ISI FOOD PROTECTION
Centre of Expertise for Applied Food Microbiology

Microorganisms in plant-based dairy substitutes
new challenges,
but also new opportunities

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our know-how
for the safety and stability of
your food products

Challenges from food trends:
from a microbiological perspective

Intrinsic parameters

often higher levels than in products of animal origin

Microbiological quality of raw material

Partly higher load of mould spores and bacterial endospores

Novel raw material and processes

limited experience regarding microbial hazards and stability

Psychological aspects

plant-based = natural = of no concern

Trend „E-Number-free“

no preservatives or other stabilising compounds

Pressure for innovation

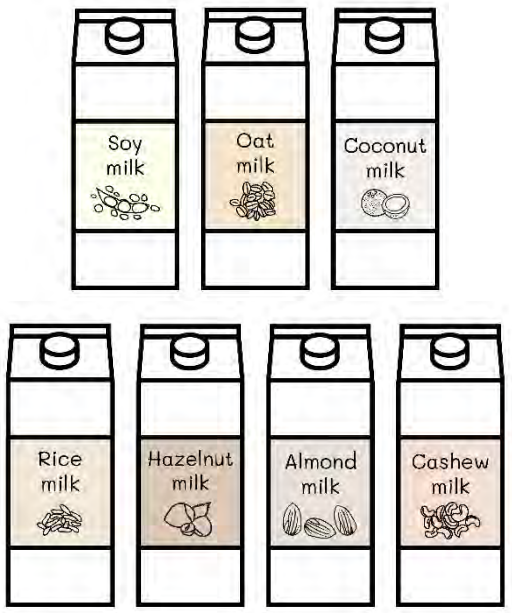
limited time for product development

Plant-based dairy alternatives (PBDA)

Categories

- **Fluid products:**
  Milk alternatives (PBMA)

Plant-based milk substitutes, or plant extracts, are water-soluble extracts of legumes, oilseeds, cereals or pseudocereals that resemble bovine milk in appearance.



- **Plant-based yoghurt-like products (PBYL)**

PBYL are generally made by fermenting aqueous extracts or flour–water suspensions of cereal, pseudo-cereals, legumes, and nut flours, or homogenized fruit pulps.

"Ingredients: *live active cultures: S. thermophilus, L. delbrueckii subsp. bulgaricus, L. plantarum, L. acidophilus, B. lactis*"

www.siggis.com

- **Plant-based cheese alternatives (PBCAs)**

"Ingredients: filtered water, coconut oil, modified potato and corn starches, potato starch, sea salt, natural smoke flavor, olive extract, beta carotene for color".

