

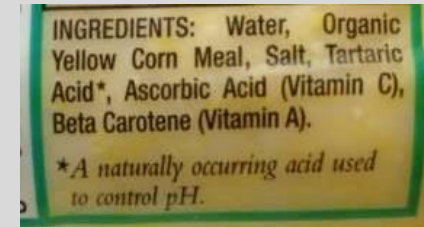


Applying enzymes in clean label formulations

Charlotte Horsmans Poulsen
Dupont Industrial Biosciences
Bertebos, 29th of August

CLEAN LABEL

- ✓ Consumers are skeptical and suspicious of ingredients they do not understand or recognize.



Clean Label Definition

- ***There isn't one!***
- A label with ingredients that are hard to pronounce, synthetic or simply unfamiliar to many people are often unwanted.
- Other interpretations include producing products without ingredients included in the various "no-lists".
- Even stricter interpretations may include only ingredients that are produced organically, not genetically modified, and/or socially/environmentally conscious.



Top 5 label descriptors among clean label shoppers

- Recognizable ingredients, as well as all natural ingredients, are descriptors sought after by all clean label shoppers
- Baby boomers, Gen-Xers and Millennials all seek products with recognizable ingredients, while 65+ consumers seeks products low in sodium, as well as no sugar added
- Millennials are the only group that calls out organic as an attribute that would greatly increased purchase interest

| TOP 5 LABEL DESCRIPTIONS THAT GREATLY INCREASE PURCHASE INTEREST | |
|--|---|
| <p><u>MILLENNIALS:</u></p> <ul style="list-style-type: none"> All natural ingredients No preservatives No artificial colors Recognizable ingredients Organic | <p><u>GEN XERS</u></p> <ul style="list-style-type: none"> Recognizable ingredients No artificial ingredients Grown without pesticides Locally grown Short ingredient list |
| <p><u>BABY BOOMERS:</u></p> <ul style="list-style-type: none"> High fiber Contains whole grains No HFCS All natural ingredients Recognizable ingredients | <p><u>MATURES:</u></p> <ul style="list-style-type: none"> No added sugar No artificial ingredients Low sodium All natural ingredients No preservatives |

| TOP 5 LABEL DESCRIPTIONS AMONG HEAVY, MODERATE & LIGHT CLEAN LABEL CONSUMERS |
|--|
| <p><u>HEAVY CLEAN LABEL CONSUMERS:</u></p> <ul style="list-style-type: none"> No artificial ingredients ✓ All natural ingredients No artificial preservatives No HFCS ✓ Recognizable ingredients |
| <p><u>MODERATE CLEAN LABEL CONSUMERS:</u></p> <ul style="list-style-type: none"> No added sugar ✓ All natural ingredients ✓ Recognizable ingredients No artificial preservatives Organic |
| <p><u>LIGHT CLEAN LABEL CONSUMERS:</u></p> <ul style="list-style-type: none"> No added sugar ✓ All natural ingredients No HFCS ✓ Recognizable ingredients Organic |

Three examples of applying enzymes to create a simpler label

- Lipases generating emulsifying effect in bread
- A lactase for fiber generation and sugar reduction in yogurt
- Achieving oxidative effect by an enzyme (HOX) in bread compared to the use of bromate



A photograph of two women sitting at a restaurant table. The woman in the foreground has grey hair and is smiling broadly, looking towards the camera. The woman in the background has blonde hair and is also smiling. They are surrounded by plates of food, including a salad, and glasses of beer. The background is slightly blurred, showing other restaurant patrons and cars parked outside.

