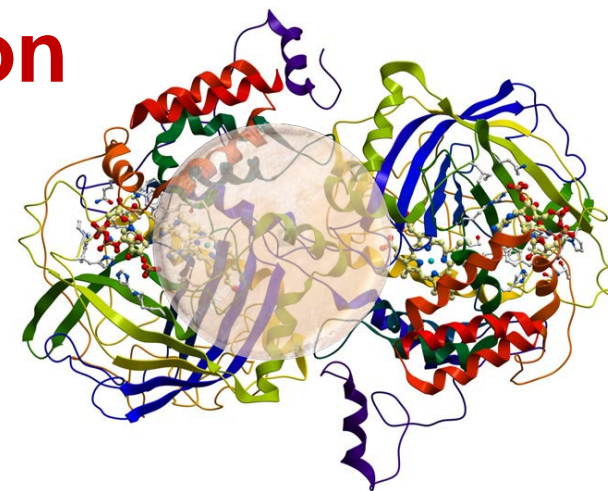


ANYA BAKING LAB
Food and Bakery Consultancy Services



Enzymes in Flat Bread Application

....a clean label solution....



Anita Srivastava, Ph.D., CFS

anitasri38@anyabakinglab.com

info@anyabakinglab.com

+1 470.558.5750

Enzymes in Flat Bread Application

Topics Of Discussion

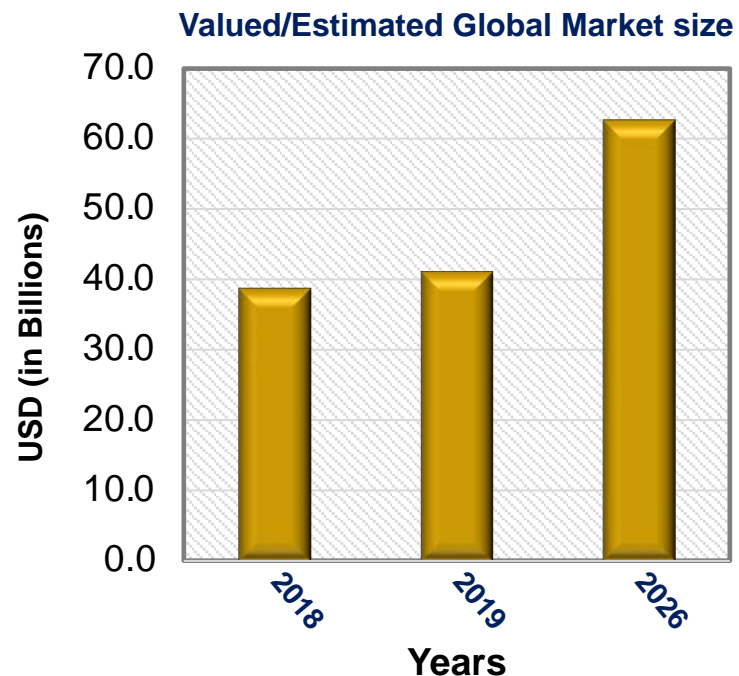
1. Flatbread Industry
2. Types of Flat Bread
3. A process Difference
4. Flat Bread Characteristics
5. Challenges
6. Enzymes and Enzyme Kinetics
7. Enzymes in Flat Bread
8. Application of Enzymes
9. Enzyme Blends & Prototype formulations



Flat Bread Market is Expanding

Flatbreads Are The Oldest Of All Bread Products

Traditionally consumed in: Middle East, North/South Africa, Indian subcontinent, Central America, China, and Europe



Expected to reach \$62.8 billion by 2026 with CAGR of 6.2%



Flat Bread Market is Expanding

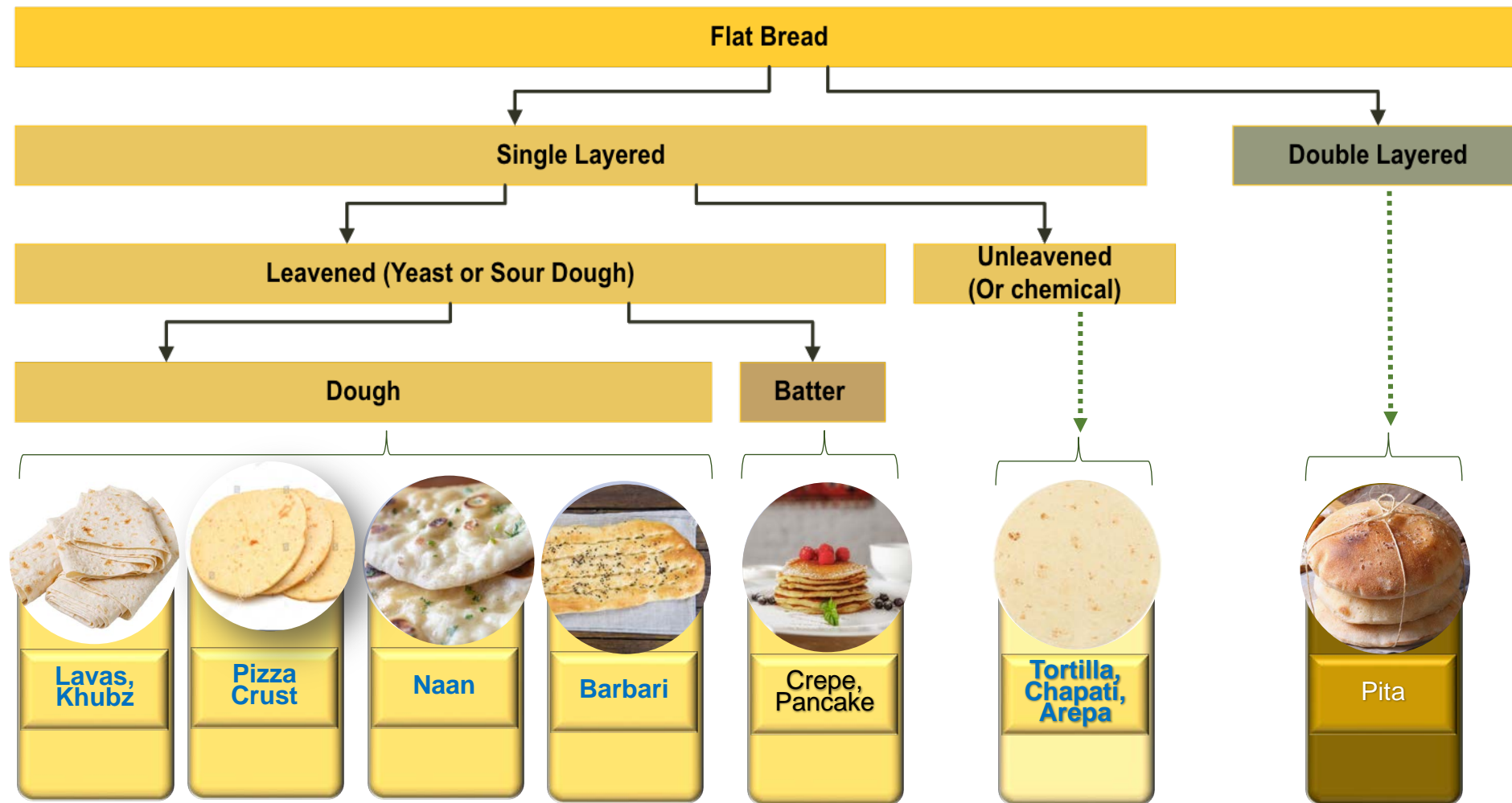
Key Driving Factors For Increasing Demand Include

1. Innovative flavors: wide variety of fillings
2. Health deliverables (Better-for-you), Diabetic Friendly)
3. Small portion size of tacos
4. Varieties (e.g., Pita, Naan, Barberi, corn tortilla, flour tortilla, wraps, etc.)

