Bakery Enzymes in Tortilla Applications (Effect of Enzymes on Tortillas)

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Tortillas

- Tortillas have become very popular baked products world wide.
- Because of versatility, functionally, and convenience as wraps.
- The low cost of tortillas, ease of preparation and range of options for customers are key drivers for strong industry.
- Customers prefer tortillas that are flexible, opaque, large and have long shelf life.
- Stale tortillas are firm and crack when folded. Thus, becoming unacceptable for customers.
- Functional Ingredients and additives such as enzymes are added to tortilla formulation to provide desirable attributes.

(Jondiko et al., 2016 & Tuncil, et al., 2016)

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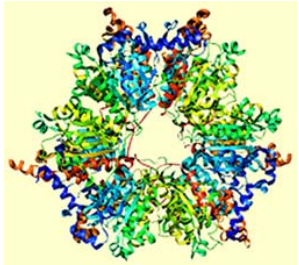
•Enzymes

- Naturally occurring components from plant, animal and microbial origins.
- They are specific, efficient and biodegradable and have unique 3-D shapes.
- Within the three dimensional structure are cavities that fit lock and key with a substrate molecule - another protein, fat or carbohydrate.
- The perfect match between the enzyme and substrate will result in a specific chemical reaction to occur to the substrate molecule.



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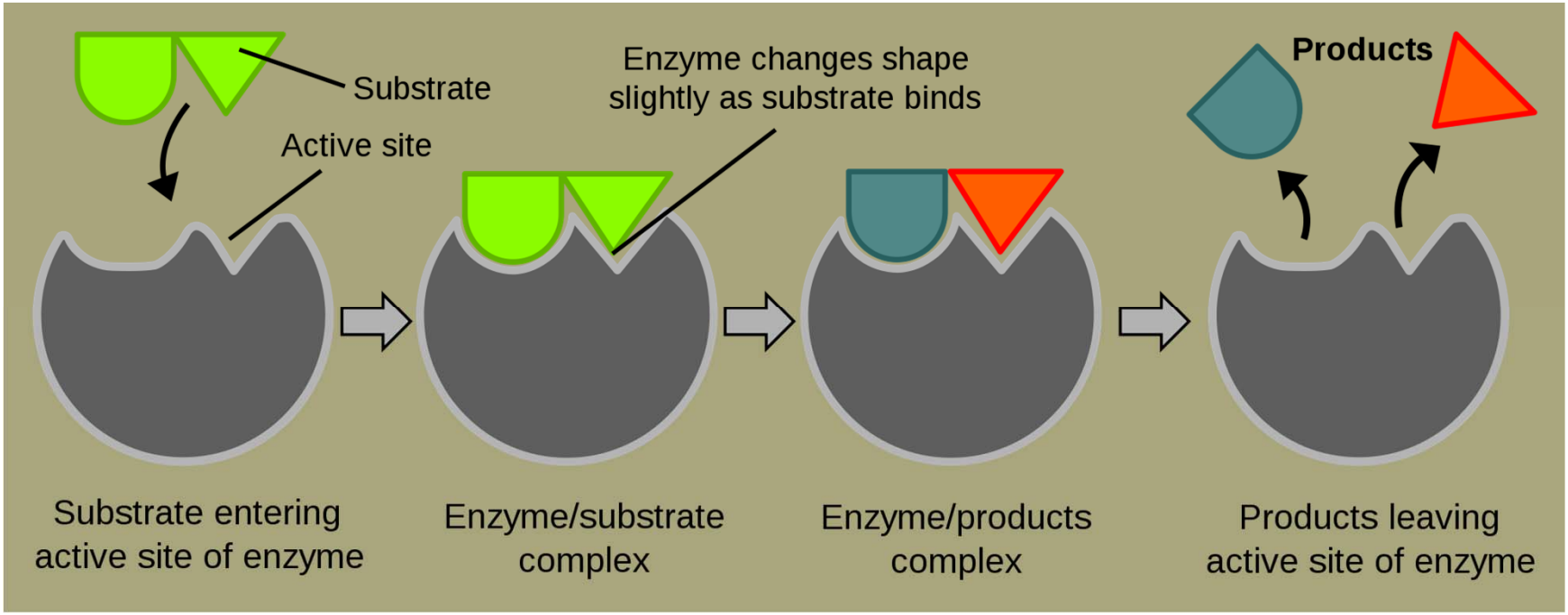
(Panesar et al., 2010 & Illanes, A., 2008)

