Getting Smart with Lipases in Baked Goods

Bread (dough) Improvers are commonly added to overcome deficiencies in bread making quality of flour. Exogenous lipases modify the natural flour lipids so they become better at stabilizing the dough. This ensures more stability when the dough is over-fermented, larger loaf volume, and significantly improved crumb structure. Lipases that have specificity towards non-polar lipids can break down dairy derived fats, releasing sharp smelling, short-chain fatty acids. While this may be desirable for cheese production, it is undesirable in baked goods. If the baked good contains a significant amount of butter or milk fat, it can take on a putrid odor if such lipases are used in the recipe. This presentation will cover chemistry of different types of lipases and how they will affect the flavor and amount of short chain fatty acid during storage. The attendees will learn how to match a lipase to a baked good to obtain dough strength and bread volume without undesirable off-flavor.

Learning Objectives

- Lipase chemistry, how they work
- How to match a lipase to a baked good
- A new lipase can overcome off-flavor

Presenter
Austin Dilek, Novozymes

Presentation Time
Monday, February 25, 2019
2:50 pm - 3:25 pm

Session
Breakout 2
GETTING SMART with LIPASES

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Novozymes North America Inc.
ASB19, February 25, 2019, Chicago IL
Agenda

Enzymes 101
Lipases in baking
  Functions

An introduction to NEW LIPASE

Wrap up
Challenges with growing population

10 Billion
Estimated population by 2050
A fresh keeping enzyme changed the industrial baking

- Less fertilizer and traction
- Less heat and electricity
- Less flour, heat, electricity and packaging
- Less driving
- 100 breads
- 6 waste breads
- Sale from primary store
- Animal Feed
- Animal production
Enzymes are found in nature

Example: Laundry with cold-water wash

A rare microorganism that express enzyme which is active in cold found in stalagmite column

These novel enzymes enable...

... cold-water wash

... significant savings of energy, CO₂ and money
Structure and functionality

Most enzymes are globular proteins

Calcium ions

Active site
Region that contains catalytic residues

Bacterial alpha-amylase
Enzymatic catalysis

Substrate

Enzyme
Enzymatic catalysis

Enzyme-substrate complex (transition state)
Enzymatic catalysis
Enzymatic catalysis

Enzyme

Products
Enzymes work in a range

Temperature effect on the enzyme activity

At pH 5.0

pH effect on the enzyme activity

At 37 °C

Enzyme dosage, Substrate availability, Inhibitors also important
What are the wants?

Consumers want a bread with
- nice and even bloom
- good shape and volume
- less additives

The industry wants a dough that is
- robust
- easy to handle
- good machinability
- right balance between elasticity and extensibility
Benefits of lipases

- **Optimal dough strengthening effects**: Higher mixing and fermentation tolerance
- **Appealing bread appearance and size**: Improved volume as well as a finer and more regular crumb appearance, sliceability
- **Clean label**: 1 kg can replace 100 to 1,000 kg of traditional emulsifiers which leads to a cleaner label and an improved carbon footprint
- **A NEW LIPASE**: Highest tolerance towards short fatty acid chains which leads to a broader usage in application including recipes containing butter

No risk of off-flavour
Volume increase during baking

Stages of gas cells

Dough consists of discrete gas cells lined with liquid films, embedded in starch-protein matrix

After fermentation, matrix does not completely enclose gas cells, leaving only liquid films

Baking increases expansion, film cannot meet demand of increased surface area – conversion to open sponge

Weaker film leads to more open bubbles and greater loss of gas retention

Modified from Gan, Z., Ellis, P.R. and Schofield, J.D. (1995)
During proving, gas bubble stability depends on the types of lipids adsorbed at the air-water interface.

- Both proteins and lipids are active at the air-water interface, but is dominated by lipids.
- FFAs are detrimental to foam stability therefore loaf volume, while polar lipids improve foam stability and the volume.
• Lipases modify naturally occurring nonpolar and polar lipids in wheat flour, such as triglycerides, lecithin, and digalactocyl diglycerides (DGDG).

  The resulting lipids have improved emulsifying properties

• They can also work in synergy when combined with other enzymes.
Lipase fact sheet

**Lipases catalyzes** the hydrolysis of fats and oils in the presence of water.

**Acts on** wheat flour lipids, egg lipids, fats and oils in baked goods

**Specificity against polar and non-polar lipids:** polar and non-polar lipases

**Works in the bowl,** inactivated in the oven

**Dough strengthening:** ↑ loaf volume, appearance, crumb structure

↓ loss during slicing

**MIGHT give off-flavor** solid fat including formulas
Lipids in wheat flour

Wheat flour lipids 2%

Non-polar lipids (50.9%)
- Triglycerides 20.8%
- Steryl esters 7.5%
- Free fatty acids 7%
- 1,2-Diglycerides 6.2%
- 1,3-Diglycerides 6.0%
- Free sterols 2.3%
- Monoglycerides 1.3%