Tortilla segment dominates the overall global market



Classification of Flat Bread



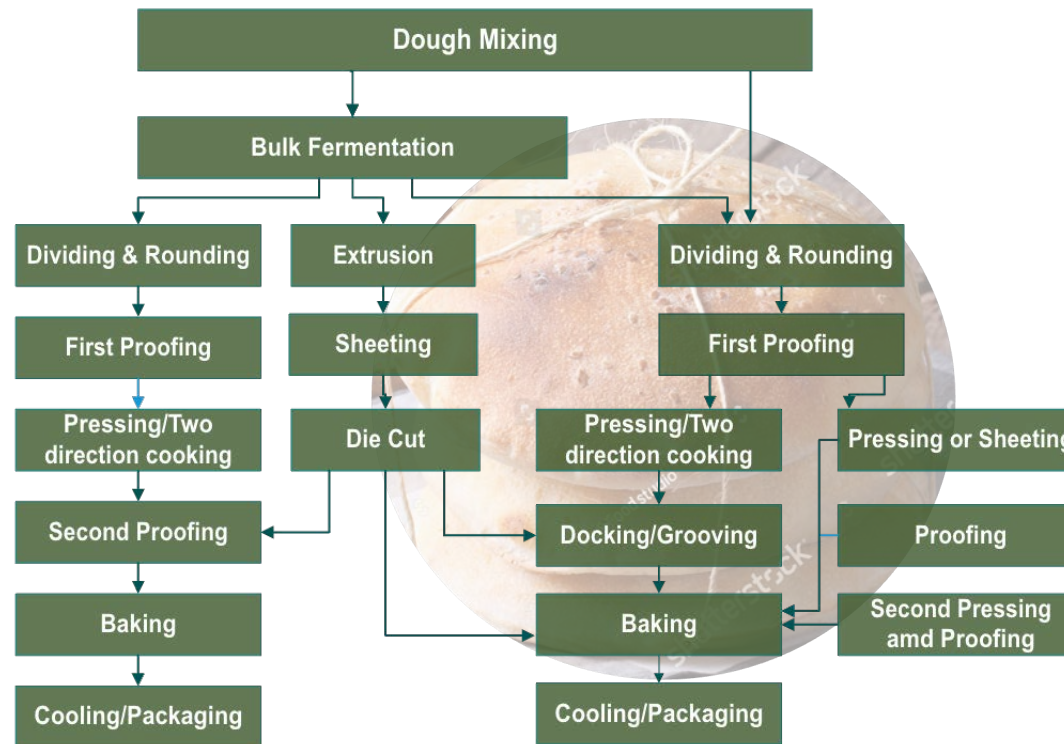
Qarooni, J. (1996).

Enzymes in Flat Bread Application

Single Layer And Double Layer: A Process Difference

Single-layered flat bread

- Dough balls are baked immediately after sheeting
- Baking is at lower temperature
- Sheeting dough pieces are docked/grooved/pressed before baking



Double-layered flat bread

- Dough Balls are proofed after sheeting
- This allows dough to relax, aerate, and develop a thin skin.
- Baking at high temperature
- Top and bottom crust separate into two layers by
- Force from the steam from free water in the dough,
- Pressure from CO₂

Source: Qarooni, J. (1996)

Characteristics of Flat Bread

Quality of Flat Bread

Single Layered

- Smooth crust, or with uniform blisters
- Uniform edges with soft texture
- Excellent rollability/flexibility/foldability
- Resistance to cracking/breaking
- No zippering; no sticking

Double Layered

- A clean separation between top and bottom layers
- Fine and uniform crumb appearance
- Preferred crumb color creamy-white.
- Softness, Resistance to cracking/breaking
- Good tearing quality

Scoring system is preferred method to evaluate flat bread



Enzymes in Flat Bread Application

Challenges in Shelf-Life of Flat Bread

- Minimal Ingredient list
- Highly price sensitive
- Shelf-life: 1 or 2 days
 - Major concern: Staling

Thanks to enzymes!!!!

- With a customized enzyme blend, commercial manufacturers can roll **Freshness**, **Convenience**, and a **Low Price** into one appealing flatbread.



What are enzymes!!!!

Enzymes in Flat Bread Application

What Are Enzymes

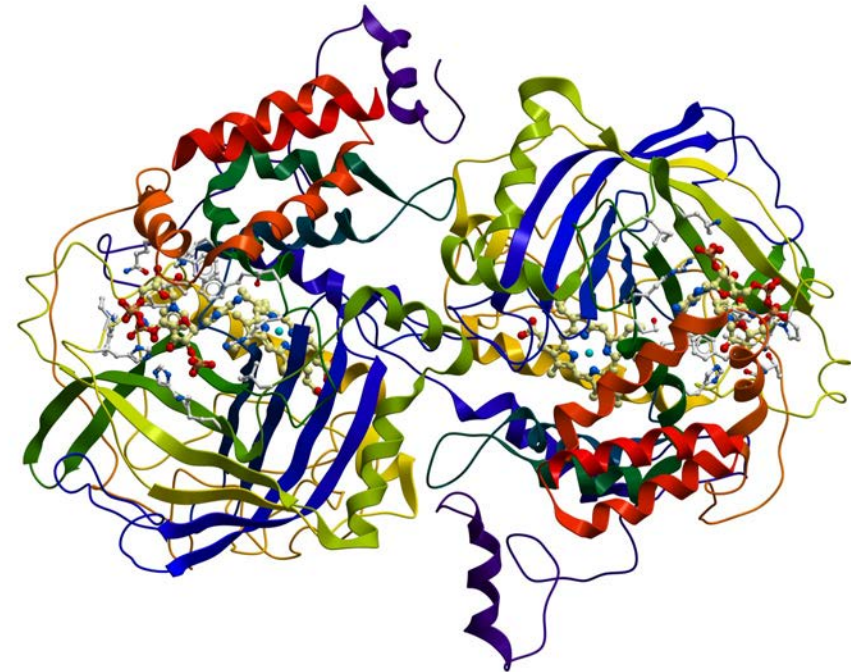
Enzymes Basics

Enzymes are proteins that function as biocatalysts

Work under mild conditions

Most of the enzymes used today are derived from microbial sources and are produced by fermentations

Proteins produced during fermentation is added in the formulation NOT the microorganism itself