•Enzymes



(Panesar et al., 2010., Arora, S., 2003 & Illanes, A., 2008)

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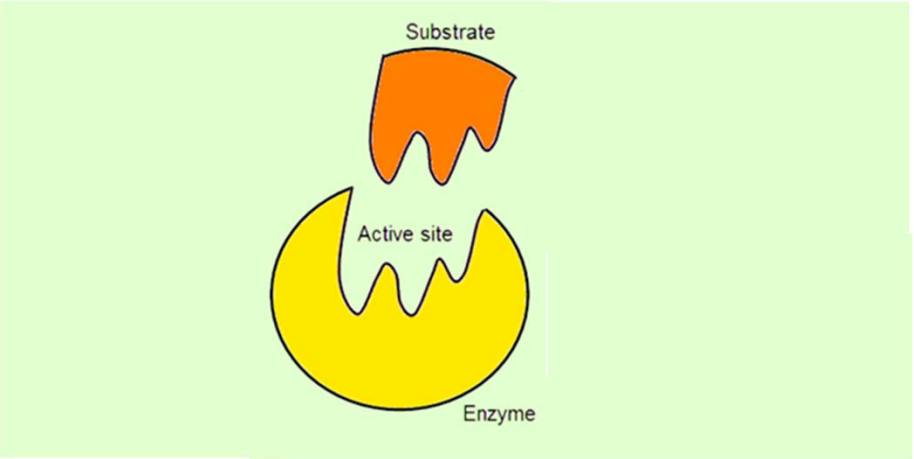
•Factors Affecting Enzyme Activity

- ❖ pH
- ❖ Temperature
- ❖ Water Activity
- ❖ Ionic Strength
- ❖ Enzyme Dosage

- ❖ Presence of different molecules:
  - ➢ Inhibitors
  - ➢ Stabilizers
  - ➢ Oxygen



(Panesar et al., 2010., Arora, S., 2003 & Illanes, A., 2008)

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•Enzymes in Bakery Products

- Wheat flour is the primary ingredient.
- Flour is mainly composed of starch and protein.
- Baking enzymes are used to alter starch and protein properties.
    - Alter flour behavior in mixing.
    - Improve dough fermentation, handling, machinability properties.
    - Enhance dough mixing tolerance and mixing stability.

More intense crust color

Improve crumb characteristics

Increase loaf volume

Extend Shelf life

(Miguel et al., 2013 & Sanz Penella, et al., 2008)

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•Enzymes in Tortillas

- Optimizing baking properties.
- Improving Product Quality.
- Staling is caused by chemical & physical changes after baking.

- **Increasing firmness, dryness and loss of product freshness.**

- Staling rate & shelf stability of tortillas depends on:
  - Formulation.
  - Processing & storage conditions.

- **Despite using optimized time & temperature, tortillas still go stale.**

(Alviola et al., 2008, Waniska, R., 1999)

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•Enzymes in Tortillas

Wheat Flour Tortillas

- Flour tortillas are mainly composed of starch and protein.
- Hence, Flour tortilla enzymes are used to alter starch and protein properties.

Gluten /Starch Retrogradation Theory

- During cooling and aging, cross links between gluten and starch are formed (interaction).

Ambient Storage (22°C)

Corn Tortillas

- Starch is the major component of corn tortillas, and only starch specific enzymes are used.

Starch Retrogradation Theory

- During cooling and aging, starch retrogrades from amorphous state to rigid crystalline state.

