Microbial Contaminants Relevant to Safety and Quality of Plant Protein-Based Dairy Alternatives

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- Independent company
- Confidential contract research for the food industry
- Roots in dairy, > 15 years non-dairy

Our markets
Market trends

• Increasing demand for plant-based products

• Consumer’s choice for dairy alternatives:
  o Casein allergy / lactose intolerance
  o Vegan, no animal products
  o Sustainability – reduce ecological footprint

Plant protein based alternatives

• Making a nutritious, tasty product with the right texture
  o Ingredient / composition choice

• Product safety
  o Allergens
  o Chemical contaminants
  o **Microbiological contaminants**
    • throughout the chain and in finished product
Dairy products – many decades of know-how

• Control of microbial contaminants – product and process specific
  o Levels and types of contaminants in raw milk
  o Processing contaminants

Farm
Feed, environment, milking installation

Factory
Control measures in place (GMP, HACCP)

Product
Pathogens / Spoilers
## Plant-based ingredients

*many sources, production processes, and qualities*

<table>
<thead>
<tr>
<th>Class or category (biological origin)</th>
<th>Protein sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legumes</td>
<td>Pea, faba bean, chickpea, lentil, mung bean</td>
</tr>
<tr>
<td>Oil containing</td>
<td>Soy, lupin</td>
</tr>
<tr>
<td>Cereals</td>
<td>Oat, wheat, barley, rice, quinoa, amaranth</td>
</tr>
<tr>
<td>Nuts</td>
<td>Hazelnut</td>
</tr>
<tr>
<td>Stone fruit</td>
<td>Almond, coconut</td>
</tr>
<tr>
<td>Oil seeds</td>
<td>Canola, rapeseed, sunflower, hemp, flax</td>
</tr>
</tbody>
</table>
Dairy alternatives

**different challenges for different product categories**

- Chain control to ensure microbial safety / quality

- Microbes in ingredients – what can be expected?
  - Levels and types?

- Product specific (drinks/yogurt-/cheese-like)
  - Heat treatments – what can be applied for products?
  - Post processing contamination?
  - Does product support growth of species present?
  - Storage conditions

Quantitative risk assessments for target microbes
Establishing microbes in different plant proteins

• Many different ingredients investigated:

• Different qualities
  o Flour, protein concentrate, protein isolate, kernels, flakes, hydrolysates

• Ingredients typically undergo heat treatment (at least 80°C)

• What are the levels?
  o Aerobic mesophilic plate count
  o Different groups of spore formers

• What are the types?
  o Isolates from highest dilutions: most prevalent species ID-ed
Determining contamination levels in different plant proteins

- Pea, faba bean, mung bean and chickpea
  - Flours
  - Isolates
  - Concentrates

- Analysis
  - Aerobic mesophilic plate count
  - Spore formers - aerobic mesophilic, thermophilic

Conclusion
- Plant protein ingredients:
  A significant fraction of the total count consists of spores (up to $\sim 3 \log_{10}/g$)

[Graph showing total aerobic counts and mesophilic spore counts in samples of raw milk and various plant protein isolates (fava, mung bean, chick pea, pea)]
Determining species present

- Representative isolates from the highest dilutions were identified

- Predominant species
  - *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens*
  - *Bacillus cereus*

- Also present:
  - Sulfite reducing clostridia (SRCs)
  - *Geobacillus stearothermophilus*
Drinks

• Ensure sterility of drinks - thermal processing

• Calculation heat treatment
  o Initial levels and types
  o Apply optimal thermal process for inactivation

• Some spores survive high heat treatments (UHT)
  o surviving thermostable spores
    • *Geobacillus* spp. (storage >45°C)
    • *B. subtilis* isolates with genetic elements that render high level heat resistant spores (ambient stable)

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Inactivation spores *C. botulinum* (I) and *C. sporogenes*

Diao et al 2014

Inactivation of bacteria and spores of *B. cereus, subtilis, Geobacillus*

Den Besten et al 2018
Drinks - neutral pH, UHT – non-sterility issues

• Commercial non-sterility:
  o One spore per packaging unit is enough!