Source: Yi Zhang et AL., 2019

Enzymes in Flat Bread Application

Enzymes Kinetics

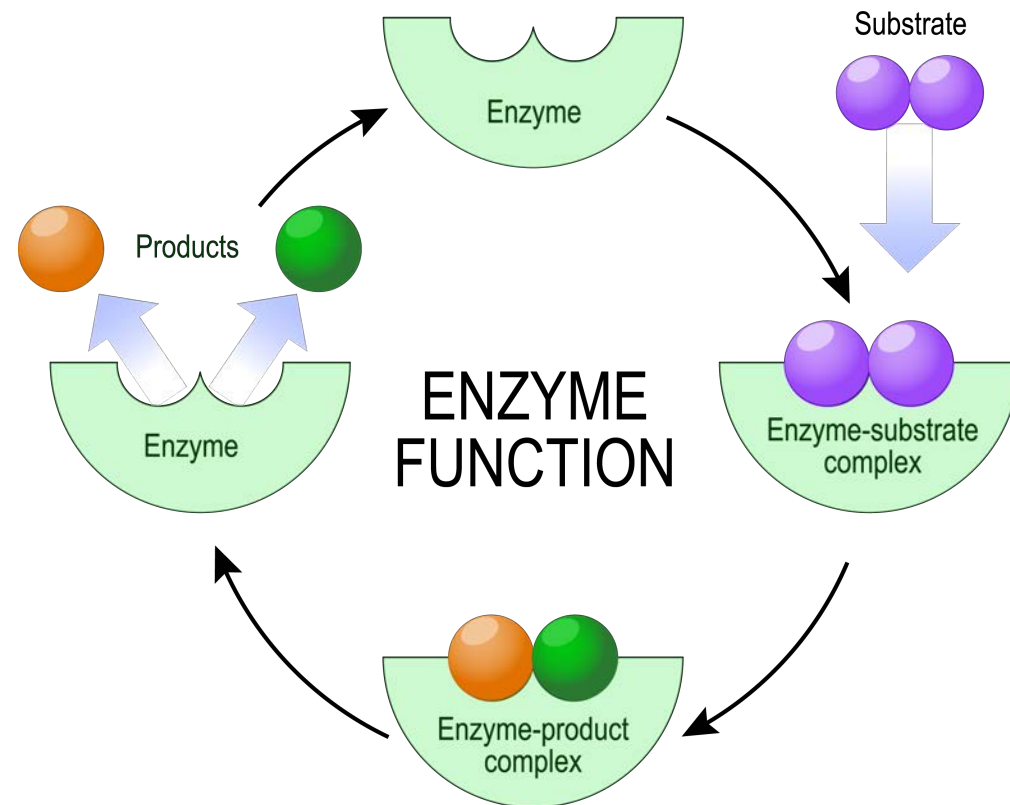
Induced Fit Model

Enzymes are very specific

They have one or more active sites

Active sites continuously reshape itself until substrate is completely bound

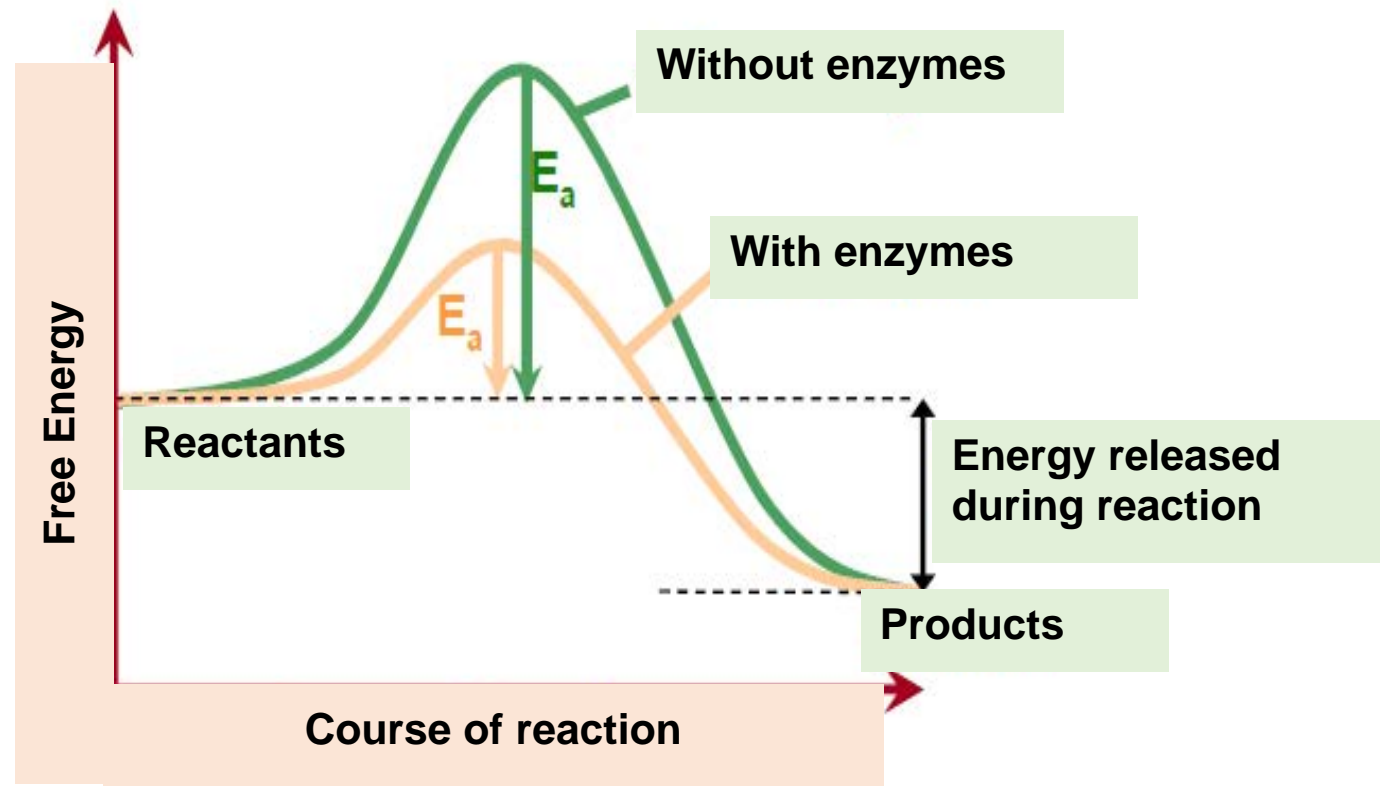
Once the reaction is complete, enzymes are free and can be reused



Enzymes in Flat Bread Application

Enzymes Kinetics

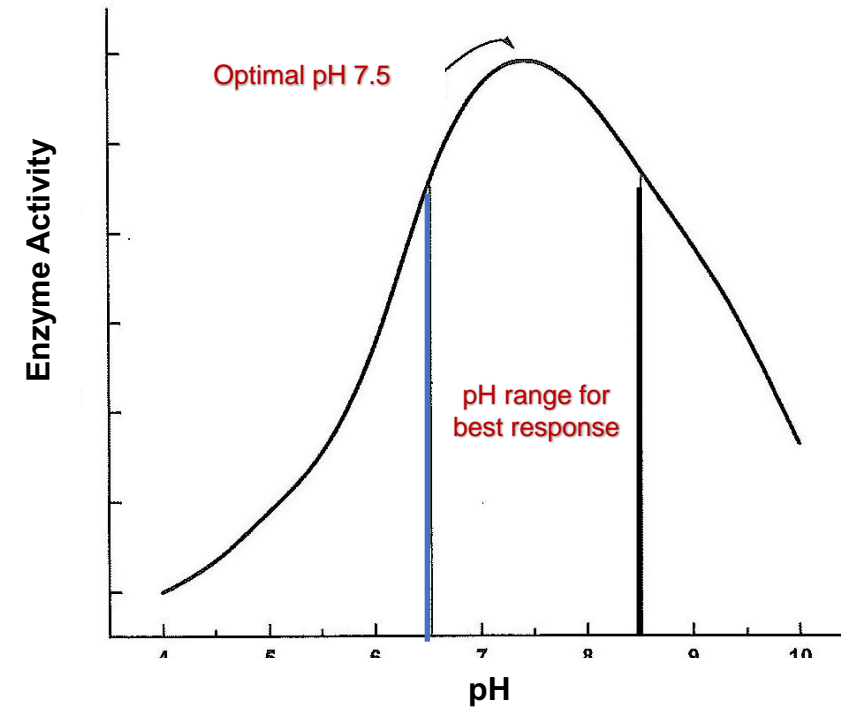
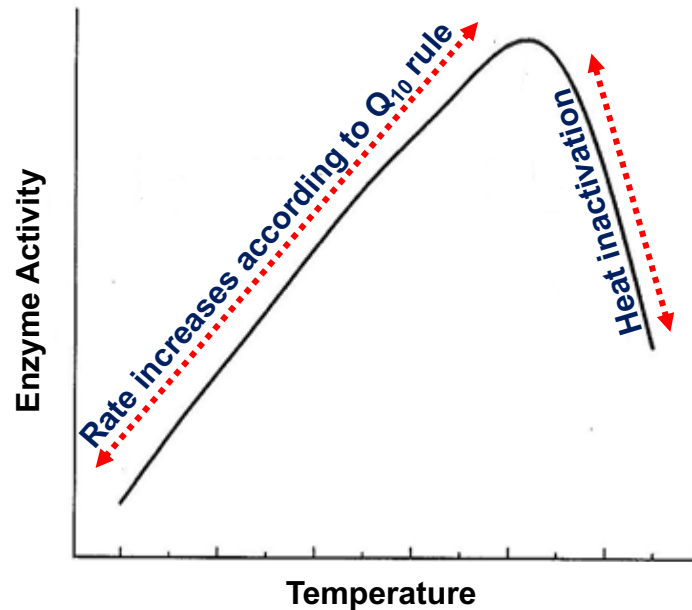
Enzymes speed up chemical processes-lower the activation energy needed for reaction to occur



Source: Oart, M.V. 2010.

Enzymes in Flat Bread Application

Factors Affecting the Enzymes



Source: Purich, D. L. 2011

Enzymes in Flat Bread Application

Factors Affecting Enzymes

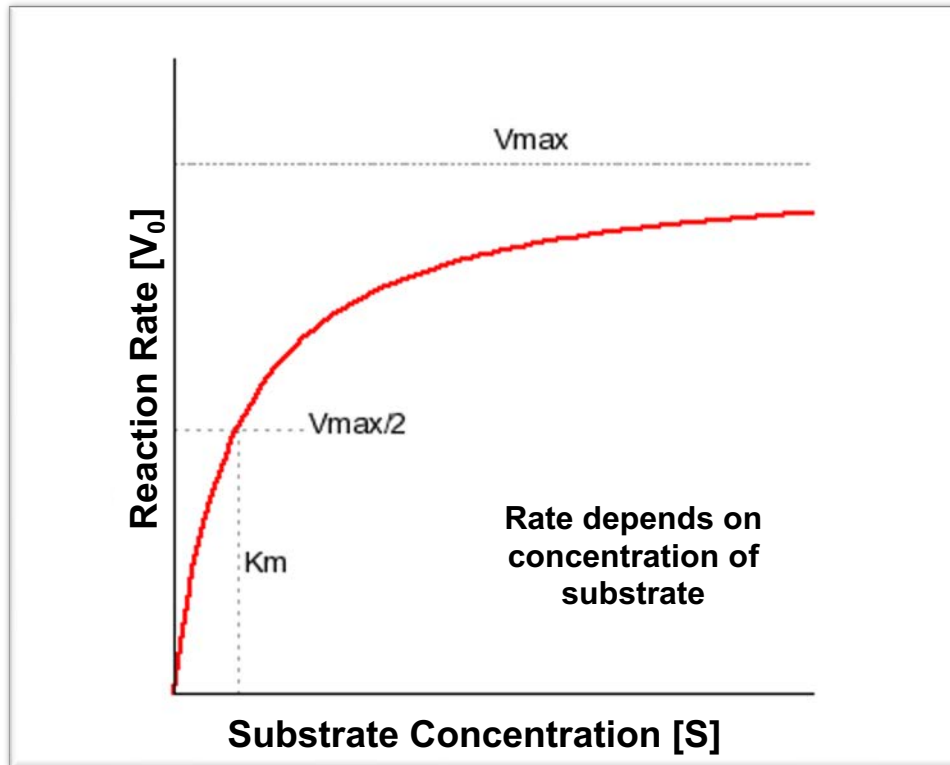
Temperature Characteristics of Starch degrading enzymes