Refrigeration Storage (3-10°C)

(Alviola et al., 2008, Waniska, R., 1999)

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•Enzymes Used in Tortillas

- Enzymes are generally classified according to their target substrate molecules.
- α-Amylases are the main enzymes used to extend shelf stability at both corn and wheat tortillas.
- α-Amylases (EC 3.2.1.1) are endo-enzymes that catalyze the cleavage of α-1,4-glycosidic bonds in the inner part of the amylose or amylopectin chain.

Oligosaccharides **α-limit dextrins**

- These enzymes can be obtained from cereal, fungal, bacterial and biotechnologically altered bacterial sources.

(Momma., M., 2000 & Miguel et al., 2013)

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•Enzymes Used in Tortillas (α-Amylases)

- Malt ingredients –
  - occur naturally in flours.
  - Seed germination produce alpha amylases.
- Fungal Amylase –
  - alpha amylase from fungal sources.
  - Mainly used to aid dough conditioning.
  - act on starches.
  - not heat stable.
- Bacterial Amylase –
  - more heat stable than fungal amylase.
  - Breaks down starch components even after baking.
  - Used to prevent staling during storage.

(Arora, S., 2003 & Miguel et al., 2013)

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•Enzymes Used in Tortillas (α-Amylases)

- Amylase from malt and fungal sources are easily deactivated by cooking and only function in the dough.
- Bacterial amylases are more heat stable and are more effective in preventing staling because they function in the finished product.
  - These enzymes are very active and have the tendency to go too far.
  - This non-stop activity can result in gummy texture and sticking of tortillas.
  - However, special types of bacteria were developed to produce amylases that work slightly different from those of unmodified organisms.

(Arora, S., 2003 & Miguel et al., 2013)

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•Enzymes Used in Tortillas (Maltogenic α-Amylases)

- Exo-acting α-Amylase:
  - Maltogenic α-amylase (glucan 1,4-α-glucanhydrolase, EC 3.2.1.133). (Maltose).
  - Maltooligosaccharide forming amylases (EC 3.2.1.60).

Act on Gelatinized Starch

Optimum Temperature (65-80 ℃)

Deactivated Temperature (90 ℃)

Effective as anti-staling Agents

(Momma., M., 2000, Korbust et al., 2002 & Miguel et al., 2013)

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•Proposed Theories for α-Amylases as Anti-staling Agents

1) The enzymatic shortening of amylopectin chain length reduces retrogradation tendencies of amylopectin.

2) The oligosaccharides (degree of polymerization (DP) 2-7) produced by exo-acting α-amylases are antistaling agents.

3) Production of low molecular weight dextrins interferes with the retrogradation of starch.

(Boyle et al., 1990, Martin et al., 1991 & Shi et al., 1995)

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•Other Enzymes Used in Tortillas

- **Hemicellulases (cleavage of β-glycosidic linkages).**
  - Hydrolyze a group of Nonstarch polysaccharides.
  - Nonstarch polysaccharides (pentosan, cellulose) hold water.
  - Water is vital for starch gelatinization & gluten development.
  - Prevent starch gelatinization & gluten formation resulting in less flexible tortillas.
    - Xylanase or endo-1,4-β-xylanase(4-β-D-xylan xylanohydrolase, EC 3.2.1.8) (Arabinixylan:Pentosans).
    - The cleavage caused by xylanase results in drastic changes in functional properties, water extractability and AX molecule weight.

(Collin et al., 2005 & Feussner, et al., 2012)

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•Other Enzymes Used in Tortillas

- **Oxidoreductases.**
  - Glucose-oxidase(β-D-glucose:oxygen:1-oxidoreductase;EC1.1.3.4) catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide.
  - Hydrogen peroxide as an oxidizing agent promotes the formation of disulfide bonds by oxidizing thiol groups (increasing crosslinking of gluten).
  - Stabilize the gluten network & improve elastic properties.

(Miguel et al., 2013 & Goesaert, et al., 2005)

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•Other Enzymes Used in Tortillas

- **Proteases .**
  - Used in wheat flour tortillas to mellow gluten activity (breaking peptide bonds):
  - Reduce dough mixing time.
  - Decrease dough consistency.
  - Reduce proofing time and dough balls resting time.
  - Enhance dough relaxation (replacement for reducing agents).

