In Situ production of Galacto-Oligosaccharides (GOS) in dairy products

DUPONT™ DANISCO® ZYMSTAR™ GOS

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South Africa

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DuPont Nutrition & Health
Dairy Innovation
1. **Overview of GOS & *In Situ* production of GOS**
   - Basic properties; β-galactosidase (lactase);
     Enzymatic mechanism; Thermodynamics;
     Optimization & Kinetics of GOS formation
   - GOS Analysis

2. **Application possibilities for *In Situ* production of GOS**
   - Products (fresh fermented; UHT; Sterilised)

3. **Features & Benefits of *In Situ* production of GOS**
   - Legislation; Consumer Segments; Features & Benefits
Overview of GOS & In Situ production of GOS
Latin phrase that translates literally to "on site" or "in position"; "natural" or "original" position or place

In Situ…?

Gal β 1-4Gal β 1-4Glc (4'-galactosyl lactose)
Gal-(Gal)n-Glc (n=1~4  β -1,4 bond)
Gal-Glc ( β -1,3 bond, etc. transformed disaccharide)
Basic Properties of Galactooligosaccharides (GOS)

1. Sweetness* = 0.3 – 0.6 X sucrose (*GOS Syrup)
2. ↓ caloric value vs. sucrose / lactose (i.e., 50% of sucrose)
3. ↓ glycaemic index of foods - Ω to α-amylase & carbohydrases
4. ↑ heat stability 160° C / 10 min (pH 7); 100 ° C for 10 min (pH 2)
5. ↑ acid stability: stable to pH 2
6. $A_w$ of GOS fibre ≈ sucrose; control moisture levels in foods
7. ↓ Freezing point in foods
8. Reported bifidogenic effect
9. Cariogenicity: Inhibit or arrest dental caries formation
10. Reported ↑ mineral absorption
11. Reported feeling of satiety
ZymStar™ GOS lactase:
- A new innovative lactase
- Produces significant levels of pre-biotic fibers (Galactooligosaccharides [GOS]) from lactose already present in the milk (in situ application)
- Reduction of lactose content by around 90%, to approx. 0.1-0.5%

vs. traditional lactase:
- Enzymes that break down lactose from milk into glucose and galactose
- Only capable of GOS production at high lactose concentration (40-50% lactose needed)
- In situ application not possible

ZymStar™ GOS offering

- In situ application: Converts lactose present in the milk into soluble fiber (GOS)
- Reduces the lactose content by 90%
- Due to lactose conversion into fiber, potential for
  - Reduced calorie level
  - Fat replacement

Traditional GOS-producing lactase

- Lactase enzyme
- Separate GOS ingredient production: high lactose concentration required
- Dairy product, standard lactose content + GOS

Traditional lactase

- Lactase enzyme
- Lactose reduced/free product
In Situ Production of GOS = 2-stage process

Hydrolyses of glycosidic bonds = 2-stage process

[ES] covalently-linked intermediate; glycosidase reversal

\[
[E] + [S] \xrightleftharpoons{\kappa_f}^{\kappa_r} [ES] \xrightarrow{k_{\text{cat}}} [E] + [P]
\]

Equilibrium Approach

- Most enzymes reactions reversible
- ↑ temp
- ↑ [S]
- ↑ S solubility ↑ reaction speed
- ↓ Aw
- ↑ Polymerization

Kinetic Approach

- *In Situ* opportunities
- [ES] is transgalactosylated on nucleophile acceptor
- Efficacy measured as $\Delta \text{glc} \& \text{gal}$ [P]
- [E], temp., [S], time & enzyme inactivation
Enzyme Mechanism of GOS production

GOS = Galactose and Glucose units = non-digestible fibers and classified as prebiotics giving increased gut health to the consumer

Enzyme mechanism

Galactosyl-enzyme

Gal Glc Glc

Lactose

β1,4

Glucose + Galactose

Galactooligosaccharide (GOS)

High affinity towards nucleophilic acceptors ensure high GOS yield
Thermodynamics of GOS generation

Galactose and Glucose are the thermodynamically preferred products (entropy) → **Enzyme inactivation** is needed in the process.
Optimization of GOS generation

Kinetic reaction control is needed (Enzyme inactivation)

Enzyme is inactivated during pasteurization process

Without enzyme inactivation, GOS are degraded to Glc & Gal

1. **Reaction**: Lactose + Lactose \[ \rightarrow \text{GOS + Glucose} \]