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

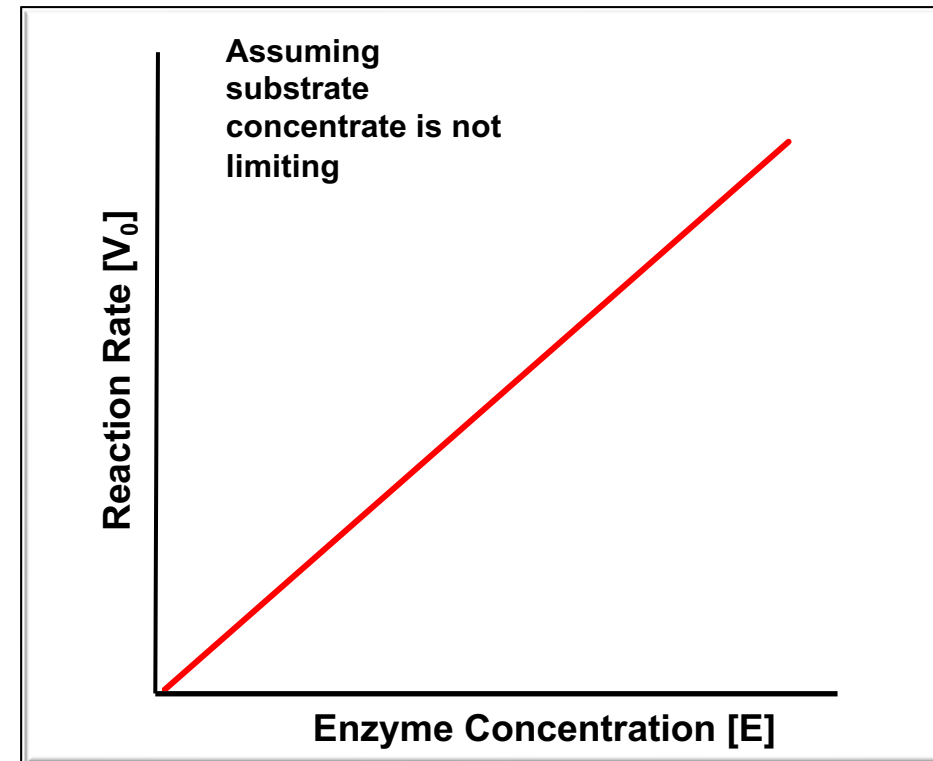
^aT_{Optimum}: Temperature of optimum activity (pH 5-7);
^bT₅₀: Temperature at which 50% of the enzyme is inactivated (pH 5-7);

Enzymes in Flat Bread Application

Factors Affecting the Enzymes



Enzyme Concentration



Enzymes in Flat Bread Application

Enzymes in Bakery

- First time enzymes were used in bakery as flour improvers
- Malt was added to flour as source of α -amylase to correct the concentration of endogenous α -amylase in the flour.
- **Diastase (amylase)** was the first enzyme purified
- Wheat flour is both the most essential ingredient and key source of enzyme substrate for the product.
- Bakery enzymes can be considered as processing aids or as additives



