

ARABINOXYLANS

Water-unextractable AX: WU-AX

Extractable AX: WE-AX

- 7 2/3 to 3/4 of total wheat flour AX
- 7 Component of the endosperm cell wall matrix
- 7 Unextractable because of covalent and non-covalent interactions with other cell wall components (AX, protein, ...)
- 7 Strong water-binding capacity

- 7 1/4 to 1/3 of total wheat flour AX
- 7 Similar to WU-AX, but water-extractable
- 7 No interaction with other components
- 7 Possibly unprocessed building-blocks of the cell wall
- 7 High viscosity

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



- Amylases - Xylanases

Lipases

- Oxidases
- Projeases
- ฟรุกรายไมรร

ENZYME TYPES & FUNCTIONALITY





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 nonpolar lipids
- Phospholipids (lecithin) and galactolipids are the predominant polar lipids

Dough Strengthening Emulsifiers





Structure of SSL



Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60) **Proposed effects (by various authors):**

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- Interaction with gluten proteins
- Stabilizing air / water interfaces by forming mono-layers



ACTION OF DUAL SPECIFICITY LIPASE ON FLOUR LIPIDS



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

ENZYME TYPES & FUNCTIONALITY



Basic Glucose Oxidase Mechanism

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



- Glucose oxidase catalyzes the oxidat **and** (β) -D-glucose to D-gluconic acid and <u>hydrogen peroxide</u> $(=H_2O_2)$.
- 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



 H_2O_2 oxidizes the sulfhydrylgroup (-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

D-xylopyranose backbone



- According to the theory of L.Hillhorst and others: Crosslinking arobinoxylan 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

- Oxideses

Proteases

-Asparaginase

ENZYME TYPES & FUNCTIONALITY



The peptide bond





Protein structure





Proteases - The main types







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



Effects are similar, but mode of action is different





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

Proteases sourced from

- Fungi
- Bacteria
- Plants (bromelain, papain)





REDUCING AGENTS

- Reversible with oxidation
- React quickly
- Consumed in reaction
- Amount of gluten weakening depends only on dose

PROTEASE

- Irreversible
- React more slowly, but continue to react until denatured
- Not consumed in reaction
- Amount of gluten weakening depends on dose, reaction time



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 @50-60C 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.



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- Xylanases
- Lipases
- Oxideses
- Projeases
- Asparaginase

ENZYME TYPES & FUNCTIONALITY



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

Source: www.irmm.jrc.be/html/activities/acrylamide/ EUacrylamidelevelmonitoringdatabase__statusJune2006.xls Institute for Reference Materials and Measurements



IUPAC name : prop-2-enamide



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 <u>might</u> be a cancer risk factor"



International Agency for Research on Cancer (IARC)



Joint FAO/WHO Expert Committee on Food Additives





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

JECFA

Joint FAO/WHO

Expert Committee

on Food Additives

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



Simplified Maillard principle



CIAA recommends asparaginase

FOOD navigator.com/europe Breaking News on Food & Beverage Development - Europe

Previous page : Asparaginase validated by CIAA in Acrylamide Toolbox

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.

Print



Asparaginase is now implemented industrially across many product categories

Product category	Reduction in Acrylamide level	Organoleptic impact
Biscuits	>80 % reduction	No impact
Rye based crackers	38 % reduction	No impact
Crisp bread	"high enough"	No impact
Honey cakes	92-93 % reduction	No impact
Cookies	68-88 % reduction	No impact
Waffles	>66 % reduction	No impact
Tortilla chips	"enough"	No impact
Corn based chips	"enough"	No impact
Potato based chips	"high enough"	No impact
Potato based extruded snack	> 80 % reduction	No impact



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!