  - Usage in corn tortillas is limited due to the disrupted structure of protein caused by high alkaline nixtamalization process.

(Miguel et al., 2013 & Goesaert, et al., 2005)

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•Enzyme Combinations

- Each enzyme has it's own specific substrate in corn & flour doughs.
- Interaction of the substrates in tortillas is complex.
- The enzyme combination can produce synergistic effect that are not seen if only one enzyme is used (even at high dosage).
- High dosage of enzyme has a detrimental effect on dough or baked tortillas.

(Di Cagno et al., 2003, Almeida, et al., 2012)

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•Enzyme Combinations

- Examples of lower dosages of enzyme combinations:

α-amylase

Xylanase

Glucose Oxidase

Maltooligosaccaride α-amylase

Fungal α-amylase

Xylanase/ Glucose Oxidase

- Achieve maximum dough consistency and stability.
- Achieve maximum shelf stability and optimum tortilla quality in terms of moistness, flexibility, and cohesiveness.

(Di Cagno et al., 2003, Almeida, et al., 2012)

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•ABI Research on Enzymes for Flour Tortillas

- We have extensively tested enzymes from different manufacturers.
- Objective: Evaluate potential functionality of enzymes in preventing staling, sticking and retaining softness and flexibility.
- Amylase A (specialized amylase with low usage rate):
  - Retains the softness and resilience.
  - Prevents staling without causing tortilla sticking and gumminess.
  - Extends shelf life.
- Amylase A works faster and requires a much lower usage rate than other enzymes tested.
- Thus, for flour tortilla applications, we use Amylase A in our BatchPak™, both for performance and cost effectiveness.

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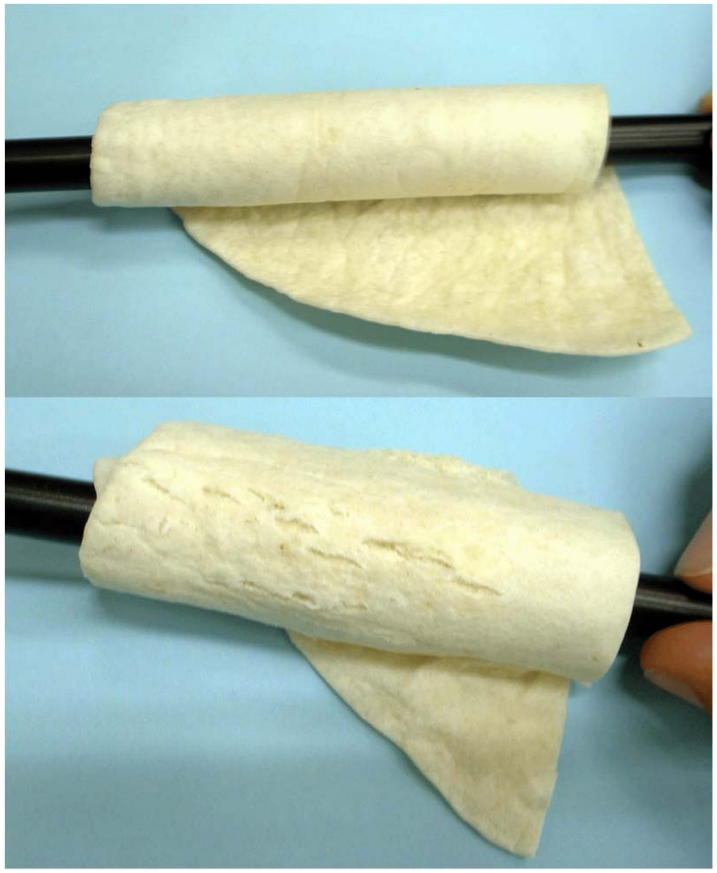
•Enzymes for Flour Tortillas