Enzymes in Flat Bread Application

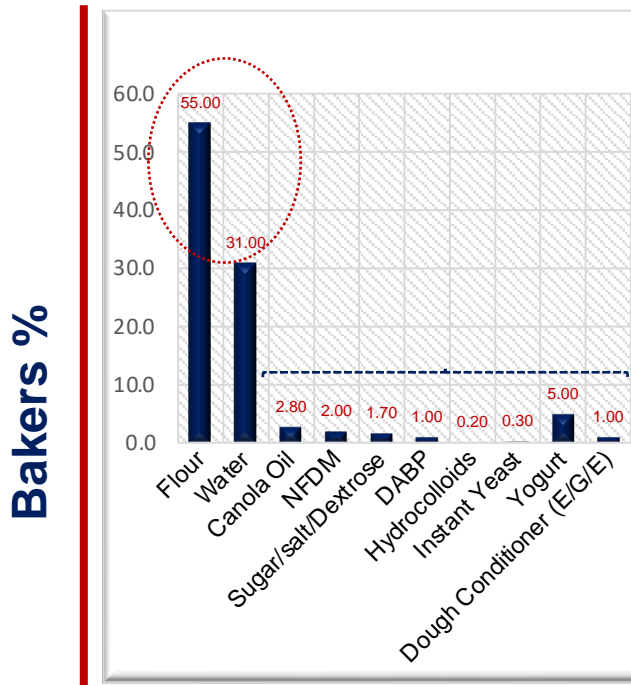
Enzymes in Bakery

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

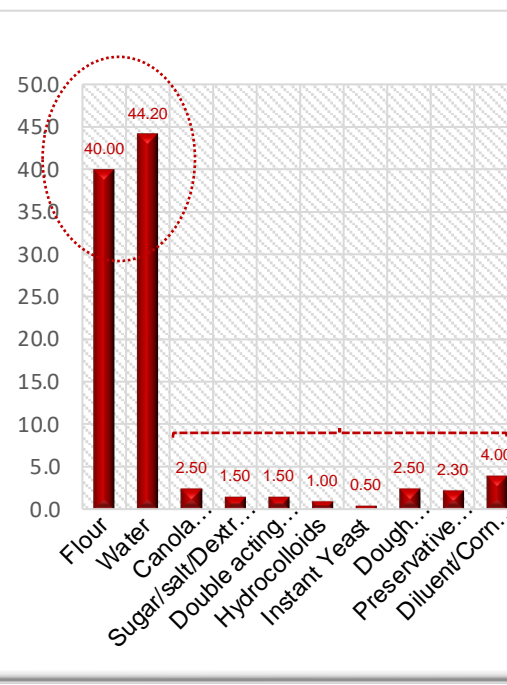
Enzymes in Flat Bread Application

Prototype Formulations

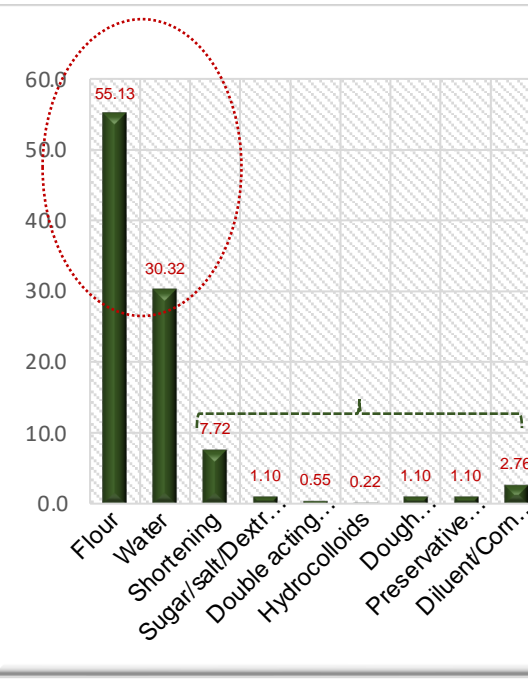
Naan



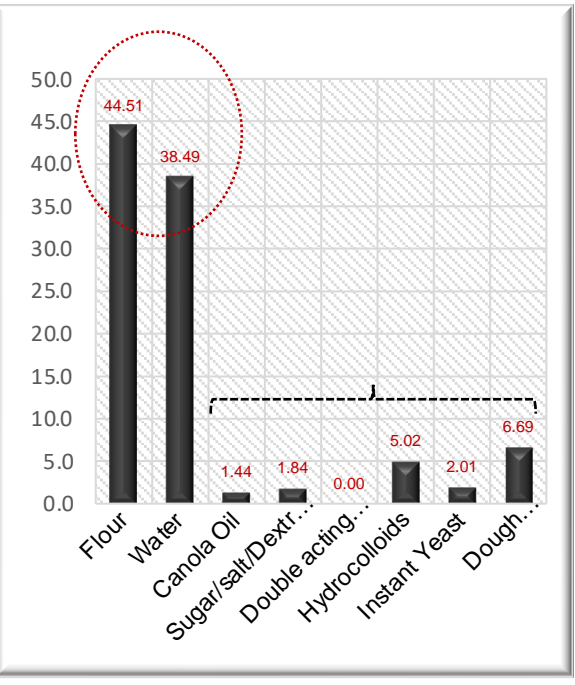
Lavash



Tortilla



Pita



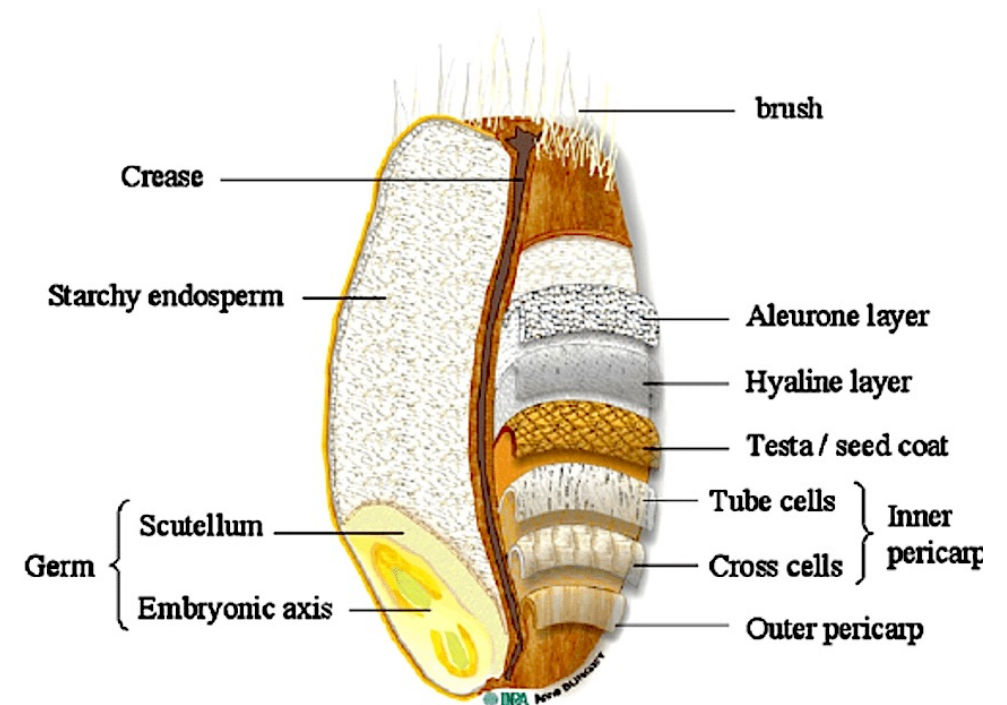
Ingredients

Enzymes in Flat Bread Application

Wheat Grain : Major Ingredient

Mature Wheat Grain has following major components

1	Pericarp (Outer Layer) Aleurone layer, outer layer of grain	Bran (14-16%) Phenolics, Vitamins and minerals
2	Starchy Endosperm	White flour (81-84%) 80% Starch, 10% Protein, Minor % NSP, Lipids
3	Germ	Embryo (2-3%) Vitamins, minerals, oil



Heinze et al., 2017

Enzymes in Flat Bread Application

Starch: Starch Retrogradation is Primary Cause of Staling

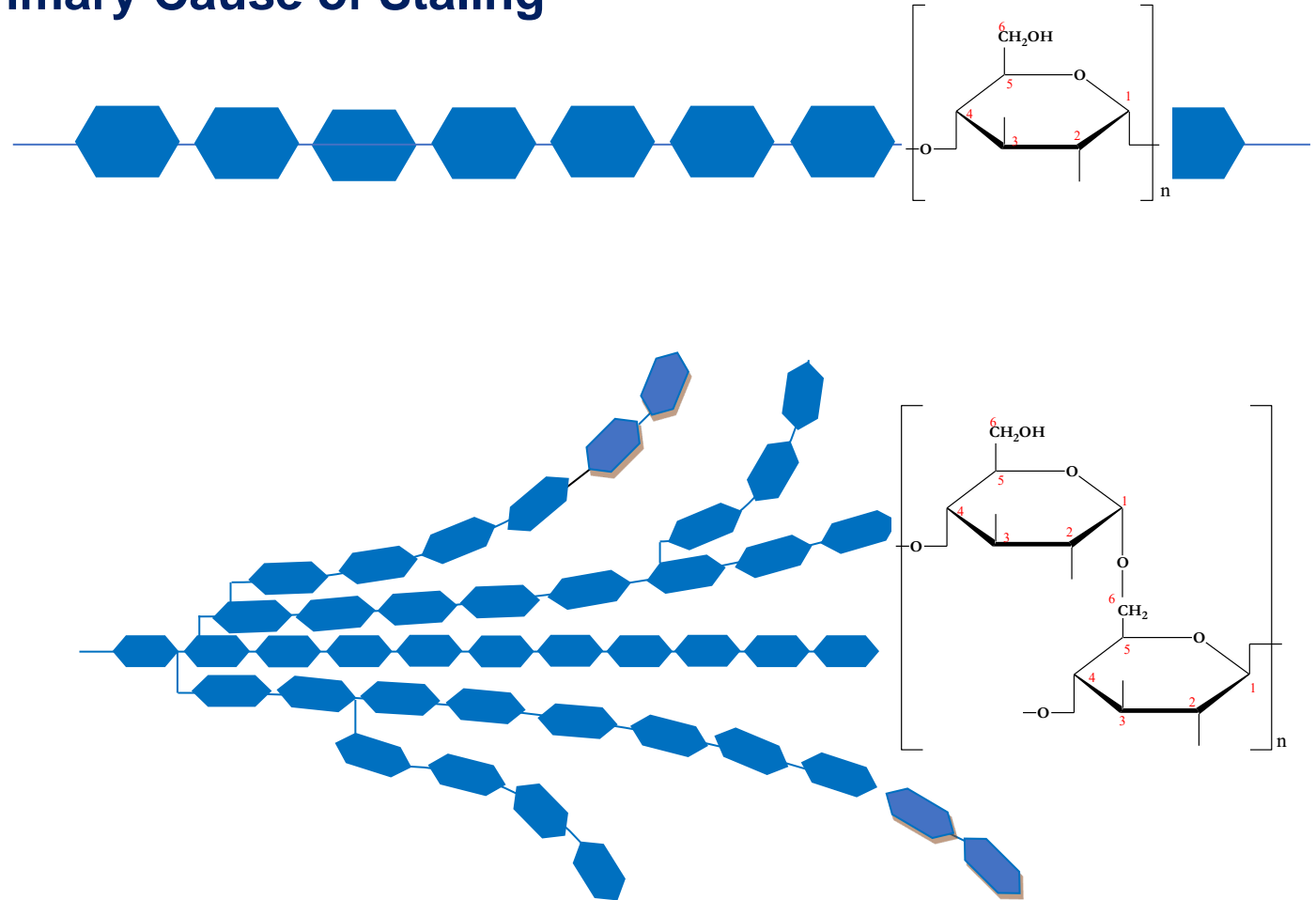
Starch has two components:

Amylose

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

Amylopectin

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



Enzymes in Flat Bread Application

Starch Gelatinization

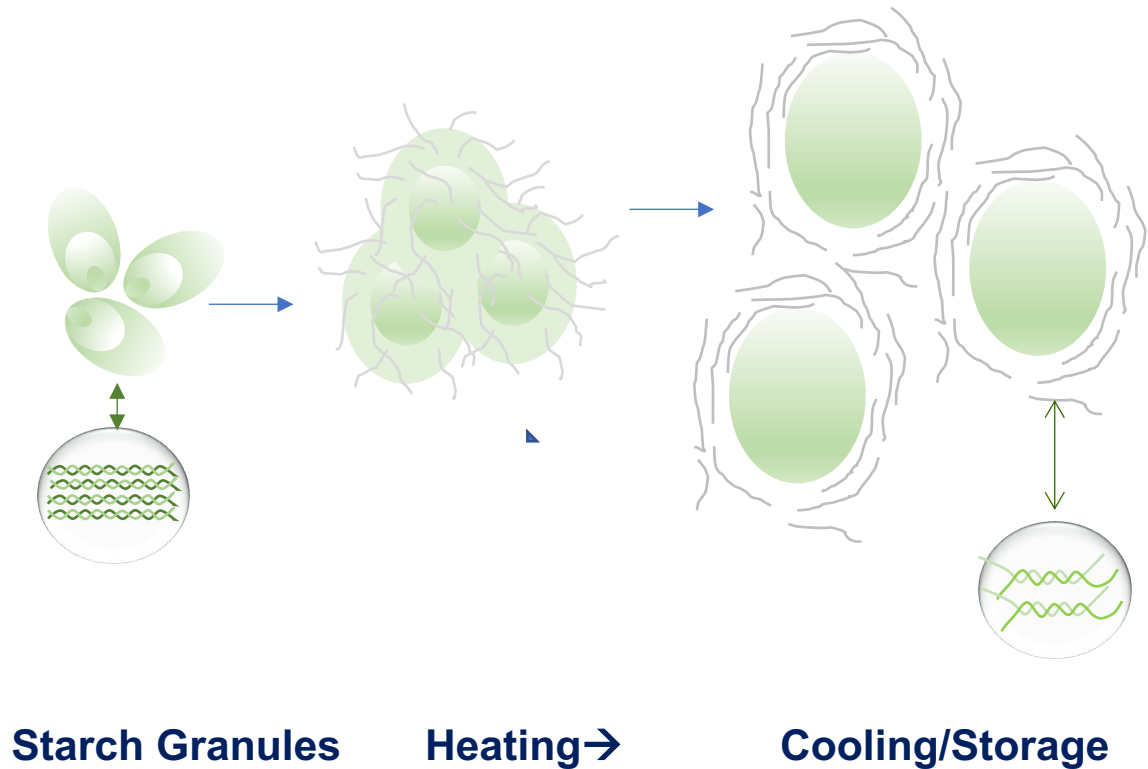
Starch Water Interaction

Gelatinization

- In the presence of water and heat starch absorb water and swells up; loses its crystalline structure, viscosity increases