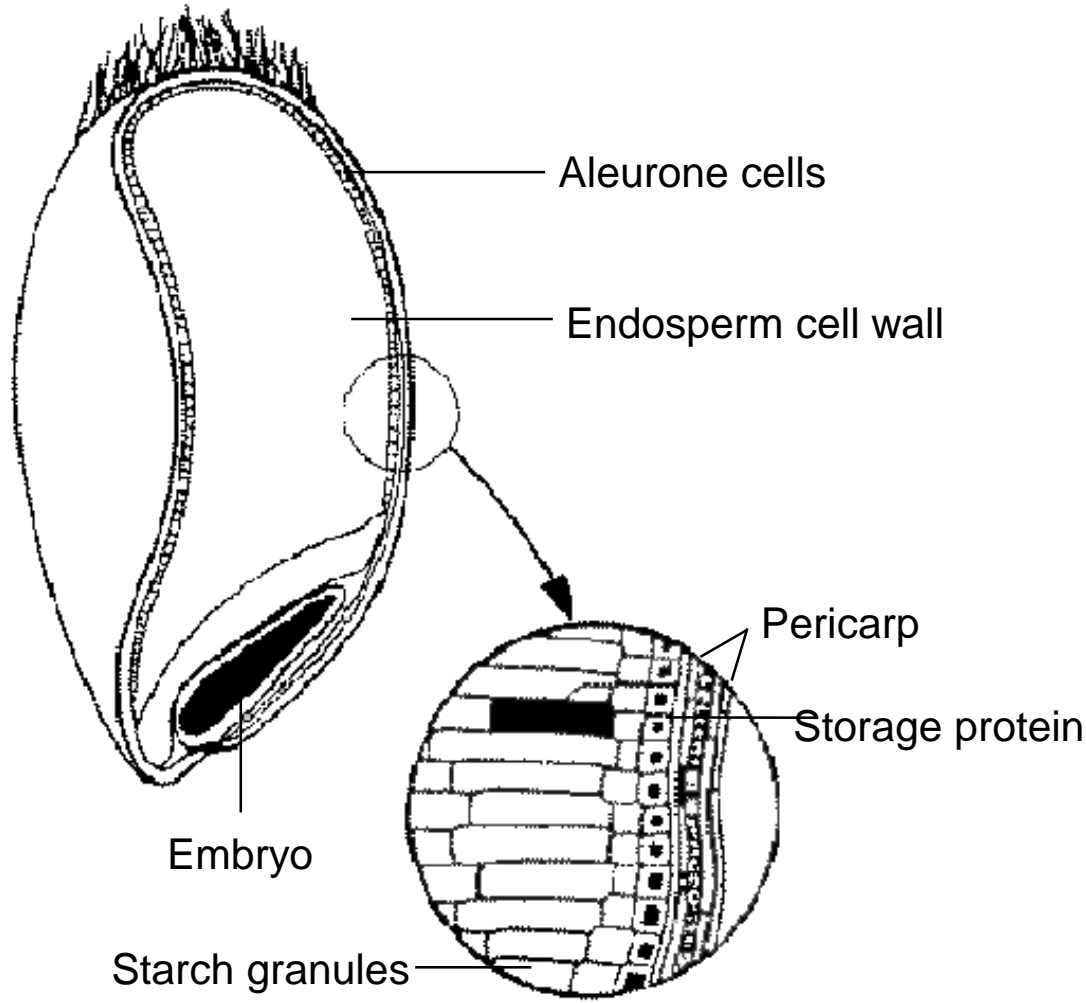
Lipases for emulsification in bread

Functionality of lipases in bread making

- **In situ generation of emulsifiers from lipids naturally present in flour**
- Creates a synergistic dough strengthening effect
- Improves tolerance to processing variations and raw materials
- Increases the volume of the final product
- Improves crumb texture

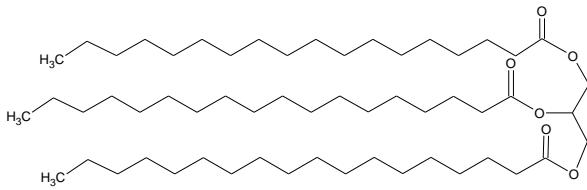


The substrate (2-3% lipid)

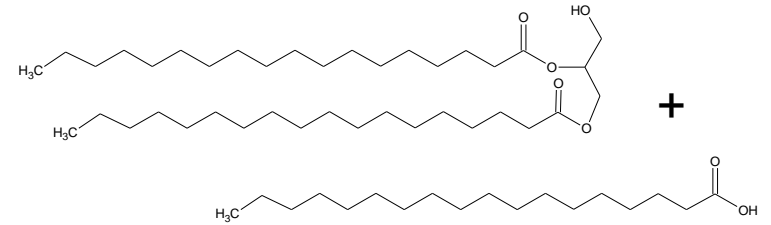
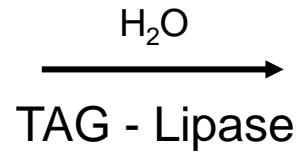


| | | |
|------------------------|--|-------|
| Nonpolar lipids | Triglycerides (TG) | 20.8% |
| | Steryl esters | 7.5% |
| | Free fatty acids (FFA) | 7.0% |
| | 1,2-diglycerides (1,2-DG) | 6.2% |
| | 1,3-diglycerides (1,3-DG) | 6.0% |
| | Other nonpolar lipids | 3.4% |
| Glycolipids | Digalactosyl diglycerides (DGDG) | 13.5% |
| | Monogalactosyl diglycerides (MGDG) | 4.9% |
| | Other glycolipids | 7.9% |
| Phospholipids | Lysophosphatidyl cholines (LPC) | 7.1% |
| | Phosphatidyl cholines (PC) | 5.8% |
| | N-acyl phosphatidyl ethanolamines (NAPE) | 4.9% |
| | Other phospholipids | 4.9% |

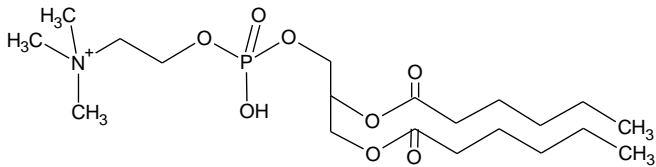
Lipases / Glycolipases / Phospholipases



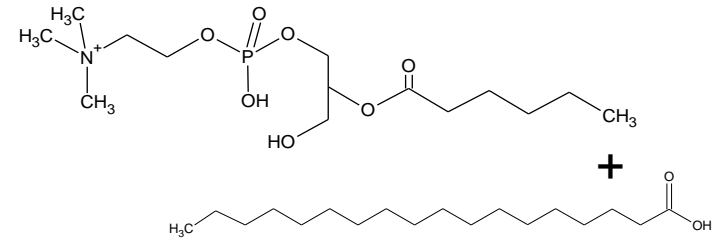
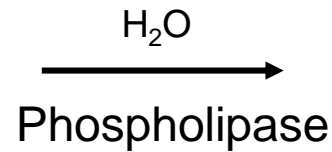
TAG



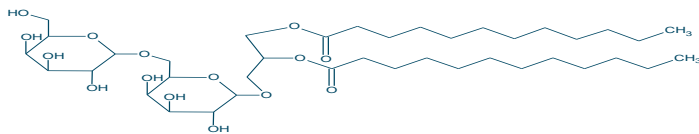
DG + FFA



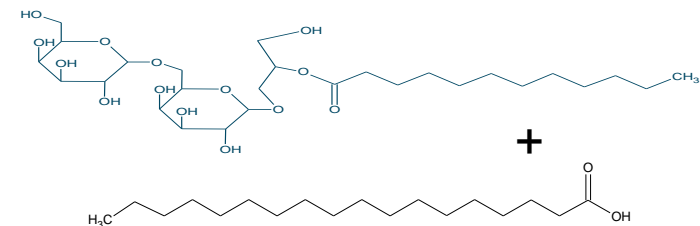
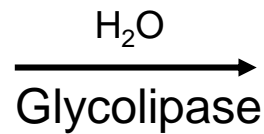
PC



