American Cheese Society 2012

REX C INFANGER

AUGUST 2012 CULTURE 201: ADJUNCT, PROTECTIVE, AND PROBIOTIC CULTURES - CHEESE ENHANCEMENT THROUGH MICROBIAL INTERVENTION

PROTECTIVE, ADJUNCT AND PROBIOTIC CULTURES
Today’s Challenges in the Food Industry

**The journey towards 2050**

### Consumer Trends

- **Food Safety**
  - Pathogen scare
  - Taste & freshness
  - Chemical scare

- **Health & Wellness**
  - Functional foods
  - Natural/organic
  - Low salt/sugar

- **Sustainability**
  - Product origin
  - Production methods
  - Recyclability & waste reduction

### Social and Political Trends

- **Regulatory**
  - Tighter regulations on health claims & microorganisms impact

- **Agricultural Changes**
  - New food pathogens
  - Animal husbandry

- **Demographics**
  - Children, Women
  - Elderly
  - Emerging countries

**Rise in natural, environmentally neutral technologies and increased attention on food waste**
Challenges

Foodborne illness (USA): 76 million sick, 325,000 hospitalized, 5,000 die per year.

Approximately 25% of the world’s food supply is discarded every year due to microbial spoilage.

Huge economic impact.

The food industry desires alternatives to chemical preservatives.

Challenge: improve microbial safety and quality of the food supply.
Figures about Dairy Waste

40 to 65% of dairy waste is generated by households in industrialized regions

Source: Global food losses and food waste - FAO 2011
Dairy Waste - Consumer Insight

*Half of the consumers declare they experienced spoilage in their dairy products at home*

Source: Danisco global survey 2011
Base: 426 respondents, informed consumers

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Increased Scrutiny of Food Borne Diseases

1 in 6 Americans (or 48 million people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases.

and

Emergence of new and more virulent strains: Salmonella heidelberg, E.coli O104:H4.
Dairy Waste - Consumer Insight

**Impact on repurchase rate**

<table>
<thead>
<tr>
<th>Product</th>
<th>Will repurchase</th>
<th>Will not repurchase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt drinks</td>
<td>63%</td>
<td>38%</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>72%</td>
<td>28%</td>
</tr>
<tr>
<td>Milk</td>
<td>77%</td>
<td>23%</td>
</tr>
<tr>
<td>Yogurt</td>
<td>79%</td>
<td>21%</td>
</tr>
<tr>
<td>Quark/fresh cheese</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>Spreads</td>
<td>83%</td>
<td>17%</td>
</tr>
<tr>
<td>Sour cream</td>
<td>88%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Source: Danisco survey 2011

© Danisco 2011
Food Protection - How do we Define It

- **FOOD PROTECTION**
  - **FOOD PRESERVATION**
    - Maintenance of organoleptic quality of food by preventing chemical (e.g., oxidation) and microbial spoilage
  - **FOOD SAFETY**
    - Minimizing of pathogen risks caused by bacterial infections throughout shelf life

- Reduction of food waste
- Protection of human health

**HOLDBAC® YM**
Food Preservation

Multiple hurdles to achieve spoilage control

- Increasing shelf life
- Sensory breakdown

Cell number

- Moisture
- pH
- Temp.
- Antimicrobials

Time

CFU: $10^6$

Inhibiting

Killing
Factors Influencing Growth

- Formula Ingredients
- Refrigeration Storage
- Modified ATM.
- Microbial Interaction
- Heat
- Moisture
- pH
- Hurdle Technology
Live Cultures: Mechanisms of Activity

Metabolite Production

Biomass Production

Fermentates

Active Cultures

Peptides (Bacteriocins)

Organic Acids

Other Molecules

Live Protective Cultures
Microbial Fermentation

These substances are naturally occurring antimicrobials which by themselves or in combination are highly effective against spoilage and pathogenic microorganisms.
Mode of Action: Organic Acids

Normal Cell State:
- $\Delta \text{pH}_{\text{in-out}} = \text{Molecular Transport}$
- $\Delta \text{H}^+_{\text{in-out}} = \text{Energy Generation}$

Organic Acid Addition:
1. Lipophilic Properties
   $\Rightarrow$ Cellular Membrane Penetration
2. Proton Delivery
   - No $\Delta \text{pH}_{\text{in-out}} = \text{Reduced Transport}$
   - No $\Delta \text{H}^+_{\text{in-out}} = \text{No Energy Generation}$
     $\Rightarrow$ Starvation

No Growth = BACTERIOSTATIC FUNCTIONALITY
## Mode of Action: Organic Acids

<table>
<thead>
<tr>
<th></th>
<th>Mol wt.</th>
<th>pKa</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetic acid</strong></td>
<td>60.1</td>
<td>4.76</td>
<td>35</td>
<td>5.2</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Propionic acid</strong></td>
<td>70.1</td>
<td>4.87</td>
<td>43</td>
<td>6.9</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Lactic acid</strong></td>
<td>90.1</td>
<td>2.74</td>
<td>0.5</td>
<td>0.06</td>
<td>0.01</td>
</tr>
</tbody>
</table>

✓ “Antimicrobial activity”: acetic ≥ propionic > lactic
Potential Advantages of Protective Cultures

Shelf life protection against normal yeast and mold spoilage

Protection against mold spoilage due to temperature abuse

Longer fermentation time may enhance protection

Reduced fat content may enhance protection – Sour Cream

Protection against different mold species at relatively high contamination levels

Little or no effect on flavor

No adverse effect on pH during storage

Protection against gas formation caused by heterofermentative lactobacilli – Fresh Cheese
Functions of Cultures

Acid Production
- Fast Acid Strains will coagulate milk in 18 hours at 69.8° F

Flavor
- Acid production of lactic oxaloacetic, acetic, pyruvic, and propionic
- Glycolitic pathway produces diacetyl
- Proteolytic pathway produces ammonia, acetaldehyde, ketones and esters
- Lipolytic pathway produces free fatty acids

Syneresis (Moisture Loss)

Protein Agglomeration (Curd Formation)

Gas Production (Eye Formation & Flavor)
Culture Growth Stages

- Lag Phase
- Growth Phase
- Logarithmic Phase
- Stationary Phase
- Death
Mesophilic Cultures (52-104 °F)

Lactococcus lactis ssp. Cremoris (Streptococcus)

Lactococcus lactis ssp. lactis

Lactococcus lactis ssp. lactis, diacetylactis (taxonomically not recognized)

Leuconostoc mesenteroides ssp. cremoris (homofermentative acid production)
Thermophilic Cultures (86-122° F)

- Streptococcus thermophilus
- Lactobacillus delbrueckii ssp. bulgaricus
- Lactobacillus helveticus
- Propionibacterium shemanii
What are protective cultures?

