## ARABINOXYLANS

<table>
<thead>
<tr>
<th>Water-unextractable AX: <strong>WU-AX</strong></th>
<th>Extractable AX: <strong>WE-AX</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2/3 to 3/4 of total wheat flour AX</td>
<td>1. 1/4 to 1/3 of total wheat flour AX</td>
</tr>
<tr>
<td>2. Component of the endosperm cell wall matrix</td>
<td>2. Similar to WU-AX, but water-extractable</td>
</tr>
<tr>
<td>3. Unextractable because of covalent and non-covalent interactions with other cell wall components (AX, protein, ...)</td>
<td>3. No interaction with other components</td>
</tr>
<tr>
<td>4. Strong water-binding capacity</td>
<td>4. Possibly unprocessed building-blocks of the cell wall</td>
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<td></td>
<td>5. High viscosity</td>
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XYLANASE As A Replacement For L-Cysteine (Thin Crust Pizza)

**TRIAL 1**
- Xylanase dose: 50ppm (based on flour weight)
- L-Cysteine eliminated (from 25ppm)

**RESULTS:**
- Dough had similar consistency to control
- Pizza crust held its circular shape after sheeting and bake
- Pizza crust had a crispy texture after baking

**TRIAL 2**
- Xylanase dose: 250ppm (based on flour weight)
- L-Cysteine eliminated

**RESULTS:**
- Dough had a slightly sticky feel, but was able to be processed
- Pizza crusts had more volume and had a better shape after pressing
- Pizza crust had a crispy texture after baking
ENZYME TYPES & FUNCTIONALITY

- Amylases
- Xylanases
- Lipases
- Oxidases
- Proteases
- Asparaginase
Lipases and Phospholipases

**Triglyceride**

\[
\text{CH}_2\text{-O-C-R}_1
\]

\[
\text{R}_2\text{-C-O-CH}
\]

\[
\text{O}
\]

\[
\text{CH}_2\text{-O-C-R}_3
\]

**Phospholipid**

\[
\text{CH}_2\text{-O-C-R}_1
\]

\[
\text{R}_2\text{-C-O-CH}
\]

\[
\text{O}
\]

\[
\text{CH}_2\text{-O-P-O-X}
\]

\[
\text{O}^-
\]

X: H, choline, ethanolamine, serine, inositol, etc.

**Lipases**

- **Phospholipase A 1**
- **Phospholipase A 2**
- **Phospholipase C**
- **Phospholipase D**

**Diagrams**

- Tri-glyceride → lipase → Monoglyceride + Free fatty acids (FFA)
Flour Lipids

- Non-starch bound lipids make up approx 1-2% of the flour’s weight
- 65% of these lipids are non-polar, 35% are polar
- Triglycerides are the predominant non-polar lipids
- Phospholipids (lecithin) and galactolipids are the predominant polar lipids
Proposed effects (by various authors):
- Interaction with gluten proteins
- Stabilizing air / water interfaces by forming mono-layers
ACTION OF DUAL SPECIFICITY LIPASE ON FLOUR LIPIDS

Digalactocyl-di-glyceride (DGDG)  Digalactocyl-mono-glyceride (DGMG)

Lecithin

Lyso-Lecithin

Triglyceride

Monoglyceride
LIPASE WITH DUAL SPECIFICITY

- Modified polar lipids mimic the performance of dough stabilizing emulsifiers

- Benefits include:
  - Cost savings by significantly reducing level of strengthening emulsifiers
  - Equal or better performance to emulsifiers
  - No acid aroma from DATEM
- Amylases
- Xylanases
- Lipases
- Oxidases
- Proteases
- Asparaginase
Basic Glucose Oxidase Mechanism

- **Basic step: Enzymatic formation of H2O2, a strong oxidant!**

![Glucose Oxidase Mechanism Diagram]

- Glucose oxidase catalyzes the oxidation of (β)-D-glucose to D-gluconic acid and **hydrogen peroxide (H₂O₂)**.
- In baking the rate of glucose conversion is dependent on both oxygen and glucose in the dough.
- **Oxygen is the limiting factor in bread baking.**
GOX Mechanism 1

• H2O2 oxidizes Gluten network directly

\[ \text{Gluconic acid} \rightarrow \text{H}_2\text{O}_2 \text{ (oxygen)} \]

H2O2 oxidizes the sulfhydryl group (-SH) of the amino acid Cysteine from wheat gluten, forming Disulfide bonds within the gluten network. \( \Rightarrow \) Dough strengthening!
GOX Mechanism 2

Oxidative gelation of Arabinoxylan by H2O2!