Lyso-PC + FFA



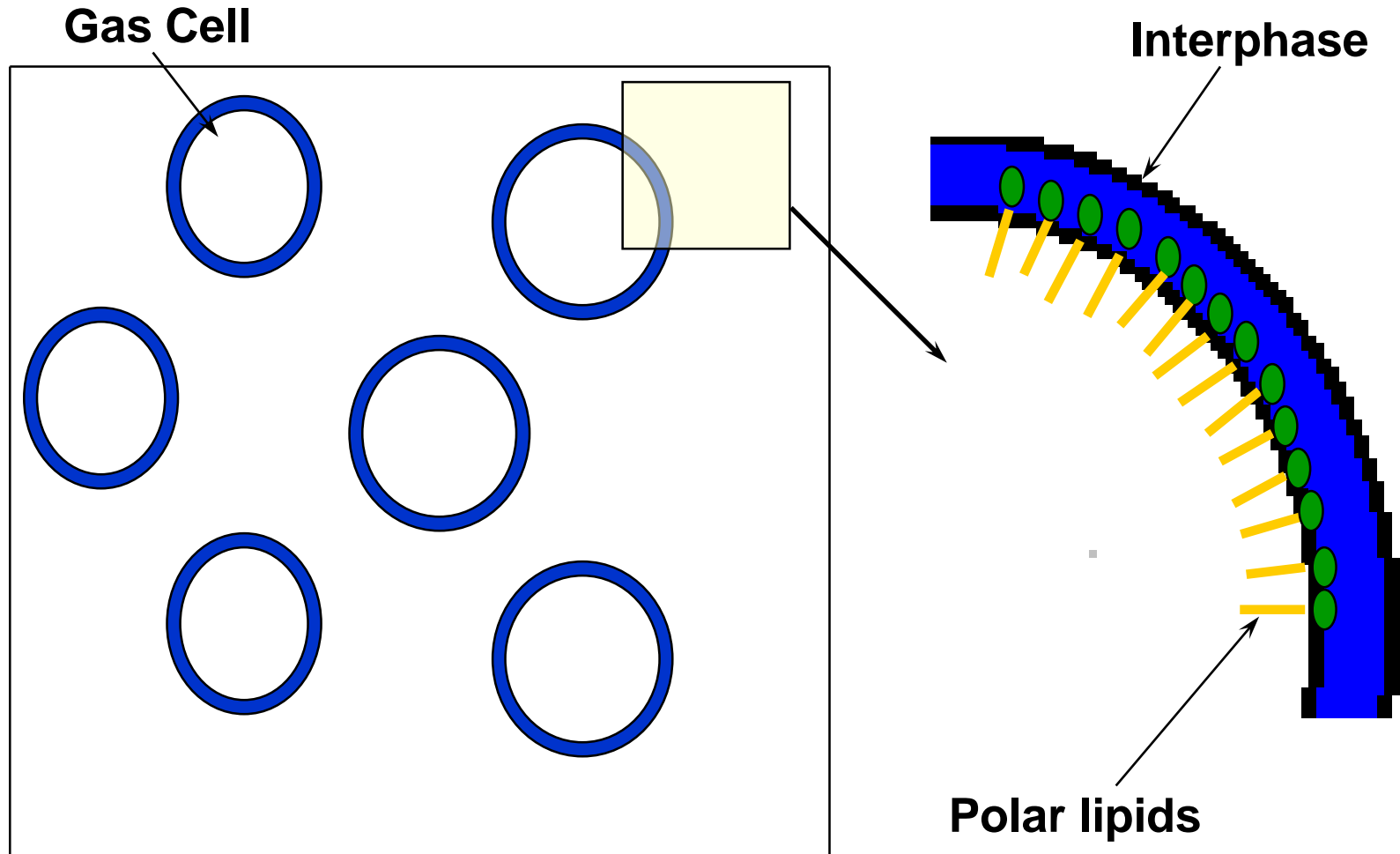
DGDG



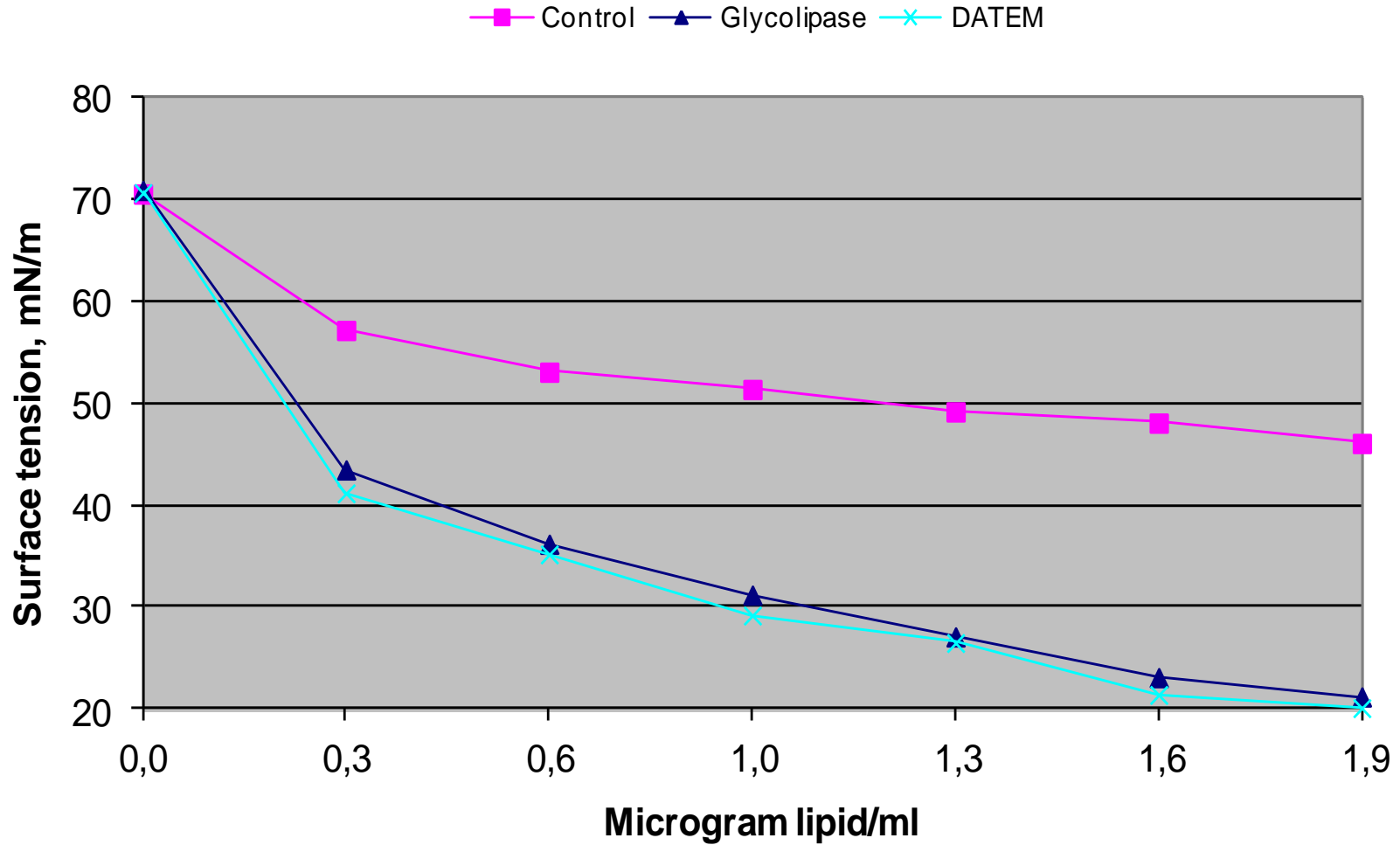
DGMG + FFA

Functionality of lipases in bread making

– Gas cell stabilisation



Surface tension



FIBERLine™ 3105 + emulsifiers vs. FIBERLine™ 3106



| Solution 1 | Solution 2 | Solution 3 |
|--|---|------------|
| 500ppm FIBERLine™ 3105 + 0.55% PANODAN® A2020 + 0.3% DIMODAN® HP 75 + 3% gluten | 500ppm FIBERLine™ 3106 + 5% gluten (No emulsifiers) Enzymes including lipase | 5% gluten |

FIBERLine™ 3106 gives a simple label

**Healthy
soft
bread
with
superior
volume**

- FIBERLine™ 3106 facilitate production of a 100% wholemeal bread with a volume and softness close to that of white wheat bread

Natural

- FIBERLine™ 3106 enables production of high-fibre bread with 100% natural wholemeal and no compromise on texture and shelf life