Retrogradation

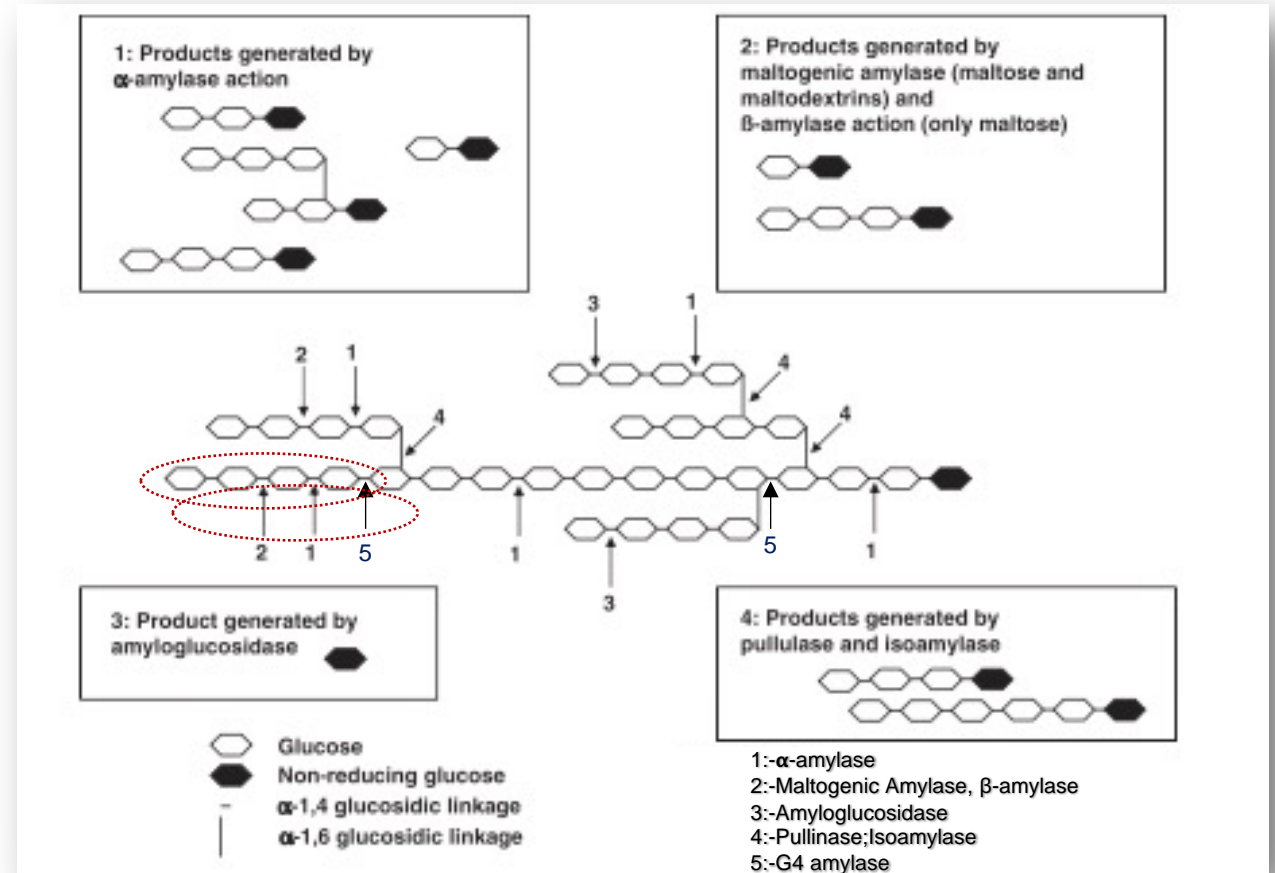
- After gelatinization starch tend to regain its crystalline structure



Enzymes in Flat Bread Application

Amylases As Antistaling Agents

Location	Enzyme	Products Produced
1	α -Amylase	Maltose/ Oligosaccharides
2	β -amylase	Maltose
3	Maltogenic amylase	Maltose and Maltodextrins
4	Amylo-glucosidase	Glucose
5	Maltotetraose (G4) producing amylase	Malto-oligosaccharides



Enzymes in Flat Bread Application

Enzyme Systems in Flat Bread

Typical features of staling

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

Enzyme based systems

- Slow down staling /retrogradation



Retrogradation (staling) is a major concern in baked products...

Enzymes in Flat Bread Application

Proteins

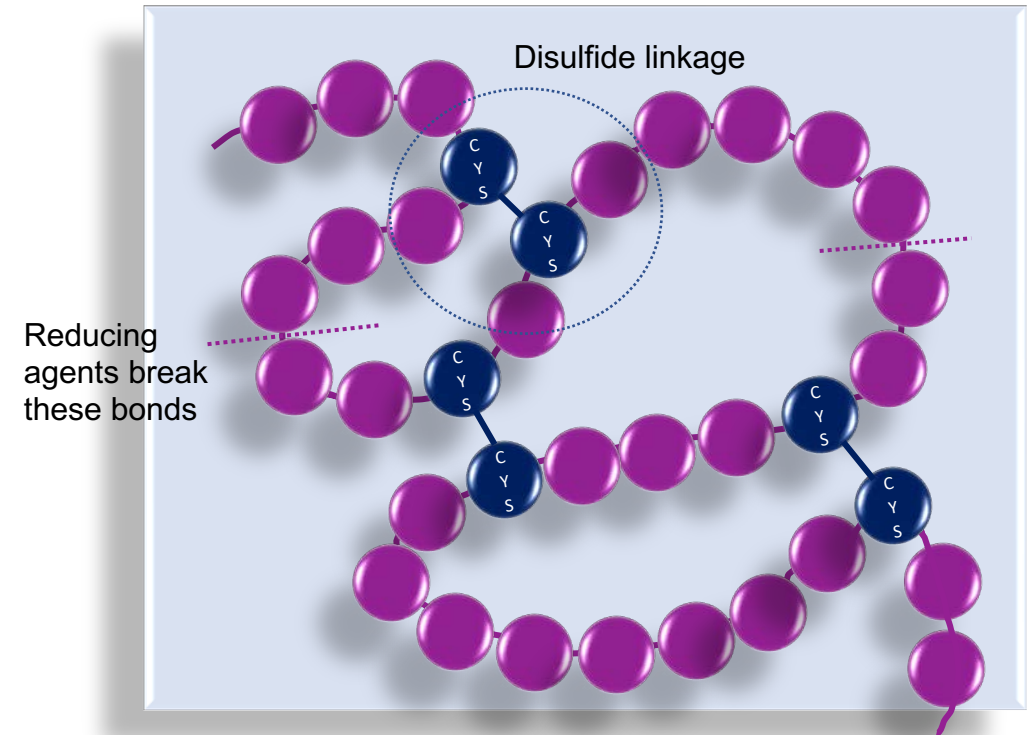
Gluten a major protein in wheat flour

Gluten protein is made with amino acids linked together with peptide bonds

Gluten is subdivided into :