Arabinoxylan

- According to the theory of L.Hillhorst and others: Cross-linking arabinoxylan via Ferulic acid to other arabinoxylan.
- The presence of oxidative ferulic acid cross-links (di ferulic acid links) makes gels more elastic and greatly increases hydration, i.e. more water binding capacity.
Commercial Flour Tortilla Trials with GOX/Fungal Amylase Cocktail

**Trial 1**
- Cocktail dose: 300ppm
- L-Cysteine eliminated (from 25 ppm)
- Sodium Metabisulfite reduced by 50%
- Absorption increased by 2%

**RESULTS**
- Dough had similar consistency to control
- Tortilla had greater “oven pop”
- Desirable fluffy texture

**Trial 2**
- Cocktail dose: 300 ppm
- Sodium Metabisulfite reduced by 50%
- Monoglycerides reduced by 50%
- Hydrocolloid blend reduced by 50%
- Baking powder increased by 16%
- Absorption increased by 4%

**RESULTS**
- Dough had similar consistency to control
- Tortillas were fluffier and had better rollability than control after 4 days

**Trial 3**
- Cocktail dose: 440 ppm
- Absorption increased by 4%

**RESULTS**
- Tortillas were fluffier and had better rollability and flexibility after 9 days of storage
- Amylases
- Xylanases
- Lipases
- Oxidases
- Proteases
- Asparaginase

ENZYME TYPES & FUNCTIONALITY
The peptide bond
Protein structure

Primary protein structure is sequence of a chain of amino acids

Secondary protein structure occurs when the sequence of amino acids are linked by hydrogen bonds

Tertiary protein structure occurs when certain attractions are present between alpha helices and pleated sheets

Quaternary protein structure is a protein consisting of more than one amino acid chain
Proteases - The main types

Aminopeptidase  Endoprotease  Carboxypeptidase

H₂N-CH-C-NH-CH-C-NH-CH-C-NH-CH-C-NH-CH-C-OH

R₁  R₂  R₃  R₄  R₅
REDUCING AGENTS vs. PROTEASE

- Weaken the gluten structure

- Effects on dough
  - Reduced mixing time and lower energy requirements
  - Increase dough extensibility, pliability, pan flow
  - Reduce dough elasticity and “buckiness”
  - Improved dough machine-ability and processing
REDUCING AGENTS vs. PROTEASE

- Effects are similar, but mode of action is different
REDUCING AGENTS vs. PROTEASE

- Commonly used reducing agents
  - L-Cysteine
  - Bisulfite salts
  - Glutathione (from inactivated yeast)

- Proteases sourced from
  - Fungi
  - Bacteria
  - Plants (bromelain, papain)
## REDUCING AGENTS vs. PROTEASE

<table>
<thead>
<tr>
<th>REDUCING AGENTS</th>
<th>PROTEASE</th>
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<tbody>
<tr>
<td>▪ Reversible with oxidation</td>
<td>▪ Irreversible</td>
</tr>
<tr>
<td>▪ React quickly</td>
<td>▪ React more slowly, but continue to react until denatured</td>
</tr>
<tr>
<td>▪ Consumed in reaction</td>
<td>▪ Not consumed in reaction</td>
</tr>
<tr>
<td>▪ Amount of gluten weakening depends only on dose</td>
<td>▪ Amount of gluten weakening depends on dose, reaction time</td>
</tr>
</tbody>
</table>
Use of Protease in Nixtamilization

US Patent 6,428,828; Jackson & Sahai; University of Nebraska, Lincoln

- Alkaline protease is used to modify traditional nixtamilization process
  - Corn is cooked in water (without lime) for 15 minutes @90°C
  - Corn is steeped in a 0.1% solution of alkaline protease @pH 10-10.5 @50-60°C for 3-4 hrs.
  - Treated corn is stone ground and used to make tortillas or dried to make masa flour. Powdered lime can be added to masa to suit taste

- Advantages
  - Significant reduction in highly alkaline waste water with high COD/BOD nejayote levels
  - Higher yield, less solids loss – allows more of the whole grain
  - Reduced water and energy consumption
  - Allows the use of hard and soft corn varieties.
• Amylases
• Xylanases
• Lipases
• Oxidases
• Proteases

• Asparaginase
Acrylamide is naturally formed in many food products

- Acrylamide is formed naturally in foods as a by-product during frying, grilling or baking at temperatures in excess of 250°F/120°C and at low moisture
- Formed as part of the Maillard rxn between the amino acid Asparagine and reducing sugars
- Acc. to JECFA, the major contributing food groups are French fries, potato chips, coffee, biscuits/cookies/pastries, bread and rolls/toasted bread

Source: Joint FAO/WHO Food Standards Programme, Codex Committee on Contaminants in Foods, February 2008


Measured Acrylamide content (ppb) in different food categories. Median, 1 quartile, and 3 quartile shown.
Why is acrylamide in food a concern?