Labelling example with
FIBERLine™ 3106

6.5% fibre

Wholemeal flour, water, gluten, vegetable fat, salt, sugar, yeast, ascorbic acid (E300)



A new enzyme for generating pre-biotic fibers and reducing sugar in dairy products

Introducing DuPont™ Danisco® ZymStar™ GOS

- DuPont™ Danisco® ZymStar™ GOS is an innovative enzymatic solution that enables dairy manufacturers to produce **dietary fibers** in the form of Galacto-oligosaccharides (GOS) in situ from existing lactose in dairy products; reducing the **lactose** level, reducing the **lactose calorie** contribution and bringing down the **total sugars** content.



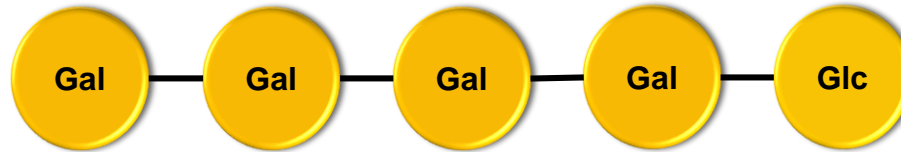
Generation of GOS fiber

Lactose content reduction

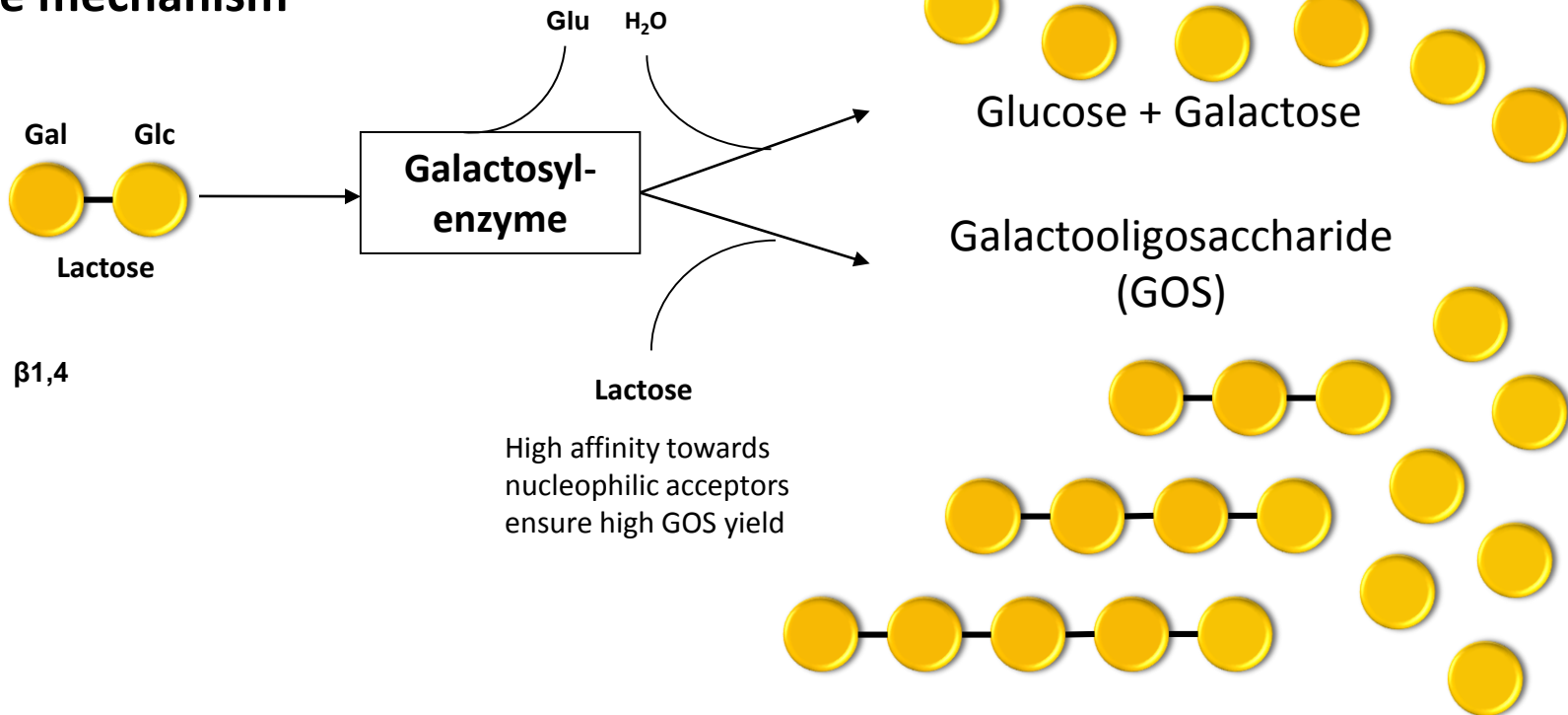
Sugars and calorie reduction

Galactooligosaccharides (GOS)