2. **Reaction**: GOS + H₂O \[ \rightarrow \text{Galactose + Glucose} \]

Maximum concentration can only be achieved through kinetic reaction control – **inactivation of enzyme** -
Kinetic of GOS formation milk at 4 – 7 °C

EU source of fiber 3 g/100 g high in fiber 6 g/100 kcal
US excellent source of fiber 3 g/100 g high in fiber 6 g/100 kcal

EU source of fiber 1.5 g/100 kcal
US good source of fiber 1.5 g/100 kcal
GOS analyses – HPLC

- HPLC-Chromatogram: In-situ GOS generation in yogurt

- GOS are analysed employing an HPLC-RI system
  - The total GOS is calculated as the sum of degree of polymerisation (DP) 3-6
Application possibilities for In Situ production of GOS
### Plain Yogurt:

<table>
<thead>
<tr>
<th></th>
<th>Lactose and other sugars %</th>
<th>Sucrose%</th>
<th>Fibers%</th>
<th>total sugars%</th>
<th>sugar reduction%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain yogurt</td>
<td>4,6</td>
<td>0</td>
<td>0</td>
<td>4,6</td>
<td></td>
</tr>
<tr>
<td>Plain yogurt + β-gal</td>
<td>2,4</td>
<td>0</td>
<td>2,2</td>
<td>2,4</td>
<td>-47,8</td>
</tr>
</tbody>
</table>

### Yogurt + sucrose:

<table>
<thead>
<tr>
<th></th>
<th>Lactose and other sugars %</th>
<th>Sucrose%</th>
<th>Fibers%</th>
<th>total sugars%</th>
<th>sugar reduction%</th>
</tr>
</thead>
<tbody>
<tr>
<td>flavoured yogurt</td>
<td>4,6</td>
<td>4</td>
<td>0</td>
<td>8,6</td>
<td></td>
</tr>
<tr>
<td>flavoured yogurt + β-gal</td>
<td>2,4</td>
<td>4</td>
<td>2,2</td>
<td>6,4</td>
<td>-25,6</td>
</tr>
</tbody>
</table>
GOS stability in yogurt – 28 days

GOS stability over **28 days of shelf life** – pasteurization at 95°C for 10 min

- 7.8 (w/w) initial lactose
- > 3.5% (w/w) GOS
- Stable GOS after enzyme inactivation (95°C / 10 min)

- Pasteurization at 95°C for 10 min ensures stable GOS over shelf life (28 d)
GOS stability in stirred Yogurt – 8 weeks

GOS stability over **8 weeks** of shelf life

- 1% fat
- 14.7% sugar (7.8% lactose)
- 3.6% protein
- 2.8% Yogurt 6051

Pasteurization at 95°C for 10 min ensures stable GOS over shelf life
Yoghurt sensory analysis

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
Residual lactose upon ZymStar™ GOS application

Significant lactose reduction 92 – 98% upon ZymStar™ GOS application

Label the product as "lactose reduced"
### Calorie reduction samples

**Scenario I: Whole milk (3.5% fat)**

<table>
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<tr>
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<th>calories/ 100 mL [kcal]</th>
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<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>Plain milk (3,5% fat)</td>
<td>31,5</td>
</tr>
<tr>
<td>Plain milk (3,5% fat) + 1.5% Inulin</td>
<td>31,5</td>
</tr>
<tr>
<td>Plain milk (3,5% fat) + β-gal</td>
<td>31,5</td>
</tr>
</tbody>
</table>

**Scenario II: Skimmed milk (0% fat)**

<table>
<thead>
<tr>
<th></th>
<th>calories/ 100 mL [kcal]</th>
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<tbody>
<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>Plain milk (0% fat)</td>
<td>0</td>
</tr>
<tr>
<td>Plain milk (0% fat) + 1.5% Inulin</td>
<td>0</td>
</tr>
<tr>
<td>Plain milk (0% fat) + b-gal</td>
<td>0</td>
</tr>
</tbody>
</table>

- Calorie reduction compared to "a similar product" with fiber between **12.5 – 23.2%**
- 1 calorie = 4.184 Joules
Legislation & Consumer Segments
Current Legislation Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972

Definition: "Prebiotics" means food components (mainly Fructo-oligosaccharides mainly from chicory, onion, garlic, asparagus, Jerusalem artichoke and soya beans as well as galacto-oligosaccharides from whey and galactosylsucrose) that escape digestion by normal human digestive enzymes and reach the large intestine where they may create conditions that will promote the growth of indigenous, colonic bacteria and are considered to be beneficial;

No claim for probiotics or prebiotics may attribute any degree of a disease risk reduction to a specific foodstuff, or ingredient thereof.