Gliadins : plasticizer: contribute to dough viscosity and extensibility

Glutenin contribute to strength, elasticity, resistance to extension



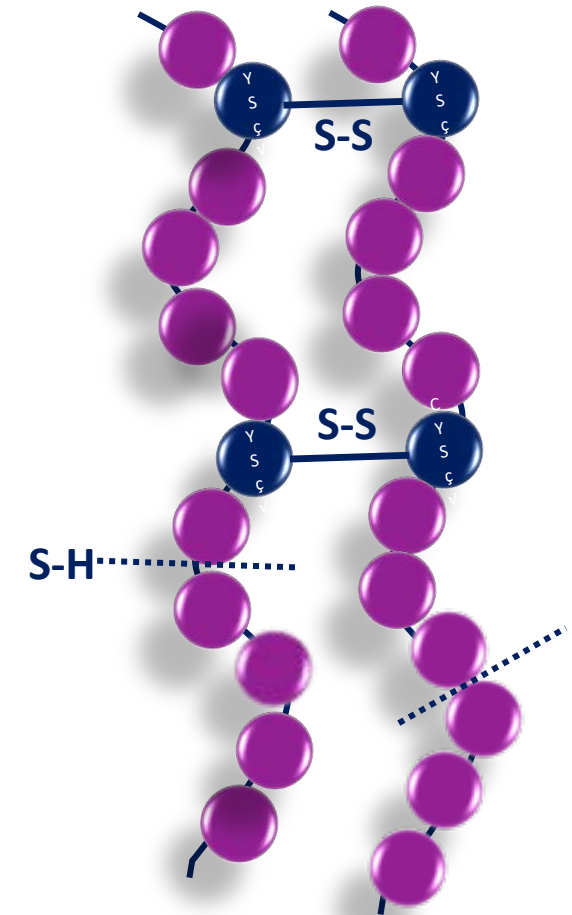
Source: ABL_FB_014

Enzymes in Flat Bread Application

Proteases/Dough Relaxers

Reducing Agents

- Weaken the gluten matrix
- Reduce dough elasticity
- Improves water absorption
- Improve softness, dough machinability and handling
- Reduces mixing time ,also called mixed-time reducers
- Reduces the size of glutenin polymers and redistribute SH/SS ratios



Source: ABL_FB_014

Enzymes in Flat Bread Application

Proteases/Dough Relaxers

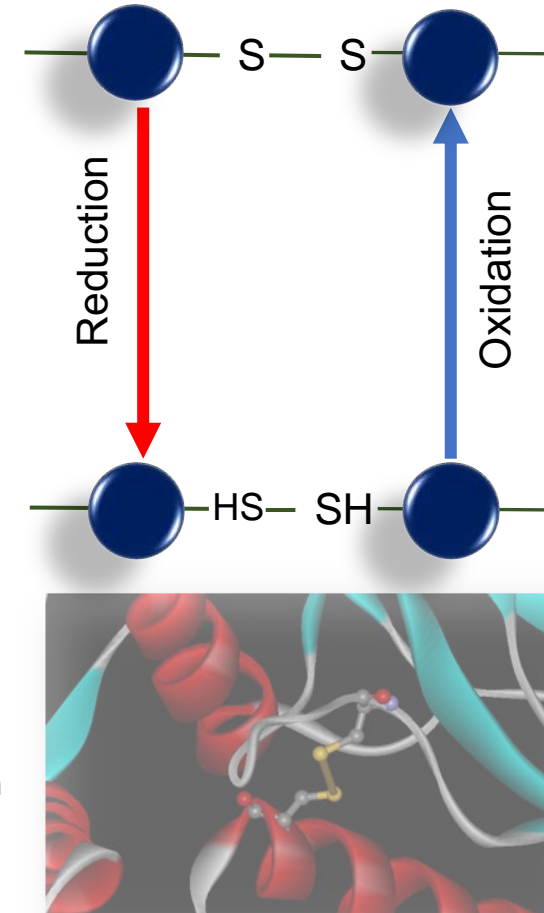
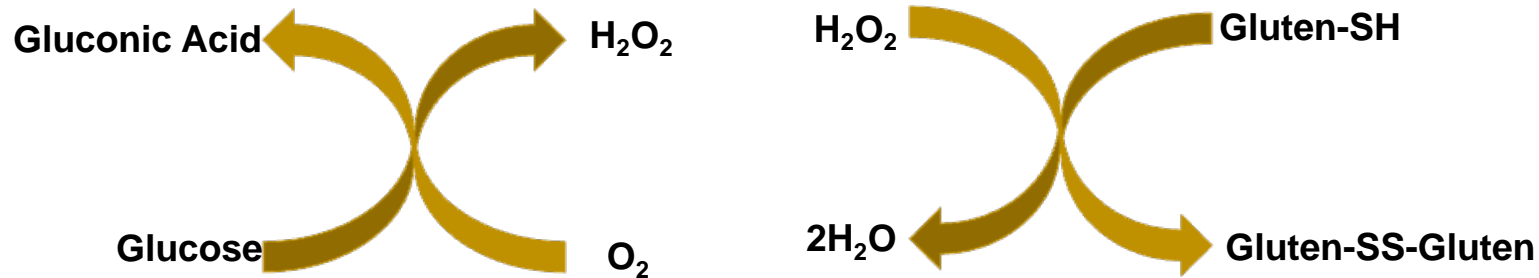
- Protease improve softness, dough machinability and handling
- Reduces mixing time and improves water absorption
- “Cleaner” replacement for L-cysteine or sodium metabisulfite



Enzymes in Flat Bread Application

Glucose Oxidase (GO)/ Dough Strengtheners

- Strong gluten matrix is created by disulfide linkages
- GO indirectly oxidize SH into SS
- By decreasing the levels of SH groups
 - Gliadin–glutenin crosslinking is developed
- This leads to the dough strengthening



Enzymes in Flat Bread Application

Transglutaminase (TG)

TG application is specially emerged in gluten free flat bread or with protein (e.g., soy flour) supplementation (Chapati/Roti)

TG catalyzes inter- and intra-molecular covalent cross-link by forming isopeptide bonds between glutamine and lysine residues of protein

Due to these cross-links high molecular weight polymer are formed

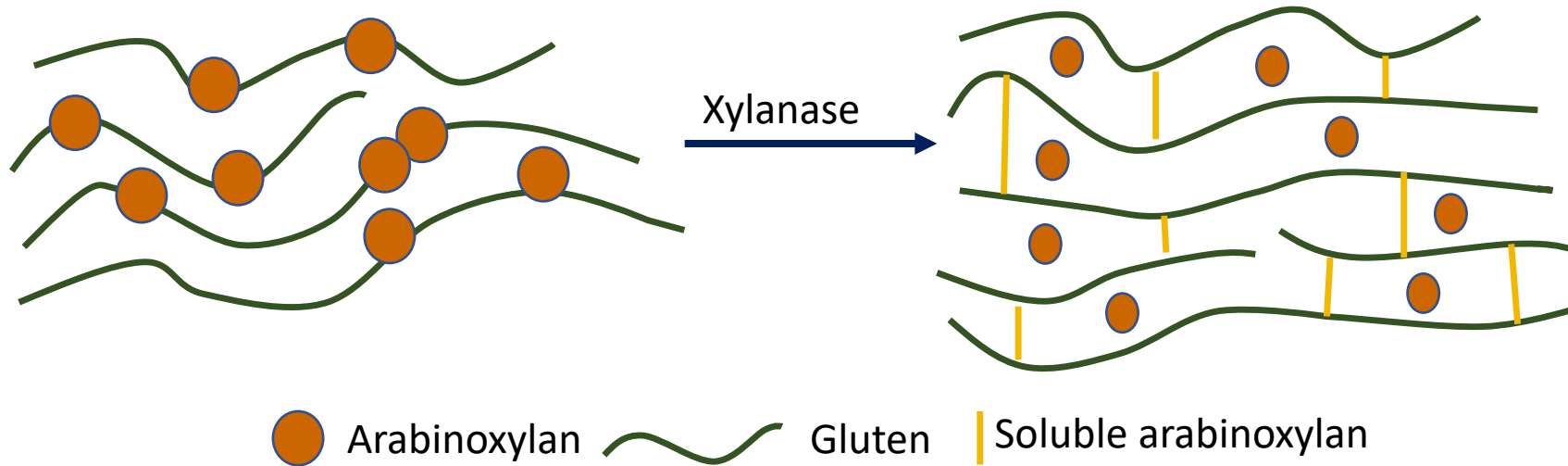
- Improves the protein network
- Increases water holding capacity
- Increases water absorption
- Viscoelastic and thermal properties