www.veganblackmarket.com

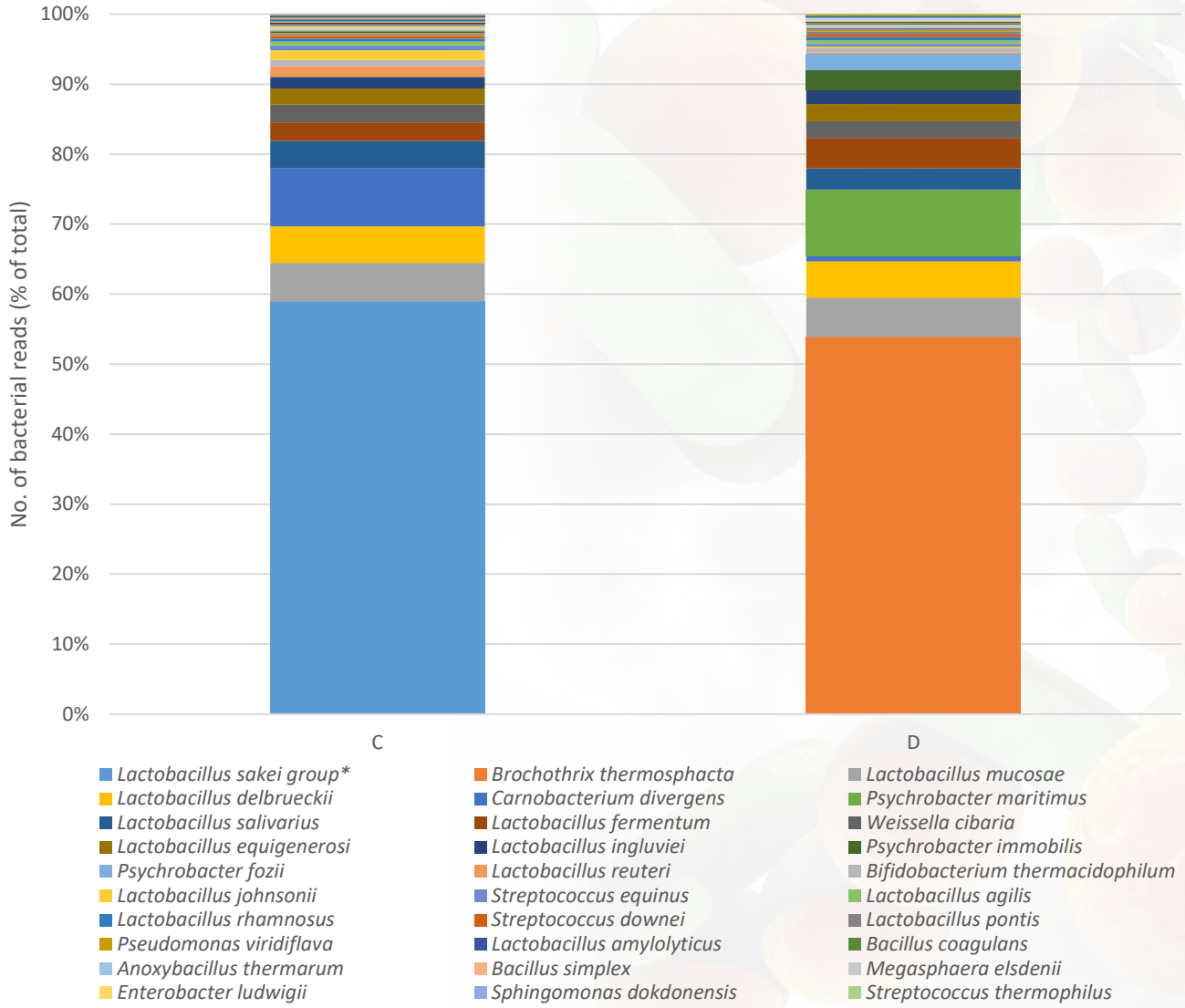
Plant-based dairy alternatives:
Principles, challenges & level of technology