Prebiotics are non-digestible food components which have a beneficial effect on the host health by selectively stimulating the growth and metabolic activities of one or a limited number of beneficial intestinal bacteria and thus improving the host’s intestinal balance.

At least 3 g prebiotic per daily serving. The amount and source of prebiotics shall be declared on the label.
Draft Legislation (R 429 for comment DOH): Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972

Definition: "prebiotics" or novel fibres mean edible carbohydrates, of which the degree of polymerization (DP) varies between two (2) to sixty four (64) monomeric units; which resist hydrolysis by mammalian enzymes that allow specific changes, both in the composition and/or activity in the indigenous human gastrointestinal microflora, which confer benefits upon host well-being and health, demonstrated by generally accepted scientific evidence to competent authorities;

Content claim: “…the food shall have at least 2 mg pure prebiotic per single serving…”

The prebiotic one or combination of the following: transgalactooligosaccharide; inulin; oligofructose; fructooligosaccharides (FOS); xylooligosaccharides (XOS); polydextrose; galactooligosaccharides (GOS).

Function Claim: “…Prebiotics such as [name of specific prebiotic] beneficially affects the intestinal flora by selectively stimulating the growth of the good/beneficial gut flora/micro-organisms / positively affects intestinal health; and An average of 6 g prebiotics is needed daily for general digestive health
### ZymStar™ GOS enzyme features and benefits

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In situ application</strong></td>
<td><strong>Enables multi claim with a single product</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Label friendly</strong></td>
</tr>
<tr>
<td></td>
<td>&quot;Natural&quot; connotation, natural perception of the consumer</td>
</tr>
<tr>
<td></td>
<td><strong>Milk origin</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Innovative image for dairy customer</strong></td>
</tr>
<tr>
<td>Lower lactose level by 90%</td>
<td><strong>Enables low/reduced lactose level claim</strong></td>
</tr>
<tr>
<td>Lower sugar/calorie level by converting lactose into fiber</td>
<td><strong>Directly enable dairy to claim “reduced sugars” in plain and fortified yoghurt/milk yoghurt/milk</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Potential for reduced calories</strong></td>
</tr>
<tr>
<td>Fiber enriching</td>
<td><strong>Enables “(good) source of fiber” claim</strong></td>
</tr>
<tr>
<td>Logistics</td>
<td><strong>Less kg to transport, less inventory space</strong></td>
</tr>
<tr>
<td>Direct purchase from enzyme producer</td>
<td><strong>Stable supply and price</strong></td>
</tr>
<tr>
<td>Technical service from application team</td>
<td><strong>Technical support on overall recipe</strong></td>
</tr>
</tbody>
</table>
**Functional Strengths**

- Possibility of multiple claims using one enzyme
- Fiber is made in situ by adding enzyme to the milk, creating stronger “natural” connotation
- Reduces total sugar level
- Changes carbohydrate composition and reduces calorie intake
- Reduces lactose as opposed to GOS available on the market which increases lactose content
- Creates GOS fiber, the main fiber used in infant formula that is widely researched to stimulate bifidobacteria*
- GOS was reported to increase mineral absorption** and induce the feeling of satiety***

**Technical Strengths**

- No impact on taste
- Stability in pH and heat ensures consistent product quality over shelf life

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*Bouhnik et al., (1997); Depeint et al., (2008); Ito et al., (1993); Shin et al., (2000)*  
**Weaver et al. (2011), Whisner et al. (2013)**  
***Overduin et al. (2012)***
In Summary:

**ZymStar™ GOS** properties are particularly suited to solve the needs of 4 consumer segments:

**Immediate opportunities:**
1. **Low sugar/calorie** products targeting consumers who seek to control their sugar intake
2. **Low-lactose & high fiber** combination claim products targeting health conscious consumers

**Investment in studies needed to demonstrate further possible claims**
3. GOS claim products targeting consumers who seek superior nutrition
4. Medical products targeting consumers that seek to prevent, control or improve health conditions
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