Definition

Protective cultures are:

- Bacterial species from food – GRAS status
- Specifically selected for ability to control unwanted microorganisms
- Selected for no adverse effect on sensory quality
- Used as viable (live) cultures
Increasing shelf life

Cell number

Moisture  pH  Temp.  Antimicrobials

Time

Static  Cidal
Food Preservation

Multiple parameters influence inhibition

- Fermentation Process/Culture
- Contamination level
- Storage conditions
- Type of spoilage organisms

+/- ANTIMICROBIAL
Protective cultures - why do they work?

- Competitive exclusion
- Organic acids
- Other natural metabolites

Protective cultures
HOLDBAC® Cultures for an All-Natural Positioning
The Perception from Consumers

- **ENRICHED Products**
  - Vitamins
  - Omega 3

- **CONTAINS**
  - Probiotics

- **NATURAL, HEALTHY Products**
  - No additives, preservatives
  - No artificial colours / sweeteners / flavours

- **ARTIFICIAL PRODUCTS**

- **STRIPPED Products**
  - Cholesterol free
  - Fat free
  - Allergy free

- **DOESN’T CONTAIN**
  - 'LESS HARM' Products

Source: Lindberg survey- Focus groups with 91 end-consumers – Nov 2010/Jan 2011

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HOLDBAC® Cultures
A natural way to protect dairy foods

- Patented live cultures
- Unique properties - control the growth of unwanted microorganisms throughout shelf life
- Isolated from food – GRAS organisms
- No additional labelling requirements in cultured products.

<table>
<thead>
<tr>
<th>Products</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOLDBAC® YM-B</td>
<td><em>Lb. rhamnosus</em></td>
</tr>
<tr>
<td>FRO &amp; LYO</td>
<td><em>P. freudenreichii subsp. shermanii</em></td>
</tr>
<tr>
<td>HOLDBAC® YM-C</td>
<td><em>Lb. paracasei</em></td>
</tr>
<tr>
<td>FRO &amp; LYO</td>
<td><em>P. freudenreichii subsp. shermanii</em></td>
</tr>
</tbody>
</table>
HOLDBAC® YM Scope of activity

CANNOT turn bad quality raw ingredients into good products

CANNOT replace Good Manufacturing Practice!

Natural
HOLDBAC® YM – do they actually work?

HOLDBAC™ YM dosage: 10 DCU / 100 liter milk
Reducing waste and returns
- extend shelf-life/minimize fluctuations

Yogurt challenge study - yeast

<table>
<thead>
<tr>
<th>Dosage</th>
<th>HOLDBAC™ YM-C added at 10 DCU / 100 l milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mould challenge</td>
<td>Pool of four yeast strains at 100 CFU / ml</td>
</tr>
<tr>
<td>Test conditions</td>
<td>Stored at 10°C for 65 days</td>
</tr>
</tbody>
</table>
Reducing waste and returns
- extend shelf-life/minimize fluctuations

Challenge study - mold

<table>
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<td>Stored at 10°C for 65 days</td>
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</tbody>
</table>
Maintain taste freshness - yogurt

Some viscosity increase can be obtained using HOLDBAC™ YM

<table>
<thead>
<tr>
<th>Dosage</th>
<th>HOLDBAC™ YM added at 10 and 50 DCU/100 l milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter used</td>
<td>YO-MIX™ 400 series</td>
</tr>
<tr>
<td>Test conditions</td>
<td>Evaluated by a trained panel after 45 d at 6°C</td>
</tr>
</tbody>
</table>
Effect of Holdbac Listeria on Munster naturally contaminated with listeria

![Graph showing the effect of Holdbac Listeria treatment on listeria contamination over time during maturation.](image)

**Note:**
- **ALC 01 treatment** on days 0, 4, and 7.
- Spraying liquid 10 cfu/ml.
- Maturation: 12°C - 15°C; 95% rel. humid.
- Washing (rind): 2x per week.

**Legend:**
- Reference without ALC 01
- With ALC 01

**Graph Details:**
- **Y-axis:** Measure for listeria contamination
- **X-axis:** Period of examination during maturation [d]
**Efficiency of the bacteriocin formed by Holdbac Listeria**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diameter Inhibitory Zone [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella infantis</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus rhamnosus</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus curvatus</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes V7</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes 4b</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes 3a</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes 1/2b</td>
<td></td>
</tr>
<tr>
<td>Listeria ivanovi</td>
<td></td>
</tr>
<tr>
<td>Listeria innocua 6a</td>
<td></td>
</tr>
</tbody>
</table>

Examination of the bacteriocin for inhibition of different microorganisms (diffusion test)
Effect of Holdbac LC on the yeast counts in Feta
Effect of Holdbac LC on yeast counts in Feta
## Molds

<table>
<thead>
<tr>
<th>Molds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium roqueforti</td>
</tr>
<tr>
<td>Penicillium candidum (album)</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
</tr>
<tr>
<td>Trichothercium domesticum (cylindrocardon sp) Mycodore</td>
</tr>
</tbody>
</table>
Yeast

Saccharomyces
debaryomyces
kluyvermyces (telomorph of candida)
candida
Mould ripening: a step wise technology

2 main steps

- pH increase up to 5.8 (D1 to 5)
  - driven by lactic acid consumption (e.g. by yeast)
  - slower inside
  - Can be influenced by dry salt (Geotrichum)
  - From this step, surface flora is critical for final product quality

- Then ripening (from day 5)
  - Change of internal pH
  - Visible growth of surface flora, lactose and lactate consumption
  - Lipolysis and fatty acids degradation – Protéolysis and amino acid degradation
CHOOZIT™ range for white moulded cheese

Yeast

*Penicillium camemberti*

*Geotrichum candidum*
Yeast functionalities

Consume residual sugars (lactose, galactose)

Consume lactate, thereby neutralising cheese surface which stimulate mould growth

Proteolysis: wide range of proteolytic activities (caseinolytic, aminopeptidasic, carboxypeptidasic…)

Contribute to lipolysis (limited)

Produce gas (potentially)

Produce flavour compounds

Interact with other microorganisms: stimulate *G. candidum growth*
### CHOOZIT™ Yeasts

<table>
<thead>
<tr>
<th>CHOOZIT™ product</th>
<th>Compo.</th>
<th>Pop. per dose</th>
<th>Assimilation</th>
<th>Fermentation /CO₂ production</th>
<th>Salt tolerance</th>
<th>Neutralising power</th>
<th>Proteolysis potential</th>
<th>Flavouring potential</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH</td>
<td>Debaryomyces hansenii</td>
<td>1D= 1.10¹⁰</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
<td>Typically used for microper sillage in soft cheese</td>
</tr>
<tr>
<td>CUM</td>
<td>Candida utilis</td>
<td>1D=5.10⁹</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>+</td>
<td>++</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>KL71</td>
<td>Kluyveromyces lactis</td>
<td>1D= 2.10⁹</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Assimilation**
- Glucose
- Lactose
- Gal

**Fermentation / CO₂ production**
- Glucose
- Lactose
- Lactate

**Salt tolerance**
- +
- ++
- +++

**Neutralising power**
- +
- ++
- +++

**Proteolysis potential**
- +
- +/-

**Flavouring potential**
- +
- +/-
*Penicillium candidum*: functionalities

Form the typical white mould cover on Brie or Camembert type cheese

Neutralisation: consume lactic acid >> pH increase

Proteolysis: complex and very active system
- Aspartyl protease ou protease acide: pH optimum 5.00 on casein
- Metalloproteases: pH optimum ph 6.00 (degradation of αS1 casein)
- Carboxypeptidases: pH optimum ph de 6.00 à 6.50
- Aminopeptidases: pH optimum entre 6.00 and 8.00 >> release free AA contribute to reduce bitterness

Lipolysis: contribute to typical white mould cheese flavour.