Polar lipids (49.1%)
- Glycolipids 26.4%
- Phospholipids 22.7%
  - Digalactosyl diglycerides 13.5%
  - Monogalactosyl diglycerides 4.9%
  - Lysolecitin 7.1%
  - Lecithin 5.8%
  - N-Acyl phosphate idylethanolamines 4.9%
  - N-Acyl lysophosphate idylethanolamines 2.9%

Modified from Pomeranz, Y. (1987)
Acts on butter, shortening with short chain fatty acid containing sources

**Produces free fatty acids (FFA)**

- Small FFA can be volatile <300 kD; Active at low quantities (ppt-ppm)
- **Interact with receptors in the olfactory system (off-flavour)**
Specificity against polar and non-polar lipids:

--Polar lipases acts on phospholipids and galactolipids to produce emulsulfier-like structures
Mechanism of polar lipases
Stabilization of gas cells during baking

The conversion of polar flour lipids leads to an increased polarity and thus the emulsifying properties are improved

Due to these emulsifying properties the lipids can reduce the surface tension between the air and water phase leading to an improved foam stability

Through the improved stabilizing effect of the inherent polar lipids, polar lipases lead to improved dough stability, larger bread volume and better crumb appearance

Modified from Gan, Z., Ellis, P.R. and Schofield, J.D. (1995)
Benefits of lipases in dough and baked goods

- Improved process tolerance in general (mixing and proofing stability), therefore easiness in handling and improved loaf volume and crumb structure

- Clean label compatible, can replace emulsifiers depending on the recipe

- Production stability irrespective variations in flour quality
NEW LIPASE improves dough strength gives higher volume and better crumb structure similar to DATEM (Diacetyl tartaric acid ester of mono- and diglycerides, also E472e)

![Bar graph showing specific volume index (ml/g) for control, NEW LIPASE at 7.5 ppm, 10 ppm, 12.5 ppm, 0.2% DATEM, 0.3% DATEM. The graph displays higher specific volume for NEW LIPASE compared to control and DATEM.]

- Control
- NEW LIPASE 7.5 ppm
- NEW LIPASE 10 ppm
- NEW LIPASE 12.5 ppm
- NEW LIPASE 0.2% DATEM
- NEW LIPASE 0.3% DATEM
NEW LIPASE can eliminate DATEM and SSL (Sodium stearoyl lactylate) in pan bread in high speed mixing process.
NEW LIPASE matches DATEM performance in white pan and whole meal bread
NEW LIPASE improves proofing tolerance, volume, bloom and crispiness in French baguette

**Specific Volume Index [ml/g]**

<table>
<thead>
<tr>
<th></th>
<th>Normal fermentation (2:00)</th>
<th>Extensive fermentation (2:30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.2</td>
<td>4.7</td>
</tr>
<tr>
<td>2.5 ppm NEW LIPASE</td>
<td>5.0 ppm NEW LIPASE</td>
<td>5.7</td>
</tr>
<tr>
<td>5 ppm NEW LIPASE</td>
<td>6.2 ppm NEW LIPASE</td>
<td>6.7</td>
</tr>
<tr>
<td>7.5 ppm NEW LIPASE</td>
<td>0.2% DATEM</td>
<td>7.2</td>
</tr>
<tr>
<td>0.3% DATEM</td>
<td>0.3% DATEM</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Notes:**
- Normal fermentation time: 2.00 h
- Extensive fermentation time: 2.30 h
NEW LIPASE gives better bloom after normal and extensive proofing time in crusty-style bread.
Example of improved bread sliceability with a lipase

Control

A lipase

Bread sliced at a core temperature of 118-122 F (48–50°C); slice thickness 12.5 mm

Factors influencing sliceability: bread core temperature, speed of slicing, changes in recipe
All pictures shown are for illustration purpose only
Lipases and off-flavour

Flour, yeast, and Maillard reaction gives desirable flavor in bread.

Lipases that have specificity towards non-polar lipids break down dairy derived fats, releasing sharp smelling, short-chain fatty acids.

This is desirable for cheese production, it is undesirable in baked goods.