- Galacto-oligosaccharides are made up of Galactose and Glucose units. GOS are non-digestible fibers and classified as prebiotics giving increased gut health to the consumer



Enzyme mechanism



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2001, p. 2276–2283
 0099-2240/01/\$04.00+0 DOI: 10.1128/AEM.67.5.2276–2283.2001
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Intra- and Extracellular β -Galactosidases from *Bifidobacterium bifidum* and *B. infantis*: Molecular Cloning, Heterologous Expression, and Comparative Characterization

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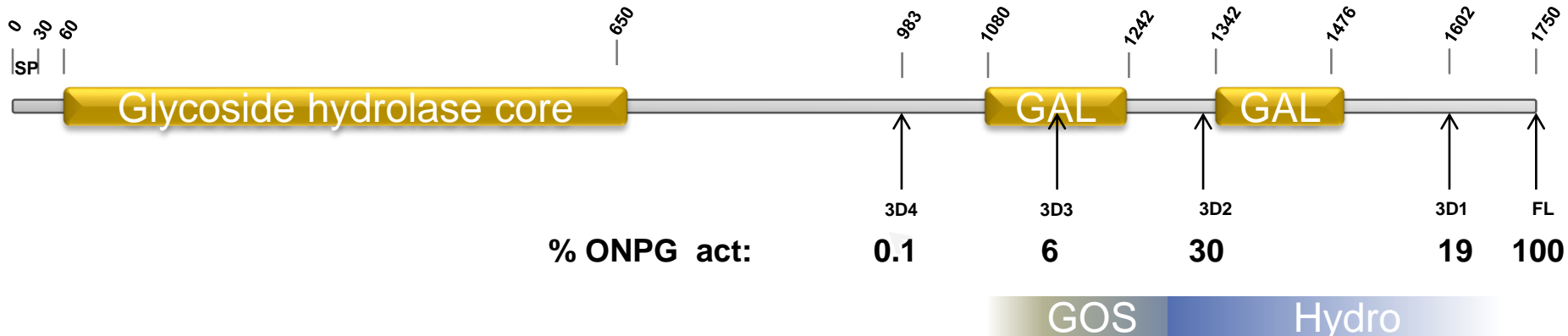
Appl Microbiol Biotechnol (2001) 57:647–652
 DOI 10.1007/s00253-001-0845-z

ORIGINAL PAPER

F. Jørgensen · O. C. Hansen · P. Stougaard

High-efficiency synthesis of oligosaccharides with a truncated β -galactosidase from *Bifidobacterium bifidum*

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 © Springer-Verlag 2001



Sugar and calorie / energy reduction Claims in EU

- **ENERGY-REDUCED**

- A claim that a food is energy-reduced, and any claim likely to have the same meaning for the consumer, may only be made where the energy value is reduced by at least 30 %, with an indication of the characteristic(s) which make(s) the food reduced in its total energy value.

- **REDUCED SUGARS**

- A claim stating that the content in sugars has been reduced, and any claim likely to have the same meaning for the consumer, may only be made where the reduction in content is at least 30 % compared to a similar product,
- “sugars” means all monosaccharides and disaccharides present in food, but excludes polyols;

“Sugars” reduction examples

Plain Yogurt:

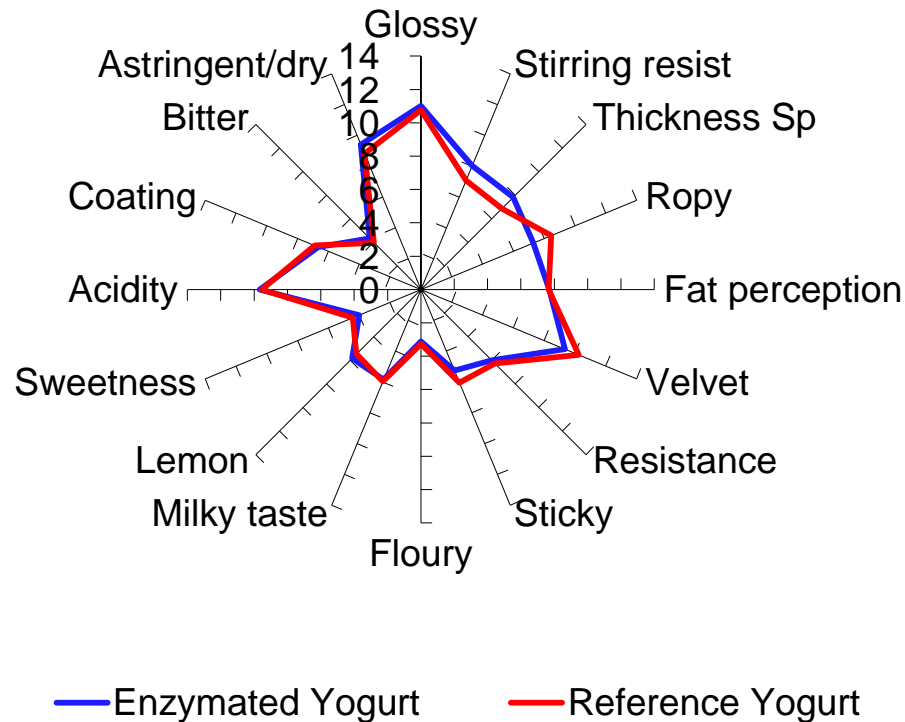
| | Lactose and other sugars % | Sucrose% | Fibers% | total sugars% | sugar reduction% |
|------------------------------|----------------------------|----------|---------|---------------|------------------|
| Plain yogurt | 4.6 | 0 | 0 | 4.6 | |
| Plain yogurt + <i>b</i> -gal | 2.4 | 0 | 2.2 | 2.4 | 47.8 |

