FERMENTATION TECHNOLOGY FOR ADDING VALUE TO YOUR PRODUCT

REDUCING FAT/SALT/SUGAR & OFF-NOTES THROUGH FERMENTATION

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Sr. Business Development Manager
NIZO - FOR BETTER FOOD & HEALTH

• Independent, privately owned Contract Research Organisation for food and health

• HQ in Ede, The Netherlands (Food Valley)

• Founded in 1948; 130 professionals
“Boosting your immune response – clinical studies that support your claim” – Expertise Group Leader Alwine Kardinaal
8 May 15.00 @ Probiotics Research Centre
OPPORTUNITIES IN THE FOOD INDUSTRY

• **Sugar, Salt and Fat reduction trending**
  Also for new protein sources

• Reductions come with challenges
  o Food safety
  o Taste
  o Texture

• Many approaches focus on sugar replacers

• What can be done through fermentation?
THE NIZO APPROACH FOR REDUCTION STRATEGIES USING FERMENTATION
THE POWER OF FERMENTATION

- Mitigate the omission of fat, salt or sugar
- Cleaner label
- Increase of nutritional value
- Boost mouthfeel
- Artisinal image: positive perception by consumer

Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products

Gerrit Smit a,b,1, Bart A. Smit c, Wim J.M. Engels c

a Wageningen University, Department of Food Chemistry, P.O. Box 8129, 6700 EV Wageningen, The Netherlands
b Undersea Food Research Centre, Department of Flavour Generation and Analysis, P.O. Box 114, 3330 AC Vlaardingen, The Netherlands
c NIZO Food Research, Department of Flavour, P.O. Box 20, 6710 Be Ed, The Netherlands

<table>
<thead>
<tr>
<th>Flavour compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methylpropanol</td>
<td>Banana, malty, chocolate-like</td>
</tr>
<tr>
<td>3-Methylbutanal</td>
<td>Malty, powerful, cheese</td>
</tr>
<tr>
<td>3-Methylbutanol</td>
<td>Fresh cheese, breathtaking, alcoholic</td>
</tr>
<tr>
<td>3-Methylbutyric acid</td>
<td>Rancid, sweat, cheese, putrid</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>Sweaty, butter, cheese, strong, acid</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>Pungent, sour milk, cheese</td>
</tr>
<tr>
<td>Ethylbutyrate</td>
<td>Fruity, buttery, ripe fruit</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>Buttery, strong</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Yoghurt, green, nutty, pungent</td>
</tr>
<tr>
<td>Methional</td>
<td>Cooked potato, meat like, sulphur</td>
</tr>
<tr>
<td><strong>Methanethiol</strong></td>
<td>“Rotting” cabbage, cheese, vegetative, sulphur</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>Bitter almond oil, character, sweet cherry</td>
</tr>
<tr>
<td>Phenyl acetate</td>
<td>Rough, lily-jasmine with metallic note</td>
</tr>
</tbody>
</table>
1. IN SILICO APPROACH: FROM MOLECULE TO STRAINS

NIZO knowledge base:
- Target flavor linked to metabolite
- Metabolite produced by enzyme
- Enzyme can be produced by bacteria
- Bacteria with specific genes only

Experimental set-up:
- Screen 43 strains for specific gene content for ability to produce enzyme
- Certain group of strains show this functionality while others not

Conclusion: successful targeted approach to find strains that can produce desired flavor compounds
2. BIODIVERSITY: NIZO CULTURE COLLECTION

NIZO holds a large proprietary culture collection:

- Over 4000 strains, of which 1849 with QPS status (GRAS)
- (Phenotypic) database for rational pre-selection of subgroup of strains
- Ready-made microtiter mother plates available for rapid screening and selection

NIZO owned strain collection open for licensing strains to be used for your desired application

### NIZO culture collection

1849 isolates with approved QPS status (and still growing)