• Spoilage by:
  o surviving thermoresistant spores
  o contamination in installations / fouling issues
    • Limited protein solubility / denaturation
    • *B. subtilis / licheniformis / circulans / cereus*

• Food safety risk in case of
  • insufficient heating *C. botulinum / B. cereus*
  • fouling *B. cereus*
**Bacillus cereus** and cereulide production in plant based substrates

- Production of cereulide after 6 days at 12°C, after 12-16h at 30°C
Surfactants by *B. subtilis* and *B. licheniformis*

- Lichenysin and surfactin from *Bacillus* spp
  - Highly heat stable (121°C, 20 min)
  - Lipopeptide, Non-ribosomally synthesized
  - Gene cluster: *srf* surfactin, *lic/lchA* lichenysin

- Isolates from plant ingredients: *lic/lchA* +

- Lichenysin production:
  - late exponential phase / high cell density locally
  - not detectable under $10^5$ CFU/mL in milk and LB
  - Levels in LB relevant (16 ug/mL toxic to Caco-2)

- Production on plant substrates?
Yogurt-like products

• Large variety commercially available, many at product development stage

• Ingredients pasteurized
  o Generally < 95 °C
  o Yeast, moulds, vegetative cells killed (e.g. *Listeria, Salmonella*)
  o Bacterial spores survive

• Target pH below 4.5
  o Acidification during fermentation by starter culture lactic acid bacteria

• Microbiological stability of fermented yogurt-like products
  o pH 4.5 + lactate present: most bacterial contaminants controlled
  o Yeast and moulds can grow – prevent contamination
Semi-hard and Cream cheese-like products

• A range of ingredients can be applied
  o based on starch
  o based on protein

• Target pH values typically 5.0 to 5.5
  o chemical acidification
  o fermentation using starter cultures

• Ingredients heated
  o yeast and moulds and vegetative bacteria inactivated
  o spores survive – *Clostridium* and *Bacillus* spp
  o post-processing contamination – slicing, extra additions?

• Potential risks:
  o Overgrowth starter during fermentation
  o Outgrowth contaminants during storage incl. post processing
Low pH products: yogurt and cheese

fermentation with lactic acid bacteria

• Fermentation of dairy
  o Dairy starter cultures – evolved in traditional milk-based fermentation
  o Conversion of milk sugar lactose -> lactic acid
  o Development of flavour and texture, antimicrobials

• Fermentation plant based substrates
  o Different substrates in plant based fermentations
  o Growth and performance different?
Acidification rates are LAB strain dependent

impact on food safety / spoilage

- Fast acidification and low pH important to prevent outgrowth of spoilage organisms
- Slow acidification comes with higher final pH
- Potential contaminant issues:
  - spore formers grow to pH of 4.6, some lower
  - *B. cereus* (cereulide toxin)
  - *B. subtilis* and *B. licheniformis* (surfactin, lichenysin)
  - *Clostridium*
- Post processing contamination
  - E.g. *Listeria monocytogenes*
Fermentation of pea-protein-enriched emulsion

**sporeformers in ingredients**

- Heat treatments of pea protein isolate
  - 100°C for 30 min + 95°C for 30 min
  - Higher heating not applied to maintain protein functionality

- Fermentation with 10 different consortia

- In various samples *Bacillus* spp came up during fermentation, incl *B. cereus*
  - Spores not inactivation

- Potential risk
  - Overgrowth of starter culture

Conclusion - Chain Control plant based products

Microbiological safety, preservation and shelf life

• Different challenges:
  o Drinks: spores (fouling/survival)
  o Yogurt: fermentation
  o Cheese: fermentation, post-processing, storage

• Control ‘by design’
  o *In silico* modelling (pathogens, spoilers) to assess risks
  o Setting relevant specifications
  o Verification
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  o Fermentation
  o Bioinformatics

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• TKI projects
  • Plant based cheese
  • Biopurification to remove off-flavours
  • Microbial contaminants plant-based