Flavour production: methyl ketones, secondary alcohols, esters, aldehydes, amines and ammonia

Interactions with other micro-organisms: very fast growth, competing with the growth of indesirable moulds.
*Penicillium candidum*: growth parameter

Temperature
- Optimum between 20 et 25° C,
- Used in between 8 et 14° C

pH
- Not very sensitive in the range pH 4.5 à 5.2

Halotolerance
- High salt resistance (dry salting or brine salting)

Nutritional need
- Sugar, lactates…
**CHOOZIT™ PC range: aspect**

**CHOZIT™ PC02**  
**CHOZIT™ PC12**  
**CHOZIT™ PC22**  
**CHOZIT™ PC HP6**  

**CHOZIT™ PC Neige**  
**CHOZIT™ PC SAM3**  
**CHOZIT™ PC VB**  
**CHOZIT™ PC VS**

*Pictures have been taken after inoculation (2µl of a solution containing 1.E+06 spores/ml) and incubation on Potatoe Dextrose Agar (PDA) for 5 days at 25° C*
## CHOOZIT™ PC: selection guide

<table>
<thead>
<tr>
<th>CHOOZIT™</th>
<th>Whiteness</th>
<th>Growth speed</th>
<th>Thickness</th>
<th>Proteolysis</th>
<th>Lipolysis</th>
<th>Other benefits</th>
<th>Target techno.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 02</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Solubilized techno. High HFD</td>
</tr>
<tr>
<td>PC 12</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC 22</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC VS</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td></td>
<td>Traditional lactic curd</td>
</tr>
<tr>
<td>PC HP6</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC NEIGE</td>
<td>++</td>
<td>+</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC VB</td>
<td>++</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC SAM 3</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>Anti-mucor</td>
<td></td>
</tr>
</tbody>
</table>

1 dose de PC = 2.10⁹ spores
**CHOOZIT™ PC range: controlling mycotoxin risk**

*Penicillium camemberti* species can produce only one mycotoxin: cyclopiazonic acid

Strain dependent feature

All our industrial strains of *P. camemberti* were tested

Not detectable or very low for all (far below toxicity level) in toxinogenic conditions

![cyclopiazonic acid concentration graph](image-url)
**Geotrichum candidum**: functionalities

Thin surface fine, white to cream colour

Influence rind look, texture and flavour

Neutralise the surface by consuming lactate, contributing to texture improvement and to stimulate acido-sensitive flora

Contribute to stabilise the rind

Contribute to protéolysis (much less than PC)

Contribute to release amino-acids and to reduce bitterness (less than PC)

Contribute to lipolysis (strain dependant feature)

Produce flavour compounds (methyl ketones, sulfury compounds...)

Photo INRA
**Geotrichum candidum** – **3 morphology** [Gueguen, 1984]

**Type 1**: yeast like
- Cream colour
  - T°C optimum: 22 to 25°C, used from 10 to 15°C
- Little mycelium, many arthrospores
  - Low proteolytic activity

**Type 2**: Intermediate

**Type 3**: Mould like
- White
  - T°C optimum: 25 to 30°C, used from 10 to 15°C
- Proteolytic

1. Yeast like
2. Intermediate
3. Moulds like
CHOOZIT™ Geo : Proteolysis

Light difference in between strains (slightly stronger for mould-like ones)

But in any case much lower than for PCs!

Quantité d’acides aminés libres en équivalent glycine (mg/g)

Transformation de l’alpha caséine S1 en alpha caséine S1-1 (%)
CHOOZIT™ Geo : Selection guide

Genre: *Geotrichum*  
Species: *G. candidum*

<table>
<thead>
<tr>
<th>CHOOZIT™</th>
<th>Format</th>
<th>Morphology</th>
<th>Growth speed</th>
<th>Flavour</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEO 13 LYO</td>
<td>Lyophilised</td>
<td>Intermediate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GEO 15 LYO</td>
<td>Lyophilised</td>
<td>Yeast like</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>GEO 17 LYO</td>
<td>Lyophilised</td>
<td>Mould like</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

* Freeze-dried formats are strongly advised for shipment outside Western Europe*
PC/GEO combination

Benefits to cheese quality:

Faster surface flora development,

Better protection against contaminants,

More regular & thinner rind,

Better stability from the rind during shelf-life,

Reduced bitterness and flavour defect (mushroom, ammonia...)

Epaisseur
# CHOOZIT for blue cheese – Selection guide

<table>
<thead>
<tr>
<th>Culture</th>
<th>PACKAGING SIZES</th>
<th>Phage alternative</th>
<th>ACIDIFICATION SPEED</th>
<th>FLAVOUR</th>
<th>GAS FORMATION</th>
<th>APPLICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEFINED HETEROFERMENTATIVE MESO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leuconostoc</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze dried</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOOZIT LM 57</td>
<td>20-50DCU</td>
<td>CHOOZIT</td>
<td>Medium</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>CHOOZIT LD106</td>
<td>50DCU</td>
<td>CHOOZIT</td>
<td>Medium</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>CHOOZIT LM 79</td>
<td>50DCU</td>
<td>CHOOZIT</td>
<td>Medium</td>
<td>++</td>
<td>+++</td>
<td>+</td>
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<tbody>
<tr>
<td><strong>BLUE MOULDS</strong></td>
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</tr>
<tr>
<td><strong>Penicillium roqueforti</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze dried</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. roqueforti CB2</td>
<td>LYO 5D</td>
<td></td>
<td>++</td>
<td>++</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>P. roqueforti PA</td>
<td>LYO 10D</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Blue grey</td>
<td>Light green</td>
</tr>
<tr>
<td>P. roqueforti PJ</td>
<td>LYO 10D</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Light green</td>
<td>Dark blue-green</td>
</tr>
<tr>
<td>P. roqueforti PV</td>
<td>LYO 10D</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

04/01/2012
CHOOZIT™ range for smear cheese ripening

Yeast:
- Consume residual sugars,
- Neutralise (bring pH up), enabling the growth of acid sensitive flora
- Contribute to proteolysis, lipolysis and to flavour development (depending on species)
- Contribute to smear look