<table>
<thead>
<tr>
<th>Bacterii</th>
<th>Bifidobacterium</th>
<th>Lactobacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. coagulans</em></td>
<td><em>B. adolescentes</em></td>
<td><em>L. casei</em></td>
</tr>
<tr>
<td><em>B. fausiformis</em></td>
<td><em>B. animalis</em></td>
<td><em>L. casei</em></td>
</tr>
<tr>
<td><em>B. kohenformis</em></td>
<td><em>B. bifidum</em></td>
<td><em>L. plantarum</em></td>
</tr>
<tr>
<td><em>B. megarum</em></td>
<td><em>B. breve</em></td>
<td><em>L. plantarum</em></td>
</tr>
<tr>
<td><em>B. pumilus</em></td>
<td><em>B. infantis</em></td>
<td><em>L. plantarum</em></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td><em>B. lactis</em></td>
<td><em>L. plantarum</em></td>
</tr>
<tr>
<td><em>G. stearothermophilus</em></td>
<td><em>B. longum</em></td>
<td><em>L. plantarum</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>other LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. hansenii</em></td>
<td>Lactococcus lactis 4x6</td>
</tr>
<tr>
<td><em>H. uvarum</em></td>
<td>Leuconostoc citreum   6</td>
</tr>
<tr>
<td><em>K. lactis</em></td>
<td>Leuconostoc lactis    20</td>
</tr>
<tr>
<td><em>K. marxianus</em></td>
<td>Leuconostoc mesenteroides 80</td>
</tr>
<tr>
<td><em>P. angusta</em></td>
<td>Pediococcus acidilactici 3</td>
</tr>
<tr>
<td><em>P. anomala</em></td>
<td>Pediococcus pentosaceus 6</td>
</tr>
<tr>
<td><em>P. reukaufii</em></td>
<td>Propionibacterium freudenreichii 77</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>Streptococcus thermophilus 134</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td><em>L. johnsonii</em></td>
</tr>
<tr>
<td><em>L. alimentarius</em></td>
<td><em>L. johnsonii</em></td>
</tr>
<tr>
<td><em>L. amylobactin</em></td>
<td><em>L. johnsonii</em></td>
</tr>
<tr>
<td><em>L. amylovoran</em></td>
<td><em>L. johnsonii</em></td>
</tr>
<tr>
<td><em>L. avilis</em></td>
<td><em>L. mecosae</em></td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td><em>L. pentos</em></td>
</tr>
<tr>
<td><em>L. buchneri</em></td>
<td><em>L. paracasei</em>  60</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td><em>L. paraplanthorum</em> 4</td>
</tr>
<tr>
<td><em>L. crispatus</em></td>
<td><em>L. pentos</em>     6</td>
</tr>
<tr>
<td><em>L. curvatus</em></td>
<td><em>L. plantarum</em>  254</td>
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<tr>
<td><em>L. delbrueckii</em></td>
<td><em>L. plantarum</em>  254</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td><em>L. plantarum</em>  254</td>
</tr>
<tr>
<td><em>L. gallinarum</em></td>
<td><em>L. sake</em>       1</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td><em>L. sake</em>       20</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td><em>L. sake</em>       12</td>
</tr>
<tr>
<td><em>L. hilgardii</em></td>
<td><em>L. sake</em>       3</td>
</tr>
<tr>
<td><em>L. homohilgardii</em></td>
<td><em>L. sake</em></td>
</tr>
</tbody>
</table>

### INNOVATING TOGETHER

[Logo: NIZO FOR BETTER FOOD & HEALTH]
3. NIZO HIGH THROUGHPUT SCREENING SET-UP

3,000 Conditions tested overnight; selection for growth
4. PERFORM IN-PRODUCT SCREENING FOR FERMENTED FOODS

• The selection of starter cultures for cheese and other fermented products is one of the key activities for influencing product functionality
• Production of 600 microcheeses (of 150 mg), fermented milk or plant substrates in one run
• Flavour, enzyme and other analysis including HPLC measurements (for e.g. peptides of interest)
• Screen strains from the NIZO culture collection or your own collection

In-product screening for functional strains closely resembles behavior in real food products saving development time and money
5. NIZO PROCESSING CENTRE

- Bioreactors up to 4.5 M³
- DSP facilities
- Extensive in-house analyses
EXAMPLES
OVERVIEW OF EXAMPLES

- Reduction of Fat
- Reduction of Sugar
- Off-flavor removal
- Reduction of Salt
- Novel protein sources
REDUCTION OF FAT USING EPS PRODUCING STRAINS