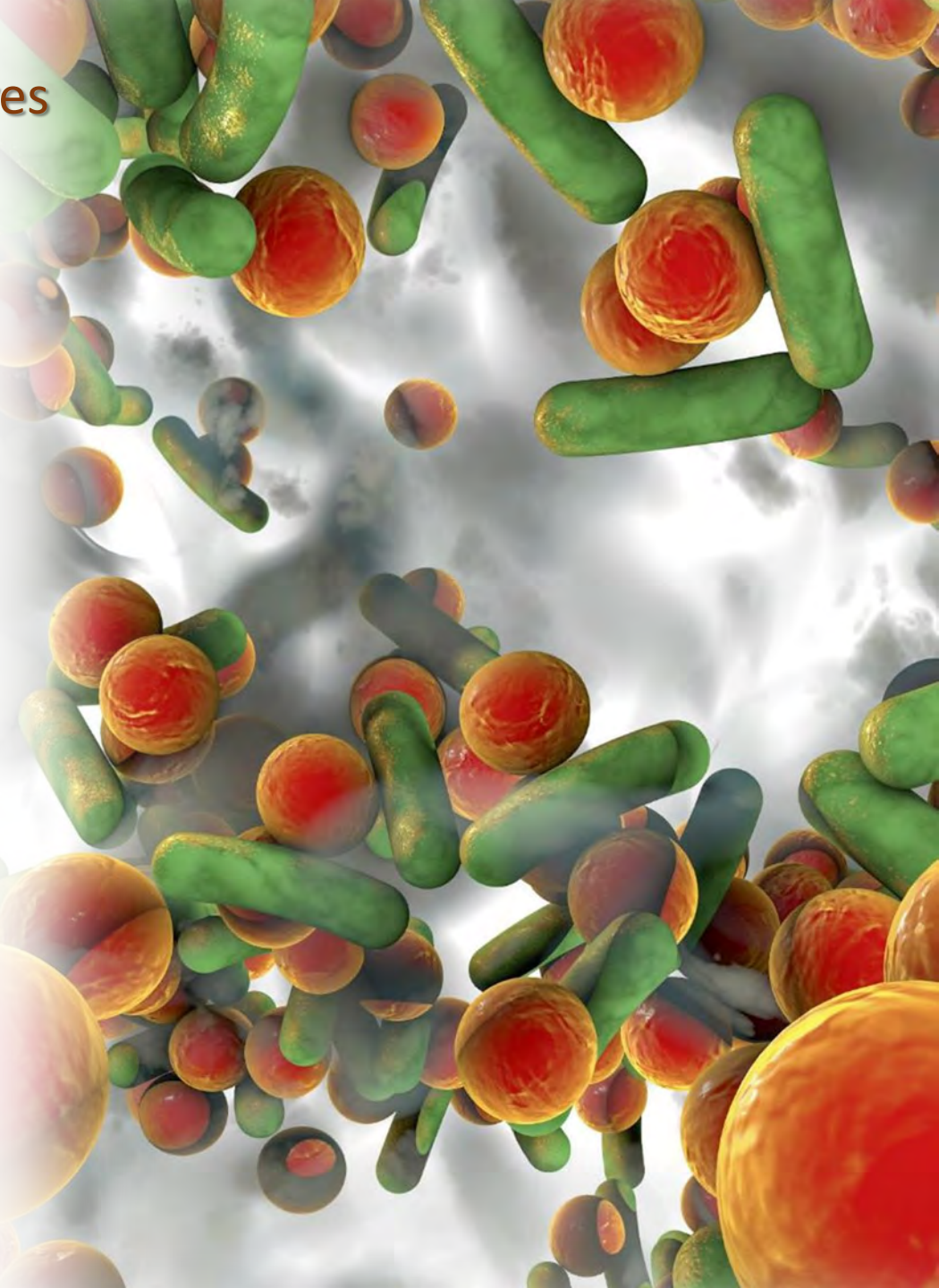
Origin	Product (analogue)	Principles	Challenges	Level of technology
Plant-based	**Milk**	Soluble proteins are extracted Interfering starch can be hydrolysed to glucose Alkaline treatment reduces unsaturated fatty acids (off flavour)	Low protein content, off flavour from free unsaturated fatty acids Presence of starches reduces emulsifying capacity	Industrial practice
	Yogurt	Fermentation with lactic acid bacteria The acidification results in protein precipitation and flavour formation	Fermentation-based flavour can differ from that of yoghurt due to raw material Off flavour of raw material	Industrial practice
	Cheese	Seeds are ground, fermented, and pressed Fermentation can hydrolyse proteins (increase digestibility) Coagulation is induced through enzymes, salts, or acid	Melting behaviour of hard cheese Off flavour of raw material	Industrial practice (traditional products) *(Experimental stage at start-up company)*
Single-cell fermentation	**Milk, yoghurt, cheese**	Milk proteins are recombinantly produced in yeast or bacteria	Only single proteins are expressed, no higher-order structures such as micelles Mutations and posttranslational modifications possible Consumer acceptance can be a problem	Experimental stage at start-up company
Insect / mussel based	**Milk, yoghurt**	Emulsifying and gelling capacity depend on the extraction method Further hydrolysis and fermentation are possible	Off flavour and off colour possible with insects Consumer acceptance for insect extracts low in Western countries	Only model-system experiments on individual functionalities