Brevibacterium:
- Mesophilic, optimal temperature: 25° C à 30° C – minimal temp.: 10-12° C
- Acid-sensitive: minimum pH = 6.00 or 5.80 for CHOOZIT™ latest generation
- Salt-tolerant: till 12% NaCl for some strains
- Strong production of sulfury flavour compounds (feet, garlic, egg smell)

Other bacteria and blends: positive interaction with Brevibacterium

Potential use in combination with Geo or PC
# CHOOZIT™ Brevibacterium

<table>
<thead>
<tr>
<th>Culture</th>
<th>Composition</th>
<th>Formats</th>
<th>1 Dose = 5.10^{10} cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOOZIT™ FR13</td>
<td>B. linens</td>
<td>Lyo 10-50-100D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ FR22</td>
<td>B. linens</td>
<td>Lyo 2-10-50D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ SR3</td>
<td>B. linens</td>
<td>Lyo 2-10-100D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ LR</td>
<td>B. linens</td>
<td>Lyo 10-100D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ LB</td>
<td>B. linens</td>
<td>Lyo 10-100-300D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ Linens W</td>
<td>B. linens</td>
<td>Fro 500g</td>
<td></td>
</tr>
</tbody>
</table>

# CHOOZIT™ Other bacteria or blends

<table>
<thead>
<tr>
<th>Culture</th>
<th>Composition</th>
<th>Format</th>
<th>1 Dose = 1.10^{11} cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOOZIT™ MVA</td>
<td><em>Staphylococcus xylosus</em></td>
<td>Lyo 2-10-100D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ MVS</td>
<td><em>Staphylococcus carnosus</em></td>
<td>Lyo 10D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ MGE</td>
<td><em>Arthrobacter nicotiniae</em></td>
<td>Lyo 10-100D</td>
<td>5.10^{10} cfu</td>
</tr>
<tr>
<td>CHOOZIT™ PLA</td>
<td>A. nicotianiae, B. linens, D. hansenii, G. candidum</td>
<td>Lyo 2-10D</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ OFR9</td>
<td>B. linens, D. hansenii, G. candidum, C. utilis, B. casei</td>
<td>Liquide</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ ARO 21 – HA</td>
<td>P. membranaefaciens, K.lactis, L. paracasei, S. carnosus, B. linens</td>
<td>Lyo 10D</td>
<td></td>
</tr>
</tbody>
</table>
CHOOZIT™ Brevibacterium – Growth & colour features

Growth of CHOOZIT™ FR13 et FR22 (latest generation) on thermophilic matrix (buffered, low pH), with same amount of yeast
Mesure de demethiolase and aminopeptidase activities: activities are expressed as total activity per biomass used in the assay (measured DO 410 nm / DO650 nm of culture at 1/10th dilution).
CHOOZIT™ Brevibacterium
– Flavour features: production of sulfury compounds (MM & DMDS) on milk+methionine
CHOOZIT™ Brevibacterium – Flavour features: non sulfury

Production de volatils par brevibacterium après croissance sur lait UHT 1/2 écrémé - 48h

Surface des pics en µV.s

- Ethanol
- 2-propanol
- Propanol
- 2-butanol
- Isobutanol
- Butan-1-ol
- 3 methyl butanol
- 2 methyl butanol
- 1 hexanol
- Acetaldehyde
- Acetone
- 2 methyl butanal
- Isoval
- Methanethiol
- DMDS
- Diacetyl
- Acetoïne
- Ethyl acetate
### CHOOZIT™ Selection guide – Smear ripened cheese

<table>
<thead>
<tr>
<th>Ferment CHOOZIT™</th>
<th>Colour</th>
<th>Sulfury flavour</th>
<th>Growth speed</th>
<th>Acid-tolerance</th>
<th>Other benefits</th>
<th>Target techno.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOOZIT™ FR13</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>5.8</td>
<td>No colour déviation during ripening</td>
<td>All technologies, esp. solubilized</td>
</tr>
<tr>
<td>CHOOZIT™ FR22</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ SR3</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>6</td>
<td></td>
<td>Traditional smear ripened lactic curd</td>
</tr>
<tr>
<td>CHOOZIT™ LR</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ LB</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ MVA</td>
<td>-</td>
<td>+++</td>
<td></td>
<td></td>
<td>Competitive exclusion – Improve surface appearance</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ MVS</td>
<td>-</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ MGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Boost brevi – act. Aminopep.</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ OFR9</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>Blend</td>
<td></td>
</tr>
<tr>
<td>CHOOZIT™ PLA</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>Blend</td>
<td></td>
</tr>
</tbody>
</table>
Probiotics represent one of the fastest growing sectors within the global functional food market

- In 2011, estimated total finished product sales was $25 billion USD.
- A strong growth rate is expected to raise this size to $32 billion by 2015.

Source: Euromonitor 2011
North America, Latin American, and Asia Pacific have strong growth forecasted

- These regions’ market sizes will significantly increase in the coming years.
- Western Europe & Japan are the most developed markets for probiotics, and growth is slower than the average.

Source: Euromonitor 2011
## Cheese examples

<table>
<thead>
<tr>
<th>Dean Foods</th>
<th>Colun</th>
<th>Paranhos</th>
<th>Interdeli</th>
<th>Agropur</th>
<th>Altiments Ultima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Said to inhibit harmful bacteria as well as promote improved</td>
<td>Cheese with Probiotics</td>
<td>Contains lactobacilli that help regulate the digestive system</td>
<td>Contains probiotics</td>
<td>Contains probiotics that contribute to a healthy digestive system</td>
<td>Contains more than 1 billion probiotics per serving to contribute to healthy digestion</td>
</tr>
</tbody>
</table>

Source: Mintel GNPD 2011

1/17/2012
Cheese: a potential “functional food”?  