If the bread contains a significant amount of butter or milk fat, it can take on a putrid, vomit-like odor if such lipases are used in the recipe.
# Fatty acids and off-flavor

## Examples of fat sources in baking: fatty acid composition

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Wheat flour</th>
<th>Rapeseed</th>
<th>Coconut</th>
<th>Butter</th>
<th>Flavour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric</td>
<td>C4:0</td>
<td></td>
<td></td>
<td>4</td>
<td>Sharp, acetic, cheese, butter</td>
</tr>
<tr>
<td>Hexanoic</td>
<td>C6:0</td>
<td></td>
<td></td>
<td>2.3</td>
<td>Sour, fatty, sweaty, cheesy</td>
</tr>
<tr>
<td>Octanoic</td>
<td>C8:0</td>
<td></td>
<td></td>
<td>7</td>
<td>Fatty, waxy, rancid, oily</td>
</tr>
<tr>
<td>Decanoic</td>
<td>C10:0</td>
<td></td>
<td></td>
<td>8</td>
<td>Fatty, waxy, rancid, oily</td>
</tr>
<tr>
<td>Lauric</td>
<td>C12:0</td>
<td></td>
<td></td>
<td>48</td>
<td>Rancid, sour, fatty, soapy</td>
</tr>
<tr>
<td>Myristic</td>
<td>C14:0</td>
<td></td>
<td></td>
<td>16</td>
<td>Fatty, coconut</td>
</tr>
<tr>
<td>Palmitic</td>
<td>C16:0</td>
<td>20</td>
<td>2</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>C16:1</td>
<td>3</td>
<td></td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Stearic</td>
<td>C18:0</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Oleic</td>
<td>C18:1</td>
<td>15</td>
<td>14</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Linoleic</td>
<td>C18:2</td>
<td>60</td>
<td>14</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Linolenic</td>
<td>C18:3</td>
<td>5</td>
<td>9</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Erucic</td>
<td>C22:1</td>
<td>45</td>
<td></td>
<td></td>
<td>45</td>
</tr>
</tbody>
</table>

Short and medium chain free fatty acids give immediate flavour.

Long chain fatty acids: no flavour, but unsaturated fat can oxidise => odourous volatiles (e.g., pungent, green, fatty aldehydes).

Oxidation flavour forms over time:
Fair correlation between sensorial perception and short-medium FFA in baked goods acids

Odour as function of butanoic acid day 1

Taste as a function of hexanoic acid, day 1

$R^2 = 0.8121$

$R^2 = 0.7981$
Sensory perception and off-flavor affected by lipases

- Off-flavor intensity of brioche bread
  - 8 ppm
  - NEW LIPASE
  - 30 ppm Lipase A
  - 30 ppm Lipase B

Sensorial off-flavor score (1-9)

Butyric acid (mg/g bread)

- Control
- 8 ppm NEW LIPASE
- 30 ppm Lipase A
- 30 ppm Lipase B

Off-flavour
n-Butyric
Butter bread (3% butter) sponge-dough process
Sensory testing shows NEW LIPASE CAN NOT be overdosed and does not produce off-flavour 4 day-old bread
NEW LIPASE does not produce more off-flavour compounds even overdosed 4 day-old bread

Total off-flavour compounds (n-butanoic acid, n-hexanoic acid, octanoic acid, decanoic acid, lauric acid)
Brioche-20% butter
NEW LIPASE is more tolerant producing off-flavour compounds in longer retardation periods

Total off-flavour compounds (n-butanoic acid, n-hexanoic acid, octanoic acid, decanoic acid, lauric acid)
Pandesal
(coconut shortening)
NEW LIPASE **does** not produce off-flavour in **Pandesal** (coconut shortening) **compare to control** at Day 7

Total off-flavour compounds (n-butanoic acid, n-hexanoic acid, octanoic acid, decanoic acid, lauric acid)
Pan Blandito (butter)
Sensory testing on Pan Blandito (butter) shows the NEW LIPASE does not produce off-flavour in Pandesal (coconut shortening) **compare to control** at Day 7.
NEW LIPASE does not produce off-flavour in Pan Blandito (butter) compare to control over time

Total off-flavour compounds (n-butanoic acid, n-hexanoic acid, octanoic acid, decanoic acid, lauric acid)
Brioche (retarded dough)
Off-flavor compounds does not increase during dough retardation with NEW LIPASE in Brioche even with overdosing

![Graph showing total off-flavour compounds (mg/kg bread) over 24 hours and 48 hours for different lipase dosages.]

- No lipase
- Optimal dosage NEW LIPASE
- 2X optimal dosage NEW LIPASE
- 3X optimal dosage NEW LIPASE

Total off-flavour compounds (n-butyric acid, n-hexanoic acid, octanoic acid, decanoic acid, lauric acid)
LIPASES

- Improves process tolerance in general (mixing and proofing stability), therefore easiness in handling and improved loaf volume and crumb structure

- Clean label compatible, can replace emulsifiers depending on the recipe

- Production stability irrespective variations in flour quality
NEW LIPASE for your enzyme tool box

- Stronger, easy to handle dough
- Satisfy clean label demands
- NO unpleasant flavour
QUESTIONS?

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