Acrylamide is a concern because the food industry’s first priority is safety and well being of consumers

Issues re. acrylamide

- Acrylamide has been classified by the International Agency for Research on Cancer (IARC, 1994) as “probably carcinogenic for humans”

- The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that levels of dietary exposure to acrylamide indicate a “human health concern” (FAO/WHO/2006)

- A 3 year EU study of acrylamide (“The HEATOX Project”) concluded in November 2007 that “Increasing toxicological evidence suggests that acrylamide in food might be a cancer risk factor”
Signs of tighter legislation?

August 2008, law suit settlement on acrylamide in California

- 5 major food manufacturers agree to reduce acrylamide
- 4 major food chains to post acrylamide warnings at their restaurants
- 2nd “wave” initiated, June 1st 2009

July 2009, JECFA approves code of practice for acrylamide reduction

- The joint FAO/WHO Codex Committee on Contaminants in Foods agrees on final adaptation of the code of practice for acrylamide in food

August 2009, Health Canada adds acrylamide to toxic substance list

- Acrylamide should be included on the nation’s list of toxic substances since current consumption levels “may constitute a danger in Canada to human life or health”
Asparaginase reduces Acrylamide, but does not impact taste, flavor or appearance

- Acrylamide is mainly formed in food as part of the Maillard reaction...
  ...and so is the desired brown crust, taste, and flavor which starchy baked and fried products are known for

- By converting Asparagine into aspartic acid, an asparaginase can effectively reduce the level of acrylamide without changing the taste and appearance of the end product
Asparaginase validated by CIAA in Acrylamide Toolbox

By Jess Halliday and Ahmed ElAmin

05/12/2007- The CIAA has included asparaginase in the new version of its Acrylamide Toolbox, a move seen to validation the efforts of companies that have developed commercial solutions using the acrylamide-reducing enzyme.

Acrylamide is a suspected carcinogen that is formed during by heat-induced reaction between sugar and an amino acid called asparagine. Known as the Maillard reaction, this process is responsible for the brown colour and tasty flavour of baked, fried and toasted foods.

The problem was discovered in 2002 by scientists at the Swedish Food Administration, and the Confederation of the Food and Drink Industries of the EU (CIAA) first drew up its acrylamide toolbox in 2005 to bring together industry understanding and intervention approaches that, in some cases are already being used by manufacturers.

The aim is to help manufacturers, including those with limited research and development resources, see which of the possible approaches could be suited to their products and processes. It updates it at intervals when new useful new methods are devised and new scientific discoveries made.
Asparaginase is now implemented industrially across many product categories

<table>
<thead>
<tr>
<th>Product category</th>
<th>Reduction in Acrylamide level</th>
<th>Organoleptic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biscuits</td>
<td>&gt;80 % reduction</td>
<td>No impact</td>
</tr>
<tr>
<td>Rye based crackers</td>
<td>38 % reduction</td>
<td>No impact</td>
</tr>
<tr>
<td>Crisp bread</td>
<td>&quot;high enough&quot;</td>
<td>No impact</td>
</tr>
<tr>
<td>Honey cakes</td>
<td>92-93 % reduction</td>
<td>No impact</td>
</tr>
<tr>
<td>Cookies</td>
<td>68-88 % reduction</td>
<td>No impact</td>
</tr>
<tr>
<td>Waffles</td>
<td>&gt;66 % reduction</td>
<td>No impact</td>
</tr>
<tr>
<td>Tortilla chips</td>
<td>&quot;enough&quot;</td>
<td>No impact</td>
</tr>
<tr>
<td>Corn based chips</td>
<td>&quot;enough&quot;</td>
<td>No impact</td>
</tr>
<tr>
<td>Potato based chips</td>
<td>&quot;high enough&quot;</td>
<td>No impact</td>
</tr>
<tr>
<td>Potato based extruded snack</td>
<td>&gt; 80 % reduction</td>
<td>No impact</td>
</tr>
</tbody>
</table>
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
Thank You!
Gracias!