Kyriakopoulou et al., 2021: Alternatives to Meat and Dairy

The microbial landscape in plant-based dairy alternatives

Complex, diverse, and different

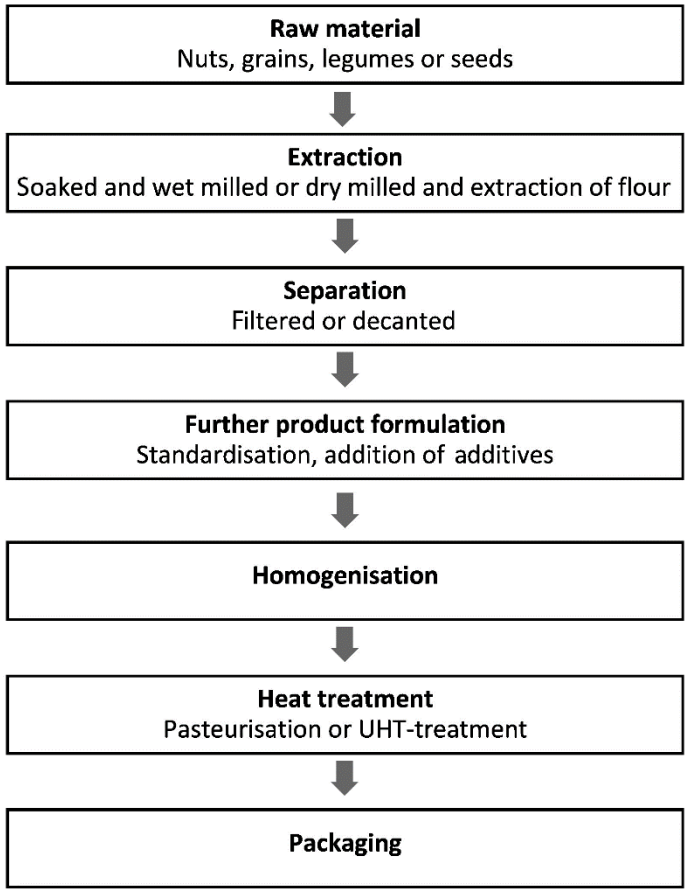




The microbial landscape in plant-based dairy alternatives

Milk alternatives

General manufacturing steps for the production of PBMAs:



Jeske et al., 2018: Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials

Microbial issues with spore-forming and thermoduric bacteria

Microbial issues in high-pressure treated products

Heat inactivation of (most) spoilage and pathogenic bacteria

Plant-based yoghurt-like products

Main ingredients and microbial starters (examples)

Main Ingredients	Starter cultures	Texture Processing, Structuring Agents
Oat protein concentrate (15 % w/w)	*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (commercial strains for yoghurt production)	Heat treatment at 90 °C for 30 min
Potato protein isolate (5 % w/v)	*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (commercial strains for yoghurt production)	High-pressure homogenization (200 MPa)
Pea protein isolate (10 % w/w)	*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (commercial strains for yoghurt production)	Heat treatment 60 °C for 60 min and high-pressure homogenisation
Soymilk (6.8 % solids)	*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (commercial strains for yoghurt production)	Concentration (heat treatment at 90 °C for 15 min)
Millet flour (8 % w/v)	*Lacticaseibacillus rhamnosus* GR-1 and *Streptococcus thermophilus* C106	Heat treatment at 90–95 °C for 60 min
Lupin protein isolate (2 % w/v)	*Lactiplantibacillus plantarum* TMW 1.460 and TMW 1.1468, or *Pediococcus pentosaceus* BGT B34 and *Levilactobacillus brevis* BGT L150	Heat treatment (140 °C for 10 s or 80 °C for 60 s) and EPS-producer LAB strain
Soy (10 % w/v)	*Lactiplantibacillus plantarum* B1-6	Heat treatment at 108 °C for 15 min
Soy, soaked soy, or germinated soy (10 % w/v)	*Levilactobacillus brevis* KCTC 3320	Heat treatment at 121 °C for 15 min

REF: Montemurro et al., 2021, Plant-Based Alternatives to Yogurt: State-of-the-Art and Perspectives of New Biotechnological Challenges

The microbial landscape in plant-based dairy alternatives

Plant-based cheese alternatives (PBCAs)

The most common spoilage microorganisms in dairy products are well know

➡Example “cottage cheese”

Psychrotrophic (gram-negative) bacteria e.g. *Alcaligenes, Achromobacter, Escherichia, Micrococci, Pseudomonas, Flavobacterium, Bacillus, Enterobacter*, and coliforms).