With Enzymes

Without Enzymes

Rollability Test

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Experimental Design of Enzyme Testing

Tortillas

Made using Amylase A (Control)

Made using sample enzymes

pH | **Moisture** | **Appearance** | **Shape** | **Color** | **Taste**

Ambient Temperature ($22^{\circ}C/72^{\circ}F$)

Storage Time (Day 8, 15, 21, 30, 40, 60)

Staling | **Rollability** | **Surface** | **Softness** | **Texture** | **Sticking**

•Comparison of Enzyme #1 Functionality versus Amylase A

CONTROL (60 Days) Rollability Test

Enzyme # 1 (30 Days) Rollability Test

CONTROL (60 Days) Staling Test

Enzyme # 1 (30 Days) Staling Test

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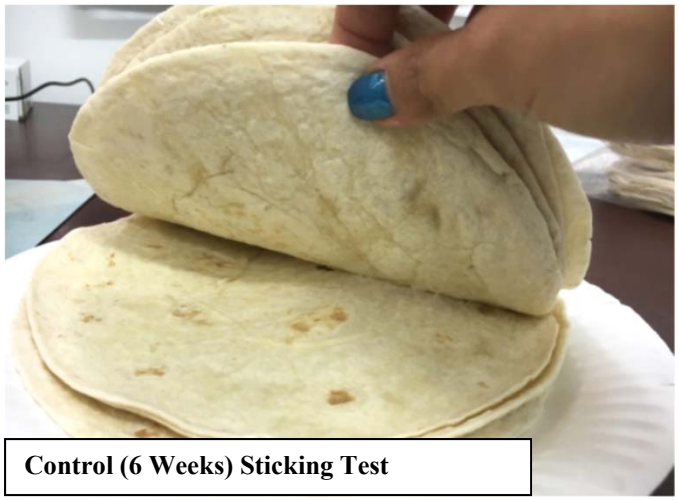
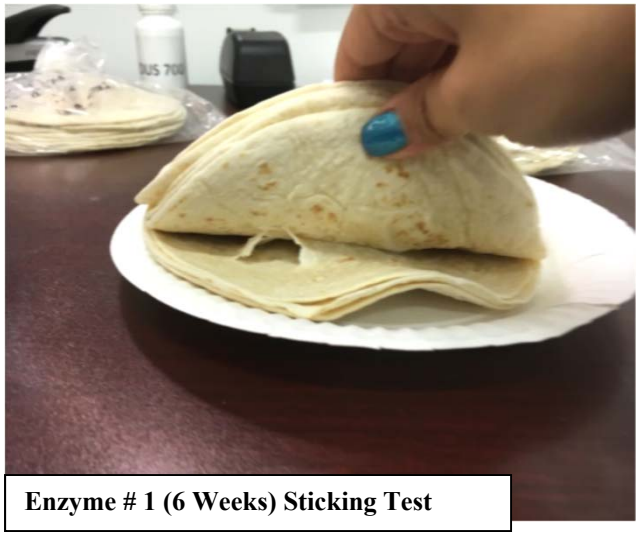
•Comparison of Enzyme #1 Functionality versus Amylase A



Control (6 Weeks) Sticking Test



Enzyme # 1 (6 Weeks) Sticking Test

- In 30 days storage test, enzyme# 1 did not show similar functionality to control in prevention of staling, sticking as well as maintaining softness, structure and resilience.
- Conclusion: enzyme# 1 activity is not comparable to control enzyme used in tortillas and may not be a good alternative.