<table>
<thead>
<tr>
<th>Research findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production of Crescenza Cheese by Incorporation of Bifidobacteria</strong>&lt;br&gt;M. Gobbetti 1, A. Corsetti 1, E. Smacchi 1, A. Zocchetti 1, and M. De Angelis 1**&lt;br&gt;1 Institute of Dairy Microbiology, Agriculture Faculty of Perugia, University of Perugia, Italy</td>
<td>When added individually, the cell counts of <em>B. bifidum</em>, <em>B. longum</em>, and <em>B. infantis</em> were $10^{8.05}$, $10^{7.12}$, and $10^{5.23}$ cfu/g, respectively at the end of the ripening (14 days).</td>
</tr>
<tr>
<td><strong>Viability of Probiotic Argentinian Fresco Cheese</strong>&lt;br&gt;(Vinderola et al.2000, Universidad Nacional del Litoral, Santa Fe, Argentina)</td>
<td>Different combinations of <em>B. bifidum</em> <em>B. longum</em>, <em>L. acidophilus</em>, and <em>L. casei</em> were evaluated as probiotic adjuncts.&lt;br&gt;Counts decreased &lt;1 log order for bifidobacteria but no decrease was detected for <em>L. casei</em>.&lt;br&gt;<em>B. bifidum</em> was the most resistant organism.</td>
</tr>
<tr>
<td><strong>Probiotic white cheese with Lactobacillus acidophilus</strong>&lt;br&gt;Kaslmoglu, A; Goencueoglu, M; Akguen, S&lt;br&gt;International Dairy Journal [Int. Dairy J.]. Vol. 14, no. 12, pp. 1067-1073. Dec 2004.</td>
<td>Survival of <em>L. acidophilus</em> during ripening of the cheese stored in vacuum or in brine was studied. On ripening in vacuum pack, <em>L. acidophilus</em> survived to numbers $&gt;10^7$ cfu/g.</td>
</tr>
<tr>
<td><strong>Incorporation and survival of Bifidobacterium sp. strain Bo and Lactobacillus acidophilus strain Ki in a cheese product</strong>&lt;br&gt;(Gomes et al. Univ. católica Portuguesa, escola superior biotecnologia, 4200 Porto, PORTUGAL)</td>
<td>During the whole storage period studied, the average numbers of <em>L. acidophilus</em> strain Ki decreased by two log cycles to $0.2 \times 10^7$ cfu/g, whereas those of <em>Bifidobacterium</em> sp. strain Bo decreased by less than one log cycle to $6-18 \times 10^8$ cfu/g. Incorporation of <em>Bifidobacterium</em> spp. and <em>L. acidophilus</em> as starters in a Gouda-type cheese is feasible and can offer an alternative and interesting route of administering them to human beings.</td>
</tr>
</tbody>
</table>
### 18 Publications available showing addition of probiotic to cheese

<table>
<thead>
<tr>
<th>Cheese Type</th>
<th>Probiotic bacteria</th>
<th>Viability (log10 CFU/g)</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emmental ripened cheese</td>
<td>L. acidophilus</td>
<td>5.9 after 90 days</td>
<td>Maintained viability. L. acidophilus can be used to give good flavour, texture, and a high level of proteolysis.</td>
<td>21</td>
</tr>
<tr>
<td>Cheddar</td>
<td>Bifidobacterium and Lactobacillus</td>
<td>7.5 after 24 weeks</td>
<td>Significant effects depending on the type of probiotic used, ripening time, ripening temperatures and their interactions on the concentration of lactic and acetic acids in the cheeses.</td>
<td>22</td>
</tr>
<tr>
<td>Cheddar</td>
<td>L. acidophilus, L. casei, L. paracasei and Bifidobacterium sp.</td>
<td>&gt;7.5 after 24 weeks</td>
<td>All probiotic adjuncts survived the manufacturing process and maintained their viability.</td>
<td>23</td>
</tr>
<tr>
<td>Cheddar</td>
<td>B. bifidum</td>
<td>&gt;7.1 after 24 weeks</td>
<td>No adverse effect on flavour, texture or appearance</td>
<td>24</td>
</tr>
<tr>
<td>Cheddar</td>
<td>B. animalis Bb-12</td>
<td>&gt;8 after 9 months</td>
<td>Partial negative effect, moisture level = 40 percent, which is slightly above the legal limit permitted for Cheddar cheese.</td>
<td>25</td>
</tr>
<tr>
<td>Cheddar</td>
<td>B. longum BB536</td>
<td>5 after 9 months</td>
<td>No adverse effect on the cheese composition.</td>
<td>26</td>
</tr>
<tr>
<td>Cheddar</td>
<td>E. faecium PR88</td>
<td>8.6 after 15 months</td>
<td>Cheddar cheese compares very favourably with fresh yogurt as a delivery system for viable probiotic microorganisms to the gastrointestinal tract.</td>
<td>12</td>
</tr>
<tr>
<td>Cheddar</td>
<td>L. acidophilus, Bifidobacterium sp., L. casei, L. paracasei and L. rhamnosus</td>
<td>≥7.6 after 32 weeks for all except L. acidophilus (3.7)</td>
<td>Cheddar cheese is a good vehicle for a variety of commercial probiotics but survival of L. acidophilus strains will need to be improved.</td>
<td>5</td>
</tr>
<tr>
<td>Cheddar-like</td>
<td>B. infantis</td>
<td>6.4 after 84 days</td>
<td>Excellent viability, metabolically active</td>
<td>26</td>
</tr>
<tr>
<td>Cottage</td>
<td>B. infantis</td>
<td>&gt;6.5 after 22 days</td>
<td>Loss of viability, metabolically active</td>
<td>27</td>
</tr>
<tr>
<td>Crescenza</td>
<td>B. infantis, B. longum and B. bifidum</td>
<td>8.05, 7.12 and 5.23 after 15 days</td>
<td>Lower viability after 14 days</td>
<td>26</td>
</tr>
<tr>
<td>Fresco</td>
<td>Bifidobacterium, L. acidophilus and L. rhamnosus</td>
<td>&lt;1 log order in 60 days</td>
<td>Cheese found to be an adequate carrier of probiotic bacteria</td>
<td>9</td>
</tr>
<tr>
<td>Minas</td>
<td>L. acidophilus, A4</td>
<td>≥6.23 after 28 days</td>
<td>Lactic acidolus remained viable during the shelf life of the product and no negative effect was detected</td>
<td>29</td>
</tr>
<tr>
<td>Gouda</td>
<td>B. lactis Bo &amp; Lb. acidophilus K1</td>
<td>6.5 after 70 days</td>
<td>Effect on cheese flavour after 9 weeks ripening</td>
<td>30</td>
</tr>
<tr>
<td>Gouda</td>
<td>L. acidophilus, L. rhamnosus</td>
<td>8.5 after in vitro simulation</td>
<td>Probiotics maintained their viability both in cheeses and during passage through the upper gastrointestinal tract.</td>
<td>10</td>
</tr>
<tr>
<td>Gouda</td>
<td>L. acidophilus, L. rhamnosus</td>
<td>&gt;4 after 4-week intervention</td>
<td>Probiotics survived the gastrointestinal transit, and enhanced immune responses was detected</td>
<td>14</td>
</tr>
<tr>
<td>Semi-hard</td>
<td>L. acidophilus and L. paracasei</td>
<td>&gt;7.78 after 60 days</td>
<td>Increase in the production of short peptides and free amino acids, and modification of peptide profiles</td>
<td>31</td>
</tr>
<tr>
<td>Semi-soft</td>
<td>L. fermentum</td>
<td>7.7 after 54 days</td>
<td>Probiotic strain survived the technological processing of cheese, retaining moderate antimicrobial and high anticoagulant activity throughout ripening and storage</td>
<td>32</td>
</tr>
</tbody>
</table>

1. L. Lactobacillus, B. Bifidobacterium and E. Emmerococcus

**Table 1.** Examples of viability of probiotics in different types of cheeses (modified after 7).
Consumption of Probiotic Gouda cheese significantly enhanced immune health markers in elderly

Consumption of 15 g probiotic Gouda cheese containing $1 \times 10^9$ CFU *L. rhamnosus* HN001 and $1 \times 10^7$ CFU *L. acidophilus* NCFM in an elderly population (mean age 85 years).