Moulds e.g. *Geotrichum, Penicillium, Mucor, Alternaria, Aspergillus, Cladosporium, Fusarium*, and *Monilia*.

Yeasts e.g. *Yarrowia lipolytica, Candida, Pichia, Geotrichum candidum, Kluyveromyces marxianus*, and *Debaryomyces hansenii*.

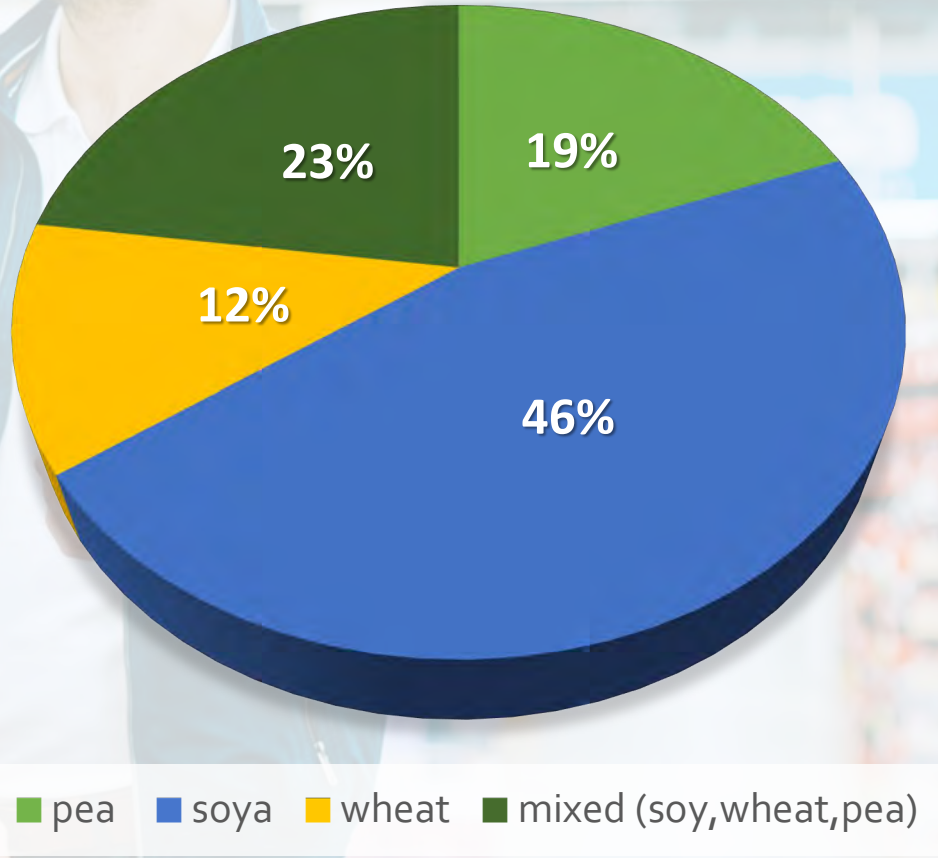
The microbial landscape in plant-based alternatives

Results of a "store check"

438 samples of plant-based alternative products from 5 European countries were taken from the shelf and evaluated at end of shelf-life

These commercial plant-based alternative products were based on:

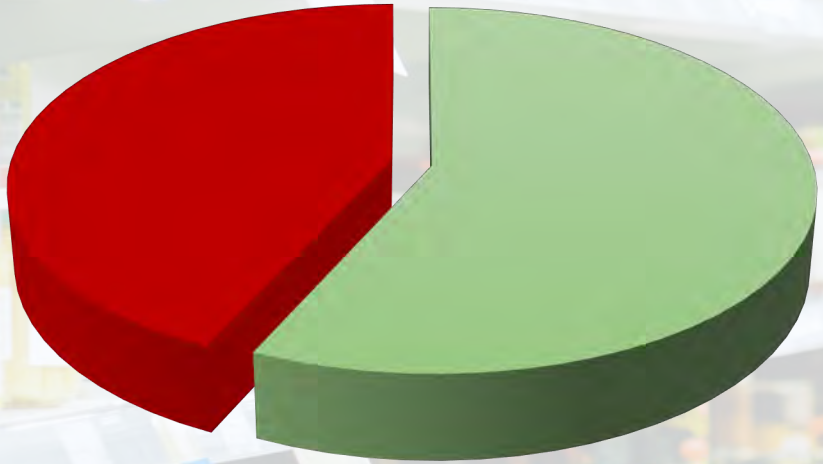
- soy/soy proteins
- mixed compositions (soy, wheat, and pea proteins)
- pea/pea proteins
- wheat/wheat proteins



The microbial landscape in plant-based alternatives

Results of a "store check"

189 samples had faults due to gas formation, slime, mould growth, off-odour, or for other reasons



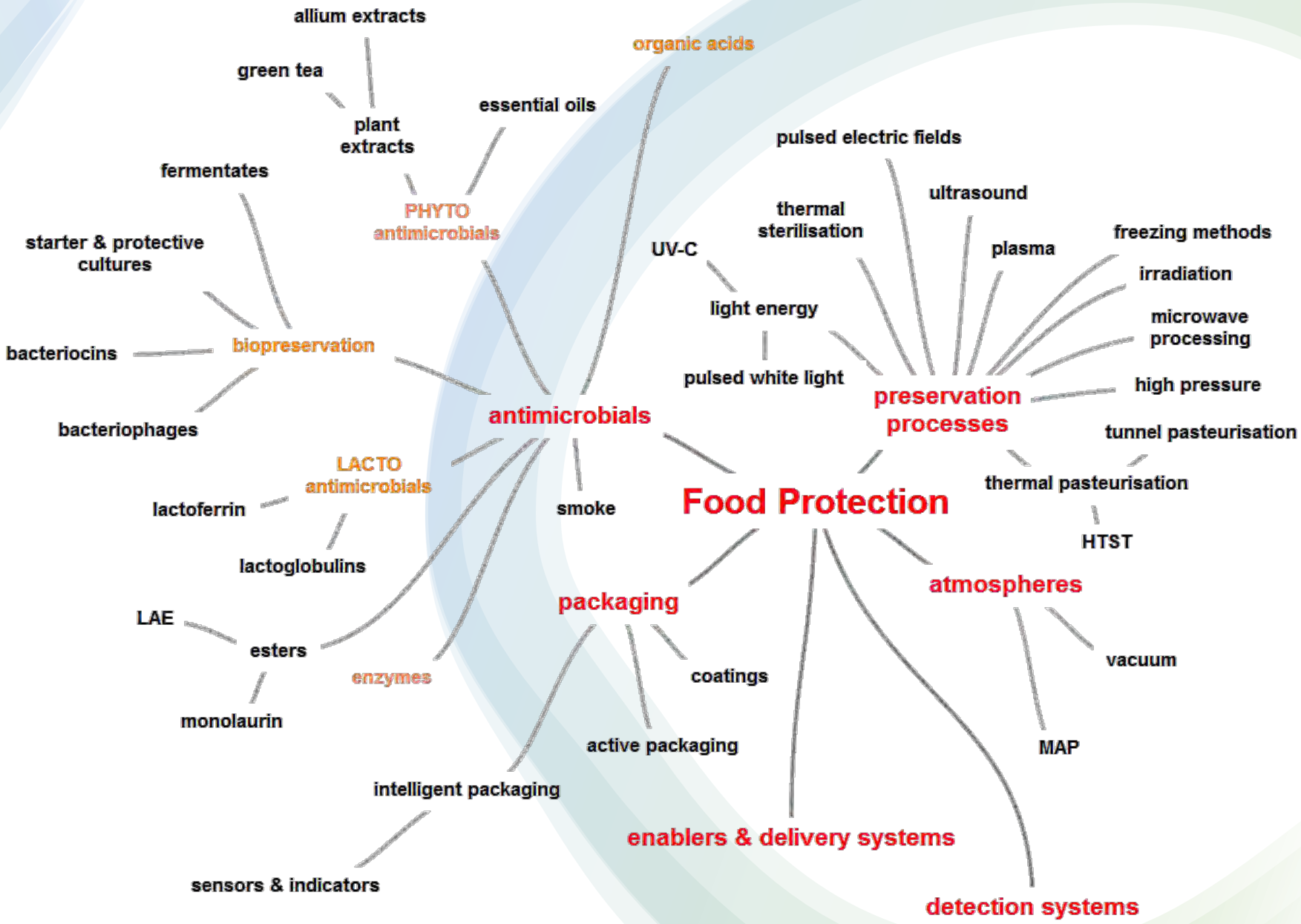
In many of these "store check" samples, we identified by 16S rRNA sequencing the following "unwanted" bacterial species:
(in high densities of > 10^6 cfu/g)