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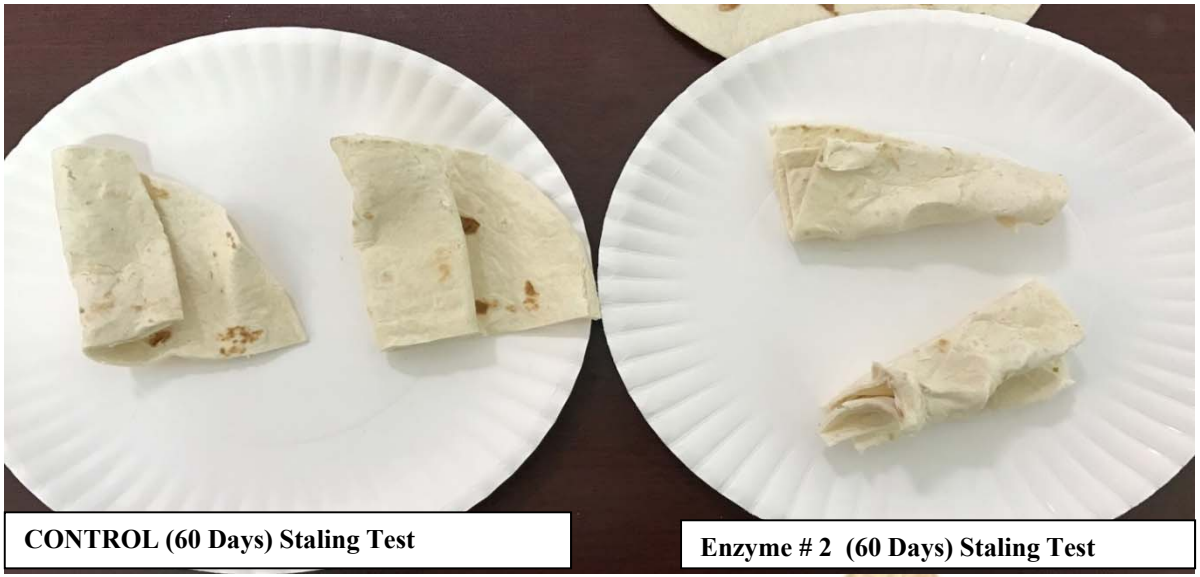
•Comparison of Enzyme #2 Functionality versus Amylase A

CONTROL (60 Days) Rollability Test

Enzyme # 2 (60 Days) Rollability Test



CONTROL (60 Days) Staling Test

Enzyme # 2 (60 Days) Staling Test

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•Comparison of Enzyme #2 Functionality versus Amylase A

Control (60 Days) Sticking Test

Enzyme # 2 (60 Days) Sticking Test

- In 60 days storage test, enzyme# 2 did not show similar functionality to control in prevention of staling; whereas, enzyme# 2 performed similar to control in preventing sticking and maintaining tortillas softness, structure, and resilience.
- Conclusion: enzyme# 2 activity is not comparable to control enzyme used in tortillas and may not be a good alternative.

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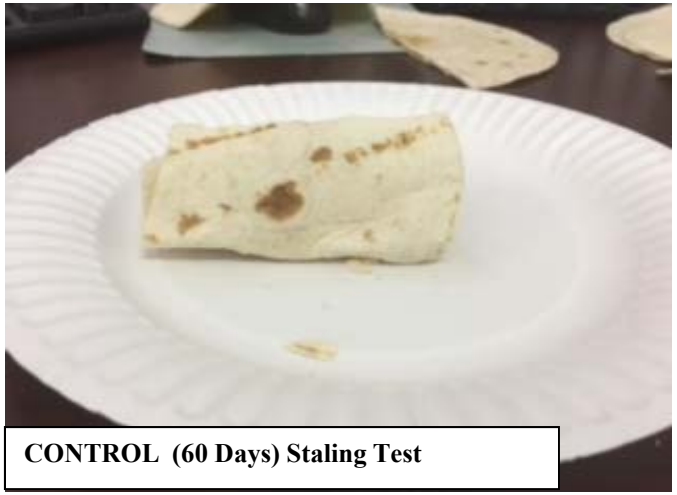
•Comparison of Enzyme #3 Functionality versus Amylase A

CONTROL (60 Days) Rollability Test

Enzyme # 3 (60 Days) Rollability Test



CONTROL (60 Days) Staling Test

Enzyme # 3 (60 Days) Staling Test

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•Comparison of Enzyme #3 Functionality versus Amylase A

Control (60 Days) Sticking Test

Enzyme # 3 (60 Days) Sticking Test

- In 60 days storage test, enzyme# 3 demonstrated similar functionality to control in prevention of staling, sticking as well as maintaining softness, structure and resilience.
- Conclusion: enzyme# 3 activity is comparable to control enzyme used in tortillas and may be a good alternative.