1/17/2012
Probiotic Cheese enhance GI microflora vs baseline and control cheese

Measured quantities of microbes in the simulator vessel fluids (modified after 10). Values are sums of microbes indicated in the 4 vessels of the simulator combined (mean copy number of the target gene ± SE).

**Significant difference from baseline p < 0.01**
Objectives:
- Evaluation of probiotic strains’ survival in gouda technology

Inoculation level used in the preliminary work
- Acidification blend:
  - CHOOZIT™ 712: 1 DCU/100l
- Probiotic strains:
  - $5 \times 10^6$ CFU/ml of milk
  - 5 DCU / 100 litres
## GOUDA cheese physico chemical comparisons

<table>
<thead>
<tr>
<th>Blend</th>
<th>Vat 1 HOWARU™ Bifido LYO</th>
<th>Vat 2 HOWARU™ dophilus LYO</th>
<th>Vat 3 L. paracasei Lpc 37 LYO</th>
<th>Vat 4 HOWARU™ Rhamnosus FRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH before Lactose removal</td>
<td>6.55</td>
<td>6.55</td>
<td>6.55</td>
<td>6.55</td>
</tr>
<tr>
<td>pH moulding</td>
<td>6.47</td>
<td>6.48</td>
<td>6.48</td>
<td>6.49</td>
</tr>
<tr>
<td>Time to reach pH 5.50</td>
<td>4h30</td>
<td>4h30</td>
<td>4h30</td>
<td>4h30</td>
</tr>
<tr>
<td>Dry mater</td>
<td>54 %</td>
<td>54.5 %</td>
<td>53.7 %</td>
<td>53.1 %</td>
</tr>
<tr>
<td>Fat</td>
<td>24.6 %</td>
<td>25 %</td>
<td>25 %</td>
<td>24 %</td>
</tr>
<tr>
<td>Fat on dry mater</td>
<td>45.6 %</td>
<td>45.8 %</td>
<td>46.5 %</td>
<td>45.2 %</td>
</tr>
<tr>
<td>pH</td>
<td>5.47</td>
<td>5.48</td>
<td>5.41</td>
<td>5.41</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.74 g/100g</td>
<td>1.75 g/100g</td>
<td>1.80 g/100g</td>
<td>2.17 g/100g</td>
</tr>
<tr>
<td>Ca/ESD</td>
<td>3.08 %</td>
<td>3.01 %</td>
<td>3.07 %</td>
<td>3.16 %</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
</tr>
<tr>
<td>Galactose</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
<td>&lt; 0.2g/100g</td>
</tr>
</tbody>
</table>

*The cheeses have very closed composition, and we don’t observe a modification of the acidification kinetic during the process.*
Gouda example – Danisco internal study
Inoculation level : 5 x10⁶/ ml of milk

Cheeses made on microfiltered milk powder in controlled bacteriological conditions

- High reproducibility
- Very good survival until 6 months

<table>
<thead>
<tr>
<th>DANISCO probiotic strain</th>
<th>Vat 1</th>
<th>Vat 2</th>
<th>Vat 3</th>
<th>Vat 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cfu / ml of milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOWARUTM Bifido</td>
<td>5,00E+06</td>
<td>5,00E+06</td>
<td>5,00E+06</td>
<td>5,00E+06</td>
</tr>
<tr>
<td>HOWARU Dophilus®</td>
<td>8,00E+06</td>
<td>4,00E+07</td>
<td>3,00E+08</td>
<td>1,30E+08</td>
</tr>
<tr>
<td>L. paracasei Lpc 37</td>
<td>1,40E+07</td>
<td>1,60E+08</td>
<td>5,00E+08</td>
<td>3,00E+08</td>
</tr>
<tr>
<td>HOWARU Rhamnosus</td>
<td>7,00E+06</td>
<td>1,30E+08</td>
<td>5,00E+08</td>
<td>3,00E+08</td>
</tr>
<tr>
<td>Cfu / g of cheese D+7</td>
<td>6,00E+08</td>
<td>2,00E+08</td>
<td>7,00E+07</td>
<td></td>
</tr>
<tr>
<td>Cfu / g of cheese D+50</td>
<td>1,00E+08</td>
<td>6,00E+08</td>
<td>2,00E+08</td>
<td>7,00E+07</td>
</tr>
</tbody>
</table>
What do we lose in the whey? HOWARU™ Dophilus

The quasi total L.acidophilus stay in the curd during the gouda technology.

Laloy and Vuillemand et al. showed that the fat content of cheese milk directly influenced the number of starter cells retained in the curd (Int.Dairy Journal 6 (1996) 729-740.)

<table>
<thead>
<tr>
<th>Population per gram or ml</th>
<th>Curd</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheying off</td>
<td>7.7 $10^6$</td>
<td>2.4 $10^5$</td>
</tr>
<tr>
<td>Molding</td>
<td>1.9 $10^7$</td>
<td>1.1 $10^5$</td>
</tr>
<tr>
<td>Salt brine</td>
<td>2.6 $10^7$</td>
<td>/</td>
</tr>
<tr>
<td>After salt brine</td>
<td>2.6 $10^7$</td>
<td>/</td>
</tr>
</tbody>
</table>
Probiotics in cheese CHEDDAR

Objectives:
- Evaluation of probiotic strains’ survival in cheddar technology

Inoculation level used in the preliminary work
- Acidification blend:
  - CHOÖZIT™ RA021: 5 DCU/100l
- Probiotic strains:
  - 1 DCU/100 litres of milk
Cheddar example – A commercial Example

- Semi industrial cheddar cheese trials
- Inoculation rate: $8 \times 10^5$ CFU/ml of milk
- High reproducibility (3 vats done per trial)
- Very good survival until 5 months

<table>
<thead>
<tr>
<th>Culture used</th>
<th>Lb count*</th>
<th>Lb count</th>
<th>Lb count</th>
<th>Lb count</th>
<th>Lb count</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. rhamnosus</td>
<td>3,40E+08</td>
<td>3,40E+08</td>
<td>7,70E+08</td>
<td>2,70E+08</td>
<td>8,00E+07</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>3,00E+04</td>
<td>5,00E+05</td>
<td>7,60E+06</td>
<td>8,60E+07</td>
<td>1,20E+08</td>
</tr>
</tbody>
</table>

* CFU/gram of cheese
Probiotics in cheese

Objectives:
- Evaluation of probiotic strains’ survival in caciotta technology

Inoculation level used in the preliminary work
- Acidification blend:
- CHOOZIT™ TA061: 5 DCU/100l
- Probiotic strains:
  - $5 \times 10^6$ CFU/ml of milk
Cheese physico chemical comparisons

Cheeses results:

<table>
<thead>
<tr>
<th>Blend</th>
<th>Commercial control CHEESE</th>
<th>HOWARU™ Dophilus LYO</th>
<th>L. paracasei Lpc 37 LYO</th>
<th>HOWARU™ Rhamnosus FRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH moulding</td>
<td>6.40</td>
<td>6.40</td>
<td>6.40</td>
<td>6.40</td>
</tr>
<tr>
<td>Time to reach pH 5.10</td>
<td>4h40</td>
<td>4h40</td>
<td>4h40</td>
<td>4h40</td>
</tr>
<tr>
<td>Dry mater</td>
<td>60.7%</td>
<td>58.8 %</td>
<td>58.2 %</td>
<td>57.2 %</td>
</tr>
<tr>
<td>Fat</td>
<td>31.4%</td>
<td>31.9 %</td>
<td>31.4 %</td>
<td>30.4 %</td>
</tr>
<tr>
<td>Fat on dry mater</td>
<td>51.7%</td>
<td>54.3 %</td>
<td>53.9 %</td>
<td>53.1 %</td>
</tr>
<tr>
<td>pH</td>
<td>5.33</td>
<td>5.33</td>
<td>5.30</td>
<td>5.30</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.40g/100g</td>
<td>1.06 g/100g</td>
<td>0.85 g/100g</td>
<td>1.08 g/100g</td>
</tr>
<tr>
<td>Ca</td>
<td>862mg/100g</td>
<td>837mg/100g</td>
<td>807mg/100g</td>
<td>838mg/100g</td>
</tr>
</tbody>
</table>

- Analysis of the cheese models have been done at 15 days
- The composition of our cheese model is close to the industrial one
- Good reproducibility between the lab vats
- Salt is slightly lower compared to the control
Survival in cheese after 90 days

### Survival in Caciotta at 12°C

<table>
<thead>
<tr>
<th>Blend</th>
<th>HOWARU® Dophilus LYO</th>
<th>L. paracasei Lpc 37 LYO</th>
<th>HOWARU® Rhamnosus FRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cfu / ml of milk</td>
<td>5.10^6</td>
<td>5.10^6</td>
<td>5.10^6</td>
</tr>
<tr>
<td>Cfu / g of cheese D+1</td>
<td>3.10'</td>
<td>2.6.10'</td>
<td>3.10'</td>
</tr>
<tr>
<td>Cfu / g of cheese D+20</td>
<td>1.10^9</td>
<td>1.5.10^9</td>
<td>1.10^9</td>
</tr>
<tr>
<td>Cfu / g of cheese D+30</td>
<td>1.3.10^9</td>
<td>1.8.10^9</td>
<td>5.10^8</td>
</tr>
<tr>
<td>Cfu / g of cheese D+60</td>
<td>1.10^9</td>
<td>2.10^9</td>
<td>8.10^8</td>
</tr>
<tr>
<td>Cfu / g of cheese D+90</td>
<td>8.10^8</td>
<td>1.2.10^9</td>
<td>6.6.10^8</td>
</tr>
</tbody>
</table>
How does it influence the peptides profiles?

Peptides Profiles:

Commercials samples have more hydrophobic peptides and less hydrophilic peptides. They are probably more bitter than probiotics trials which have a lot of hydrophilic peptides coming from an advanced proteolysis.
Conclusions

Very good survival of the probiotic strains all the cheese examples during ripening. Using selected Danisco HOWARU™ probiotics together with cheese application expertise allows you to get $10^8$ to $10^9$ live probiotics per gram after 5/6 months shelf-life at 12°C, therefore a minimum of $10^9$ live probiotics per serving of 10 g of cheese. The three lactobacillus grow to $1.10^8$ to $10^9$ cfu/g and stay at this level until 6 months in the gouda technology.

Cheddar and gouda are excellent carrier of the Danisco health promoting bacteria

Caciotta is a good vehicle for those probiotics strains thanks to the favourable composition and the low constraints

The taste profile was not affected but more investigations need to be done in order to control the exact flavour however we have observed protection against bitterness.

CFU: Colony forming unit
CONCLUSION / How much to consume?

Using selected DuPont HOWARU™ probiotics together with cheese application expertise allows you to get $10^8$ to $10^9$ live probiotics per gram after 5/6 months shelf-life at 12° C, therefore a minimum of $10^9$ live probiotics per serving of 10 g of cheese.

CFU: Colony forming unit
Mode of Action: Peptide (Bacteriocins)

Cell Wall Binding

Cellular Membrane Destabilization

Molecular Leakage

Cellular Lysis = BACTERICIDAL FUNCTIONALITY

No Growth = BACTERIOSTATIC FUNCTIONALITY
Mesophilic Cultures (52-104 °F)

*Lactococcus lactis ssp. Cremoris* (Streptococcus)

*Lactococcus lactis ssp. Lactis*
Thermophilic Cultures (86-122°F)

*Streptococcus thermophilus*
Nisin

1953: First commercial nisin preparation

1969: joint FAO/WHO expert committee recognised nisin as a safe and legal food additive

Authorised as a food additive in > 50 countries world-wide.

GRAS status

- Producer strain regarded as safe (food-grade)
- Non toxic
- No cross-resistance related to therapeutic antibiotics
- Degraded during digestion
- Heat stable at low pH
Nisin: Mechanism of Activity

Vegetative cells

- Adsorbs to cytoplasmic membrane, forms transient pores
- Low molecular weight compounds leak from cell causing loss of energy
- pH gradient across the membrane dissipated
- Collapse of proton motive force

Heat-resistant endospores

- Spores affected after germination, preventing outgrowth
- Does not normally kill bacterial spores
## Antimicrobial – Nisaplin

<table>
<thead>
<tr>
<th>Product</th>
<th>Comp.</th>
<th>Key Properties</th>
<th>Applications (US)</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisaplin®</td>
<td>nisin preparation</td>
<td>Against Gram + bacteria</td>
<td>Pasteurized Cheese Spreads</td>
<td>10,000 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liquid Egg Products</td>
<td>600 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sauces (+/- meat or poultry)</td>
<td>600 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-standard salad dressings, dips, spreads</td>
<td>600 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chilled Soups</td>
<td>400 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chilled Soups with Meat</td>
<td>400 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RTE Processed Meats</td>
<td>400 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Natural, Imitation, Cream Cheese</td>
<td>350 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RTE Vegetarian Sides</td>
<td>300 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Select “Fruit” Juices</td>
<td>50 ppm</td>
</tr>
</tbody>
</table>

Canada - Not Allowed

Mexico – Limited (Ripened and processed cheeses)
Natamycin

Natural antifungal

Produced by *Streptomyces natalensis* (soil bacteria).

Used commercially as a food preservative since 1970s, eliminating or reducing yeast and mold and preventing mycotoxin formation.

Very powerful; no development of resistant strains.

DuPont’s brand: Natamax®
Structure of Natamycin

Empirical formula $\text{C}_{33}\text{H}_{47}\text{NO}_{13}$. Molecular weight: 665.7

Amphoteric. Isoelectric point 6.5.