Yogurt + sucrose:

| | Lactose and other sugars% | Sucrose% | Fibers% | total sugars% | sugar reduction% |
|----------------------------------|---------------------------|----------|---------|---------------|------------------|
| flavoured yogurt | 4.6 | 4 | 0 | 8.6 | |
| flavoured yogurt + <i>b</i> -gal | 2.4 | 4 | 2.2 | 6.4 | 25.6 |

Sensory analysis of product prototypes

Exemplary spider-graph of a Dairy product tasting



- No difference in sweetness comparing an enzymated to a non-enzymated Dairy product
- No off-flavors were detected (lipase or e.g. protease)
- Stirring resistance and Thickness on spoon may be increased

A woman with dark hair, wearing a light blue jacket over a white top, is looking at a can of food in a grocery store aisle. She is holding the can with both hands. In the foreground, the back of a young child's head and shoulders are visible; the child is wearing a light green striped shirt and is pushing a shopping cart. The store shelves are stocked with various products, including jars of jam and boxes of cereal. In the background, other shoppers and a shopping cart are visible, along with a sign that says '5' hanging from the ceiling.

**Achieving oxidative effect by
Hexose oxidase (HOX)**

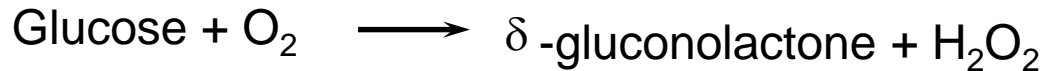
Functionality of oxidative enzymes in bread-making

- Better dough handling properties
- Improved dough tolerance
- Improved bread quality
- Improved crumb structure
- Improved bread volume
- Can supplement chemical oxidants
- Gluten replacement