- Fat in foods impacts viscosity and mouthfeel

- Extracellular polysaccharides (EPS) are longchain sugars that increase viscosity in yoghurt

NIZO screened for Lactic Acid Bacteria that can be added to yoghurt cultures leading to low fat, but thick and smooth yoghurt
Recently pili were found to be present on the surface of lactococci
Overexpressing pili led to increased viscosity and gel hardness of fermented milk
Represents a new concept to alter texture

Naturally occurring LAB strains that play a role in dairy fermentation with pili formation capacity can improve low fat variants

Tarazanova et al. 2017 Front. Microbiology
Tarazanova et al. 2018 Microb. Biotechnology
WO 2017/157430 AI
REDUCTION OF SUGAR

ENHANCEMENT OF SWEETNESS BY FERMENTATION

• Important in numerous products – drinks, condiments, sweets a.o.
• A limited number of these products are fermented
• Many sweeteners are produced through fermentation
• Can sugar be reduced/sweetness increased by fermentation in situ?

Critical Reviews in Biotechnology

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Recent advances in biological production of erythritol

Dorota A. Rzechonek, Adam Dobrowolski, Waldemar Rymowicz & Aleksandra M. Mirończuk

Review

Nutraceutical production with food-grade microorganisms

Jeroen Hugenholtz a, b, Eddy J Smid b

a Wageningen Centre for Food Sciences, PO Box 20, 6710 BA Ede, The Netherlands
b NIZO Food Research PO Box 20, 6710 BA Ede, The Netherlands
1. In situ production of sweetener
2. Production of sweetener in side stream
3. Conversion to sugar with higher sweetness impact
4. Production of sweet tasting molecules (not classical sweeteners)
5. Off flavor removal to reduce addition of sugar for masking purposes (examples, also for plant proteins)
PREVENT FORMATION OF BITTERNESS
USING ENZYMATIC CAPACITY OF STRAINS

• Peptidase N is a major peptidase involved in bitter C-peptide degradation:

\[ \beta\text{-casein} \rightarrow \text{C-peptide} \]

Use as marker peptide

By screening on C-peptide degrading capacity debittering strains were identified

• Debittering cultures are successfully marketed by starter culture company
• Similar concept can be applied to products where sugar is added to mask off flavors
OFF FLAVOR REDUCTION
BY FERMENTATION OF SOY

- Soy products have beany off flavor
- Sugar often acts as masker
- Fermentation improves overall flavor profile reducing the need for masking

Screen GRAS strains for removal of hexanal and other off flavor molecules in plant based products is a potential route.
Salt reduction has an impact on flavor and safety

- Fermentation allows to produce complex flavor profiles
- Complex flavor profiles can mask salt reduction
- Strain choice and application regimes are important
HIGH QUALITY, LOW SALT
USING COMPLEX FLAVORS

- FrieslandCampina was searching for a natural solution to lower the salt concentration in cheese

Together with NIZO, in-situ formation of compounds replacing functions of salt was realized

- Tailoring of starter and adjunct cultures was applied to compensate for low salt concentrations
- Results enabled Friesland Campina to introduce low salt Milner cheese (40% less salt)
NEW PROTEINS GIVE NEW CHALLENGES

• Soy beans in yoghurt or milk often have a ‘beany’ taste and ‘dry’ mouth feel.

• Strains from the NIZO culture collection were selected for good growth on soybean and great taste.

In collaboration with Kikkoman a great tasting soy milk-yoghurt drink was developed now sold in Japan
NIZO is a world renowned CRO in the area of fermentation for food & health

NIZO offers fermentation approaches for fat, salt, sugar and off-notes reduction strategies for foods and novel protein sources

We work from ‘molecule to benefit’ and ‘benefit to molecule’ using our expertises

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Enzymes</th>
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1. In silico  2. NIZO culture collection  3. High throughput Screening  4. Micro models  5. NIZO Processing Centre
THANKS TO THE NIZO FERMENTATION TEAM

Discovery

QA, Data and chemical analytics

Clinical Validation

Production

Business support

Meet us at booth # F140
INNOVATING TOGETHER