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•ABI Research on Enzymes for Flour Tortillas (Summary)

- In our ABI flour tortilla BatchPak™, enzyme functionality has been carefully optimized to provide the best results.

- Thus, aside from ensuring tortillas that remain flexible and rollable over extended storage, we can assure that sticking will not occur as well.

- We do this by supplementing the BatchPak™ with other thoroughly tested functional ingredients.

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•ABI Research on Enzymes for Flour Tortillas (Summary)

- Our Enzyme technology can be delivered in two ways:
- Enzymes in complete BatchPak™ formulations specifically designed to your own preference.

- Special blends of enzymes and other functional ingredients such as AB&I Flour Tortilla Improver to supplement your current formulations.

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•Enzymes for Corn Tortillas

- Corn tortillas are basically made from nixtamalized corn and water.
- Through nixtamalization, corn starch has undergone pre-cooking.

- Most of the components from corn kernels go into the masa dough.
- Corn gluten is not as abundant nor as functional as wheat gluten. Thus, corn tortilla structure mainly depends on corn starch gel networks.

(Serna-Saldivar, S., 2015 & Bueso-Ucles et al., 2003)

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•Enzymes for Corn Tortillas

- Corn tortillas stale faster than flour tortillas due to higher starch concentration and lower fat content in corn tortillas.

- Thus, while flour tortillas begin to lose flexibility after about 5 days, this will occur on corn tortillas a day after baking.

(Serna-Saldivar, S., 2015 & Bueso-Ucles et al., 2003)

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•Enzymes for Corn Tortillas

- The role of amylase in corn tortillas is even more significant.
- Since starch in corn tortillas had been pre-gelatinized, it is a lot more sensitive to amylase activity than starch granules in dough of flour tortillas.
- Using an aggressive amylase will cause wet and sticky masa that will be difficult to sheet.
- An aggressive amylase in the tortilla can also cause the tortilla to disintegrate over a short time.

(Serna-Saldivar, S., 2015 & Bueso-Ucles et al., 2003)

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•Enzymes for Corn Tortillas

Corn Tortillas 8 Days After Baking

No Enzymes

With Enzymes

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•ABI Research on Enzymes for Corn Tortillas (Summary)

- For corn tortillas, we prefer to use Amylase B.
- It is a milder form of amylase compared to Amylase A.
- It does not cause adverse effects on masa dough consistency.
- It is effective in controlling the staling process in corn tortillas.
- While amylase activity helps maintain softer corn tortilla texture, it can also cause crumbliness. This effect can be corrected with use of other ingredients.

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•ABI Research on Enzymes for Corn Tortillas (Summary)

- As in flour tortillas, ABI formulates to optimize the benefits of using enzymes by supplementing it with other carefully selected functional ingredients.
- **AB&I Shelf Extend.**
- **SuperSoft®.**
- **AB&I Corn Tortilla Softener.**
- **AB&I Corn Tortilla Conditioner GC.**
- **AB&I Corn Tortilla CL Blend.**

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Conclusions

- Bakery enzymes can be used in both Flour and Corn Tortillas to improve product attributes.

- Enzymes open opportunities for tortilla manufacturing to create and promote healthier versions of tortillas and deliver a cleaner ingredient statement.

- AB&I R&D team delivers enzyme technology in multiple and various ways.

- BatchPakTM , and AB&I Flour Tortilla Improver for Flour Tortillas.
- AB&I Shelf Extend, SuperSoft®, and other AB products for Corn Tortillas.

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Our goal is to develop products the market demands with improved functionality and taste that meet and exceed customer preferences.

Acknowledgement

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TORTILLA INDUSTRY ASSOCIATION TIA EUROPE TECHNICAL CONFERENCE

Questions?

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