Mechanism of hexose oxidase reaction in dough

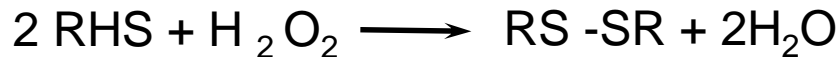
Hexose oxidase reaction



or



Oxidation in gluten



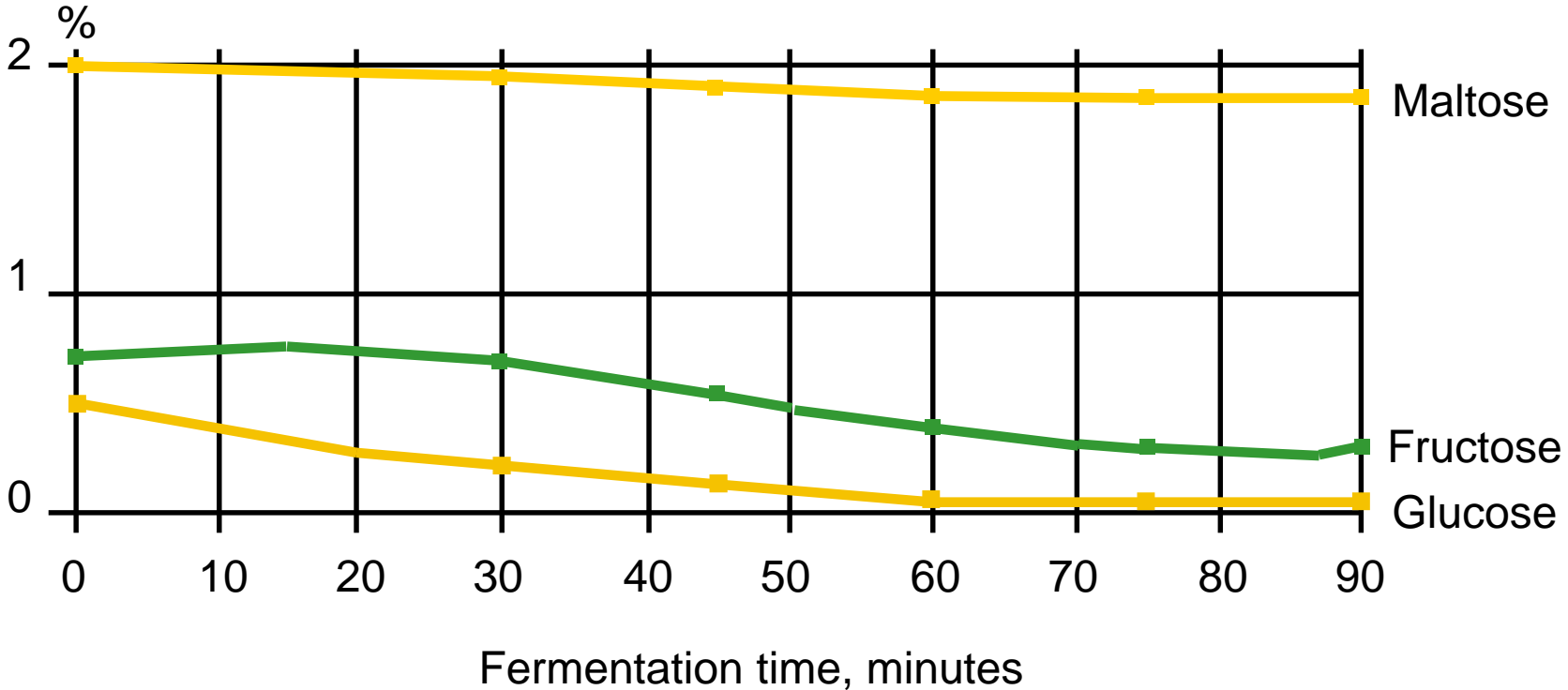
R: Protein molecule

HS: Thiol groups

S-S: Disulfide bonds

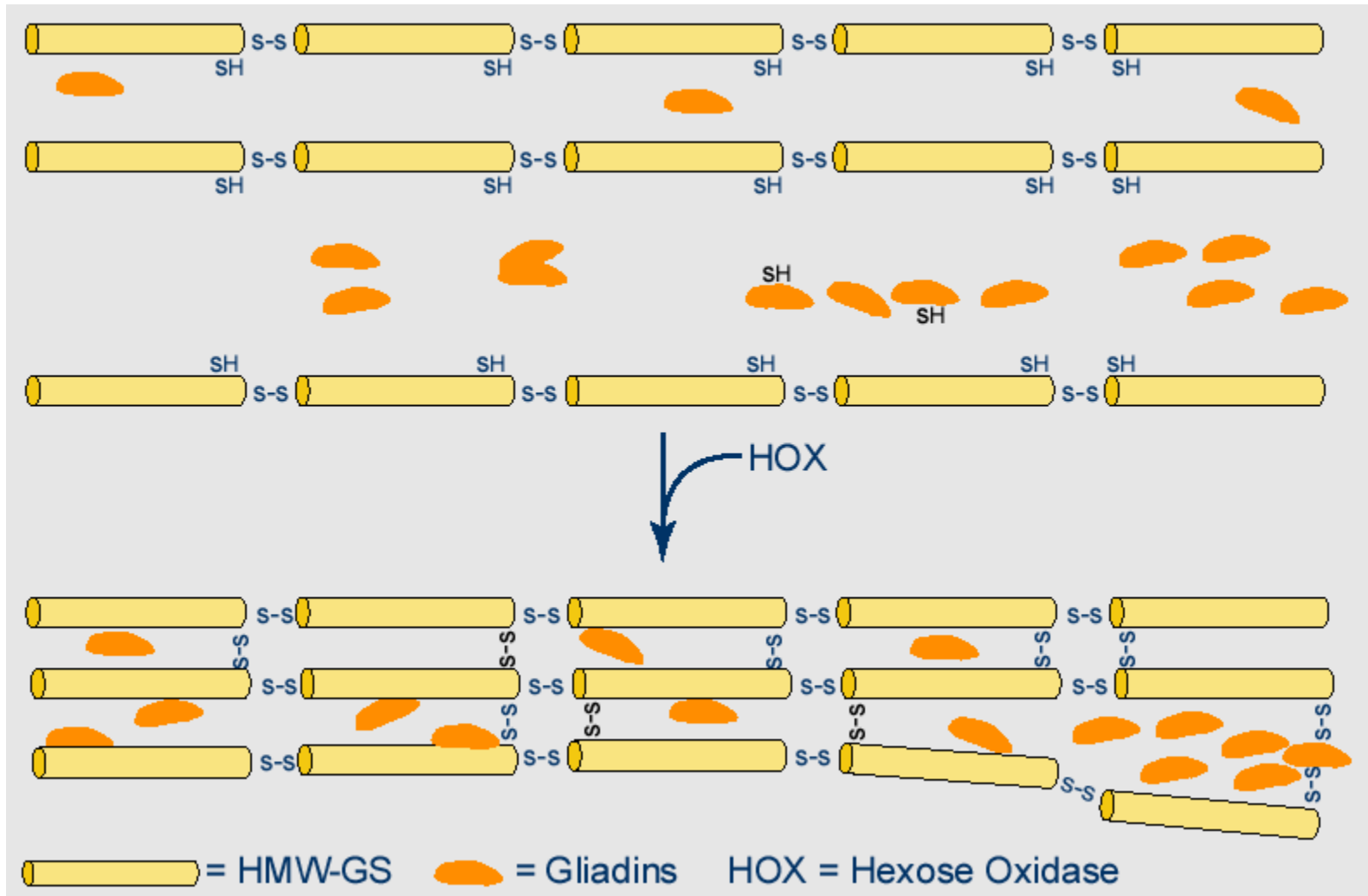
Carbohydrates in bread

- as a function of fermentation time

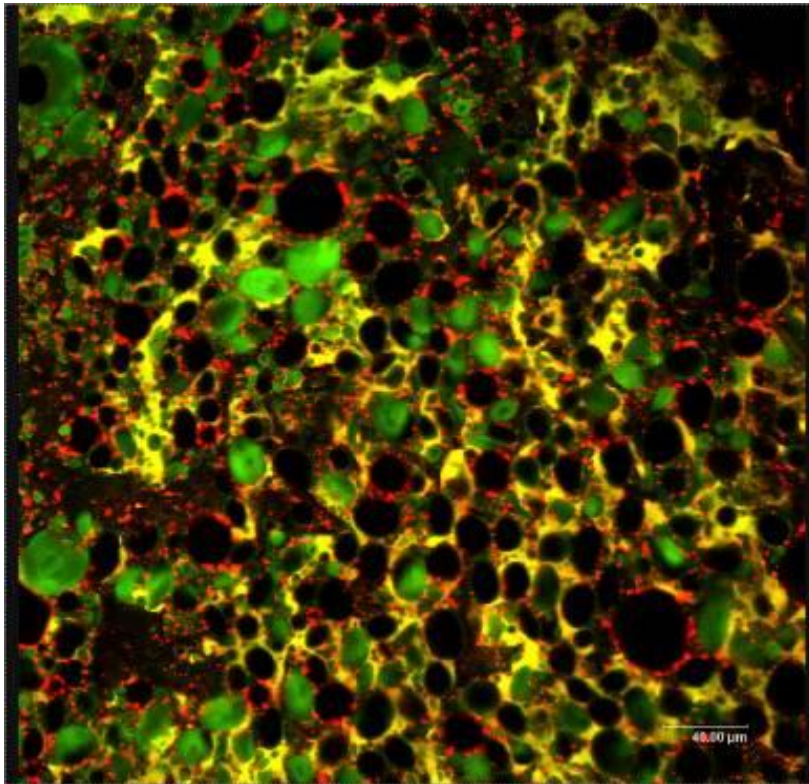


Ref: Agnete Dal Thomsen, *Næringsforskning*, Årg. 32, 105-107, 1988

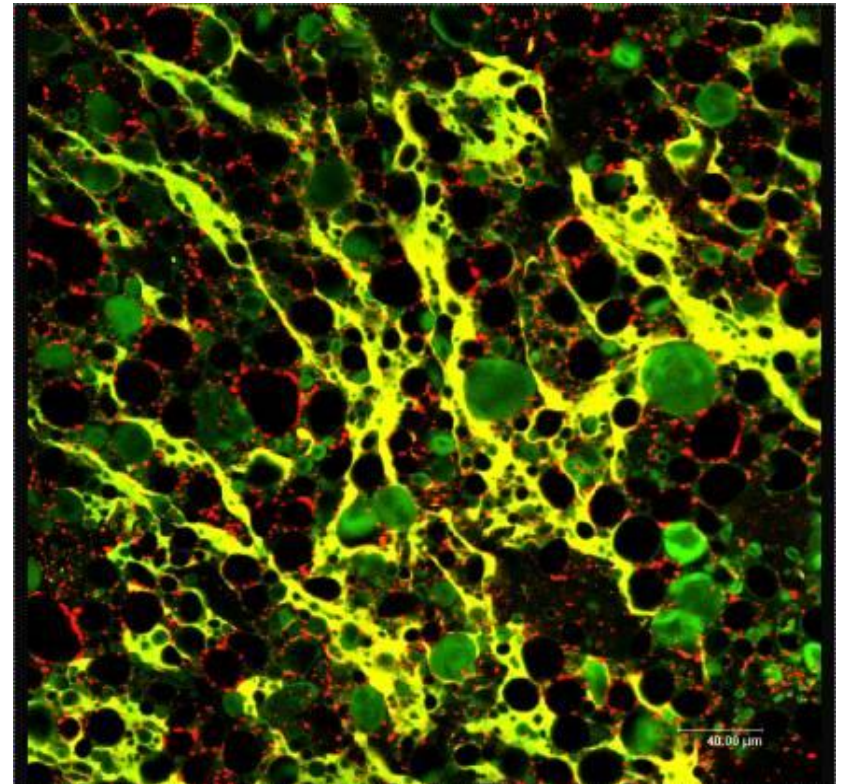
Oxidation of gluten network by Hexose oxidase



Confocal laser scanning microscopy

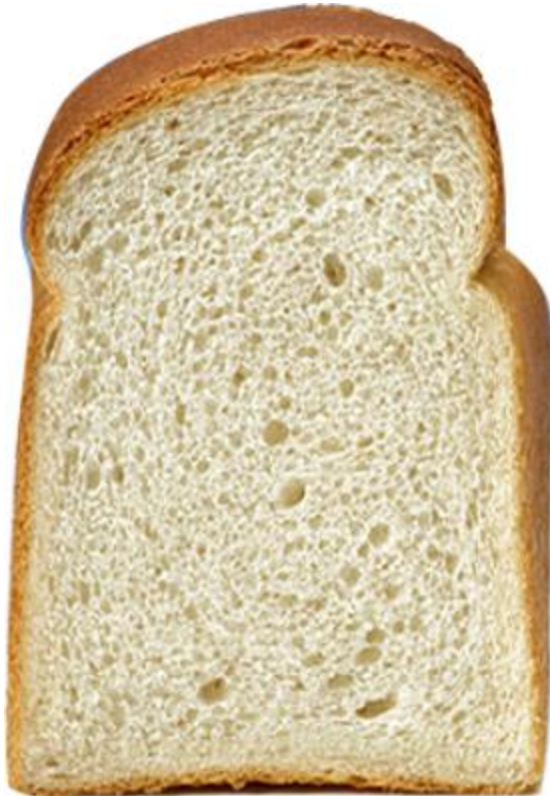


Dough without
GRINDAMYL™ SUREBake

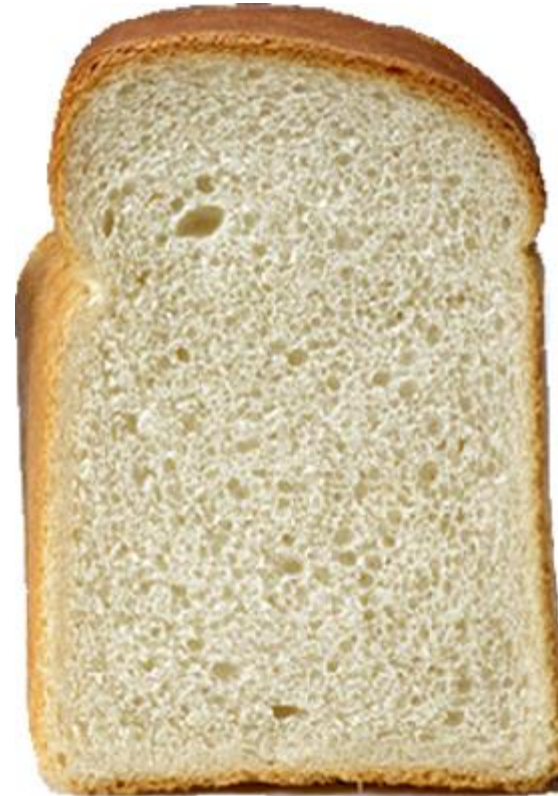


Dough with
GRINDAMYL™ SUREBake

US no-time dough