Neutral aqueous suspensions relatively heat stable (50 - 100° C).

Stability affected by exposure to UV light, extreme pH values, peroxides, oxidants, chlorine and heavy metals.

Low water solubility: approx. 30 - 50 ppm natamycin.
Heat and pH Stability of Natamycin

Dry powder: Very stable - over 1 year at 20 °C

Heat stability of aqueous suspensions

- Ambient temperature - stable
- Optimum heat stability at pH 5 - 9
- Heat-cool cycle (72 and 10 °C) gave no loss in activity
  over 10 cycles, even in brine (20% NaCl)

pH stability

- Optimum range: pH 5 - 7
- Active over range pH 3 - pH 9,
  but stability may be compromised at low pH
Natamycin: Dairy Applications

Typically applied to cheese surfaces. Effective levels 7-10 ppm (Max. 20 ppm) or 2-4µg/cm². Applied as aqueous suspension spray or dip.

Effective antimycotic in fresh cultured dairy (yogurt, sour cream, cottage cheese, cream cheese @ 5-7ppm). Applied directly to formulation.
# Natamycin Versus Sorbate

<table>
<thead>
<tr>
<th>Natamycin</th>
<th>Sorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Chemical</td>
</tr>
<tr>
<td>Fungicidal</td>
<td>Fungistatic</td>
</tr>
<tr>
<td>No effect on bacteria</td>
<td>Bactericidal</td>
</tr>
<tr>
<td>No migration into food</td>
<td>Penetrates into food</td>
</tr>
<tr>
<td>No flavor</td>
<td>Bitter flavor</td>
</tr>
<tr>
<td>Effective at 1-20 ppm</td>
<td>Effective at 1000 – 2000 ppm</td>
</tr>
<tr>
<td>Effective at pH 3 - 9</td>
<td>Effective only at acidic pH</td>
</tr>
</tbody>
</table>
Natamycin Against Yeast in Yogurt

Natamax™ achieves total inhibition of yeast (*S. cerevisiae*) growth at 8°C.
Natamycin Inclusion in Yogurt Formulation

![Graph showing the effect of natamycin on the growth of Rhodotorula mucilaginosa CBS8161 in live yogurt at 8°C. Minimum detection 1.0 x 10^2 CFU/g.](image)

*Figure 3. The effect of natamycin on the growth of *Rhodotorula mucilaginosa* CBS8161 in live yogurt at 8°C. Minimum detection 1.0 x 10^2 CFU/g.*
Thank you for your attention

Any questions?

In May 2011, Danisco was acquired by DuPont
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Adjunct Cultures

Lactobacillus plantarum
Lactobacillus casei
Lactobacillus rhamnosus
Lactobacillus para-casei
Brevibacterium linens (corynebacteria)
Micrococci
Ripening what does it mean? for cheese …

Ripening general definition:

Giving an appearance, a texture and a flavour at an acid curd

Two key technological steps

- Curd formation: Acidification
- Ripening: pH neutralisation
  Enzymatic actions
  Flavour surface and aroma formation

The physico-chemical curd consistency is essential for the ripening control

Ripening don’t allow to correct a technological or an acidifying problem
Ripening what does it mean? for cultures …

- A wide diversity of micro-organisms can play a role:
  - Lactic acid bacteria, Propionibacteria
  - Yeast
  - Moulds (white and blue moulds)
  - Brevibacteria and other red smear bacteria

- A wide range of functionalities
  - Proteolysis, Peptidolysis, Lypolysis
  - Catabolism of sugars, amino acids and fatty acids into flavour
  - Gas production
  - Surface covering and colouring

- Micro-organism can act by growing mainly in the cheese curd or mainly on the surface
Ripening: a balancing act

Cultures provide a wide range of functionalities
No micro-organism, no starter cultures has all functionalities
Ripening: a balancing act
Combination offer a wider functionality range

- Acidification
- Color modification
- Nutrition
- Reduction capability
- Surface covering
- Flavour
- Texture
- CO₂ production
- Micro-organisms selection
CHOZIT™ Adjunct Cultures for hard and semi-hard cheese

- Lactic acid bacteria with a strong enzymatic and flavour forming potential:
  - *Lactobacillus helveticus*
  - *Lactobacillus delbrueckii lactis*
  - *Lactobacillus casei*
  - *Lactobacillus paracasei*
  - *Lactobacillus rhamnosus*

- Gas forming cultures:
  - *Leuconostoc*
  - *Propionibacterium*
CHOOZIT™ TR160: the sweetest choice

Sensory profile of 9 months old Cheddar cheese added with CHOOZIT™ TR160, as evaluated by a trained panel.

Free proline levels in cheese at 3 months

ANOVA significant difference with p<0.05.
CHOOZIT™ Flav 54: Lift up your cheese flavour

Comparative chromatogram of large and potentially bitter peptides extracted from Cheddar cheese at 8 weeks of ripening clearly showing reduced peptide levels in the cheese with CHOOZIT™ FLAV 54 added.

Sensory profile of 9 months old Cheddar cheese added with CHOOZIT™ FLAV 54, as evaluated by a trained panel.
CHOOZIT™ Eyes
Fast and robust culture for great tasting cheese

Sensory profiles of semi-hard cheese (ripened 7 days at 12-14°C +11 days at 21°C) produced with CHOZIT™ Eyes 1 FRO and CHOZIT™ Eyes 2 LYO, as evaluated by a trained panel.
## Back up: overview adjunct

<table>
<thead>
<tr>
<th>Product</th>
<th>Culture</th>
<th>Principle Flavour Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOOZIT TR160</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Sweetest</td>
</tr>
<tr>
<td>CHOOZIT FLAV 43</td>
<td><em>Pediococcus acidilactici</em></td>
<td>Creamy, sweet</td>
</tr>
<tr>
<td>CHOOZIT FLAV 54</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Savoury, sweet, de-bittering</td>
</tr>
<tr>
<td>CHOOZIT LH100</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Rounded savoury</td>
</tr>
<tr>
<td>CHOOZIT LH01</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Sweet savoury</td>
</tr>
<tr>
<td>CHOOZIT LH11</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Strong savoury</td>
</tr>
<tr>
<td>CHOOZIT LF302</td>
<td><em>Lactobacillus casei</em></td>
<td>Meaty savoury</td>
</tr>
<tr>
<td>CHOOZIT LF304</td>
<td><em>Lactobacillus helveticus &amp; casei mix</em></td>
<td>Sharp savoury</td>
</tr>
<tr>
<td>CHOOZIT helv.A</td>
<td><em>Lactobacillus helveticus</em></td>
<td>Sweet savoury</td>
</tr>
<tr>
<td>CHOOZIT 220 to 240</td>
<td>Undefined Lactococcus</td>
<td>Slow maturing traditional savoury</td>
</tr>
<tr>
<td>PROBAT 222</td>
<td>Undefined mesophile</td>
<td>Nutty, open texture for use in Red Leicester</td>
</tr>
</tbody>
</table>