Source: Basman, 2002

Enzymes in Flat Bread Application

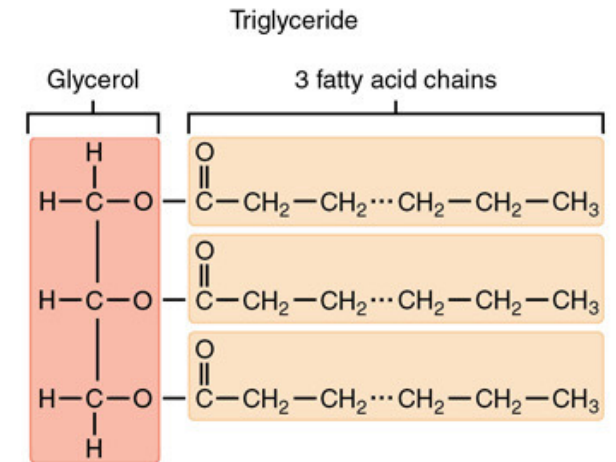
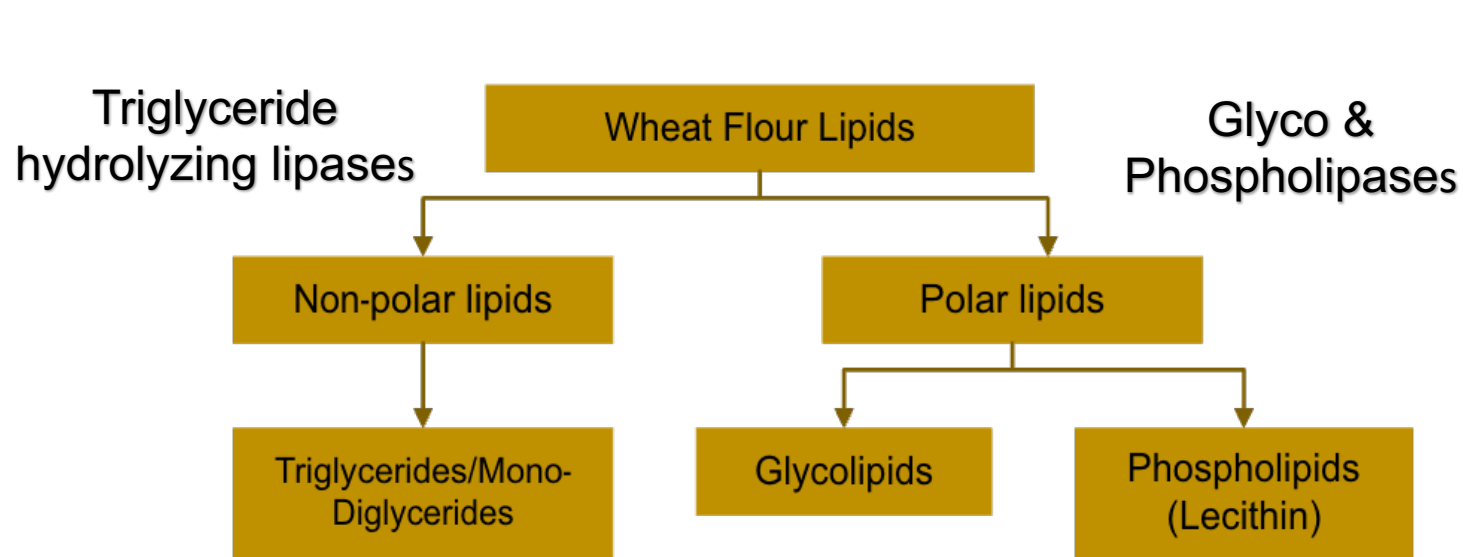
Xylanase



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

Enzymes in Flat Bread Application

Lipids



- Natural wheat lipids comprise 2% to 3% of wheat flour
- Typically stuck to starch granules or proteins in the dough.

Source: ABL_FB_020

Enzymes in Flat Bread Application

Lipases, Glycol & Phospholipases

- Lipase enzymes are esterases that break chemical bonds in lipids

Lipase: 1,3-Specific

Hydrolyze non-polar lipids, i.e., 1,3 ester bonds of triglycerides

Assures better dough consistency and stability, reduction in dough stickiness

Lipase: Broad substrate specificity

Modify triglycerides and polar lipids

Improve dough rheology, machinability, increase dough strength and stability
Also help as antisticking agent in Tortilla



Enzymes in Flat Bread Application

Enzyme Formulation

1. Application, Screening
2. Optimization: Formulation/Process
3. Analytical and Shelf-life



Enzymes in Flat Bread Application

Application/Screening and Selection

1. Finished Product and Process
- 2. Enzyme Concentration (Units/g or Dilution levels)**
3. Accurate weighing [Added at very low concentration (ppm)]
4. Enzyme application rate (Enzyme Overdose)
5. Enzyme synergy (Enzymes are mostly used in combinations to give synergistic effects)



Enzymes in Flat Bread Application

Enzyme Blend Formulation

1. Enzymes compatibility/Usage levels
2. Enzymes deactivation temperature
3. Order of mixing
4. Enzyme optimization is performed at several levels
 1. Lab scale
 2. Mimic commercial trials
 3. Shelf-life studies



Enzymes in Flat Bread Application

Analyticals and Shelf-Life

Analytical

- Sensory : Organoleptic, Texture
- Quantitative Analysis: Texture analysis, Flour Analysis , Dough Rheology, Starch characterization



Source: ABL_FB_23,24,25

Enzymes in Flat Bread Application

Thank You

Anya Baking Lab

Consulting Services |Leavened & Flat Breads (Tortilla, Naan, Pita, Lavash)

- Product Innovation & Process Design ;
- Ingredient application works (Enzymes, Emulsifiers, Antimicrobials, Cost Optimization; Ingredient procurement and optimization ; Collaborative research studies;
- Analytical Lab support: Shelf-life Studies, Texture analysis, Flour Analysis , Dough Rheology, Starch characterization, Microbiology

Atlanta, GA 30080, USA

Email: anitasri38@anyabakinglab.com;

info@anyabakinglab.com

Phone: +1470.558.5750

Enzymes in Flat Bread Application



References

1. <https://www.alliedmarketresearch.com/flatbread-market>
2. Qarooni, J. (1996). Flat Breads. In: Flat Bread Technology. Springer, Boston, MA.
3. Yi Zhang, Timothy Geary, Benjamin K Simpson. 2019. Genetically modified food enzymes: a review, Current Opinion in Food Science, Volume 25, Pages 14-18,
4. Oart, M.V. 2010. Enzymes in bread making. In: Enzymes in Food Technology (Eds. Oart, M.V, Whitehurst, R. J). Willi Blackwell, Blackwell Publishing Ltd, Iowa pp. 17-33
5. Purich, D. L. 2011. Factors influencing enzyme activity. In: Enzyme Kinetics: Catalysis and Control: A Reference of Theory and Best-Practice Methods 1st Edition, Elsevier Science Publishing Co Inc, US, pp 379-483
6. Austin 2006
7. Hammer, R. J. (1992). Enzymes and the baking industry: Friends or foes? In *Cereal Chemistry and Technology: A Long Past and Bright Future*, ed. P. Feillet, Institut National de la Recherche Agronomique, 9th International Cereal and Bread Congress, Paris, 1992.
8. Plant Foods Hum Nutr (2000) 55: 15. <https://doi.org/10.1023/A:1017237631105>
9. Heinze k.m., 2017. From Grain to Granule: The Biomechanics of Wheat Grain Fractionation with a Focus on the Role of Starch Granules Delcour, J.A., Joye, I.J, Wilderjans, B.P.E., Brijs, K., Lagrain, B. 2012. heat gluten functionality as a quality determinant in cereal-based food products, Ann. Rev.
10. Zobel HF, Kulp K. 1996. The staling mechanism. In Baked Goods Freshness, ed. RE Hebeda, HF Zobel, pp. 1–64. New York: Marcel Dekker, Inc.
11. Prof.K.Loshe, Enzymes in Baking
12. Ward et al. 2013. Natural Variation in Grain Composition of Wheat and Related Cereals. *Agric. Food Chem.* 2013, 61, 35, 8295–8303
13. Anon (1988) Veron FD SUPER and Veron ESL — *New Developments for Modern Baking Technique*. Enzyme-Report, Roehm-Enzymetechnologie, p. 13.
14. Delcour, J.A., Joye, I.J, Wilderjans, B.P.E., Brijs, K., Lagrain, B. 2012. heat gluten functionality as a quality determinant in cereal-based food products, Ann. Rev. Fd.Sc.Tech, Vol3, 469-492
15. Haarasilta, S., Vaeisaenen, S. and Pullinen, T. (1991) A new generation dough conditioner — combination of oxidative and hydrolytic enzymes. *76th AACC Annual Meeting*, October 1991, Poster (Abstract no. 146).
16. Schofield et al, 1991
17. <https://feedase.com/role-of-debranching-enzymes-in-nsp-degradation/>
18. Anderson, C. and Simsek, S. (1991) What Are the Characteristics of Arabinoxylan Gels? Food and Nutrition Sciences, 9, 818-833
19. Pomeranz, Y. 1985; Functional properties of Food Component. Academic Press Inc. Orlando, Fl.
20. <https://www.bakingbusiness.com/articles/58877-pro-tip-lipase-enzymes-can-provide-clean-label-emulsification>
21. Basman, A., Köksel, H. & Ng, P.K. Effects of increasing levels of transglutaminase on the rheological properties and bread quality characteristics of two wheat flours. *Eur Food Res Technol* **215**, 419–424 (2002)
22. <https://www.kpmanalytics.com/products/functional-rheological/mixolab-2>
23. <https://www.perkinelmer.com/category/rva>
24. <https://textureanalysisprofessionals.blogspot.com/2015/03/texture-analysis-in-action.html>