- ***Bacillus*** *subitilis, safensis, mojavensis, licheniformis, paralicheniformis, pumilis, halotolerans*
- ***Virigibacillus*** *salarius*
- ***Brochothrix*** *thermosphacta*
- ***Enterobacter*** sp.
- ***Enterococcus*** *faecalis, mundtii, casseliflavus*
- ***Staphylococcus*** *epidermidis, warneri*
- ***Erwinia*** *persicina*

How to protect plant-based alternatives?

All roads leads to Rome,

..but not all of them towards a stable and safe product



Biopreservation using protective cultures

- Protective cultures are microorganisms which are intentionally added to food raw materials or during food processing with the aim of inhibiting growth of unwanted bacteria, yeasts or moulds and thereby improve the microbiological safety and/or quality of the final food product.

- In contrast to classical fermentation, the characteristics of the food product (taste, smell, colour, texture) should not be perceivably changed by the protective culture.

1990

2000

Launch of the first generation of bioprotective cultures

2010

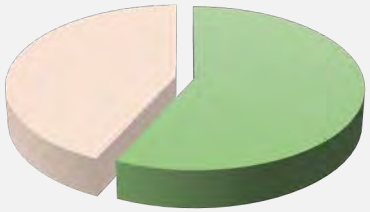
Approx. 50% of „fresh dairy“ products are produced using protective cultures against spoilage

2020

BioProtection 2.0: First commercial application in non-fermented food products (e.g. salads)

BioProtection 2.0:

249 of 438 samples did not show any noticeable abnormality



(such as off-smell, colour, discolouration, slime formation, blown packages,...)

In only 59 samples, total cell count was below 10^4 cfu/g at the end of shelf-life.

From these "non-spoiled" store-check samples we isolated and identified 122 QPS / food-grade bacterial strains, e.g.:

Lactobacillus curvatus, sakei, fuchuensis

Leuconostoc citreum, mesenteroides, pseudomesenteroides, gelidum

Pediococcus acidilactici

Lactococcus raffinolactis

Weissella hellenica

Carnobacterium viridans, divergens

Challenge tests with a pool of **spoilage bacteria** and with ***Listeria monocytogenes***

ISI FOOD PROTECTION

- Founded end of 2009
- Highly specialised on applied food & plant microbiology: Cross-industrial & services along the food value chain
- L3* classified food safety laboratories & food pilot plant
- International customer portfolio
- Accredited according to ISO 17025
- Comprehensive strain collection of food spoilage microorganisms as well as of food pathogens (e.g. *Salmonella*, *E. coli* 0157, *Campylobacter*, *Listeria monocytogenes*)
- Approval for working with *Clostridium botulinum*

ask

Many thanks for your attention!

www.isifoodprotection.com