90 U/kg flour
Hexose Oxidase



30 ppm
Potassium Bromate

Conclusion: Enzymes used in food processing are catalysts for

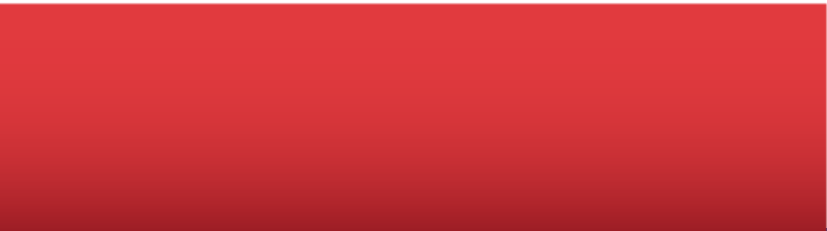
Food with improved properties

- **Enhancing health & nutritional value of products**
- **Producing soluble fiber content / bioavailability**
- **In-situ generation of ingredients based on food raw material constituents**
- Improving food texture & taste

Reduced costs & increased yields

- Improving food production stability, efficiency & safety
- Overcoming raw material variations
- Enabling more stable and palatable products
- Reducing food waste and energy consumption

All the above can help enable **CLEAN LABEL** solutions in different aspects of the concept.



The miracles of science™