NIZO

FOR BETTER FOOD & HEALTH

PERFECTING DAIRY AND MEAT ALTERNATIVES

NIZO's integrated "pyramid approach"
using an innovative high-throughput screening toolbox

Alternative proteins

An ever-evolving playing field

Non-animal proteins

Plant


Soybean


Oat

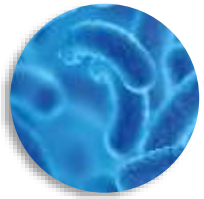

Faba bean


Chickpea


Green leaves

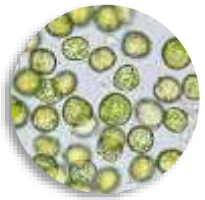

Almond

Microbial/single cell


Bacteria


Fungi


Yeasts


Microalgae

Precision ferm./cell ag.


Whey proteins


Egg proteins


Meat


Casein


Seafood

Photos by Sanjay Acharya, Shihmei Barger, Kristina D.C. Hoeppner, K.R. Harsha, Luis Molinero, Stefan Malmesjö, Mirjam van de Velde and others.

KEY CHALLENGES IN ALTERNATIVE PROTEINS

Are you facing or struggling with any of these?

Regulatory compliance & health claims

Astringency

Off-taste

Spoilage & food safety risks

Consumer expectations of dairy and meat alternatives

4 levels of consumer expectations

Benchmark set by

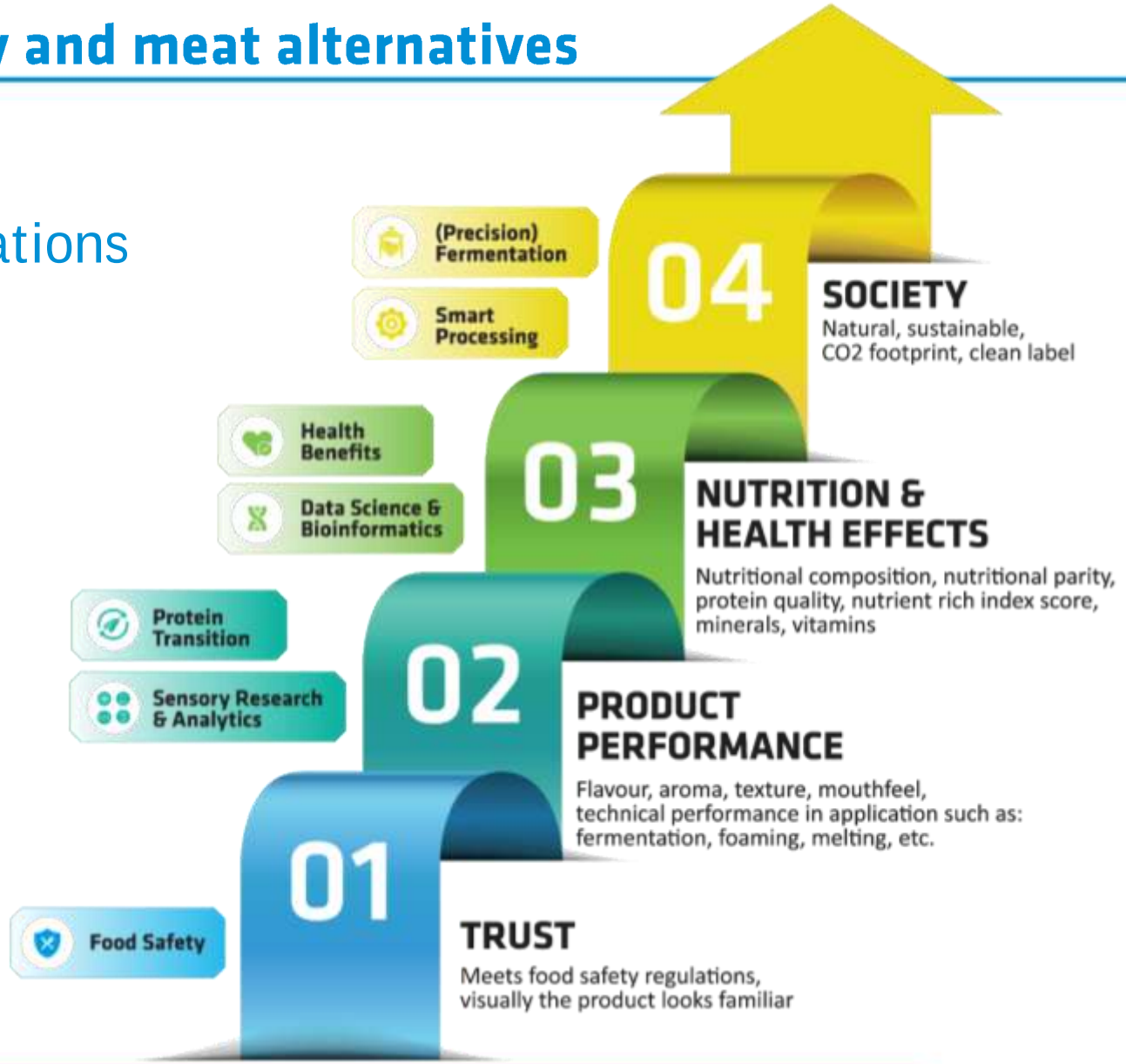
Whey proteins

Egg proteins

Meat

Casein

Seafood



NIZO high-throughput screening toolbox for integrated approach

Pick 'n mix

Health benefits

Taste

DAIRY & MEAT ALTERNATIVES (plant, microbial, precision fermentation)

Astringency

Food safety & Shelf life

04 SOCIETY
Natural, sustainable, CO_2 footprint, clean label

03 NUTRITION & HEALTH EFFECTS
Nutritional composition, nutritional parity, protein quality, nutrient rich index score, minerals, vitamins

02 PRODUCT PERFORMANCE
Flavour, aroma, texture, mouthfeel, technical performance in application such as: fermentation, foaming, melting, etc.

01 TRUST
Meets food safety regulations, visually the product looks familiar

- Enabling to meet diverse consumer expectations
- Integrated approach including multiple product characteristics
- Tailored to your specific product and business needs

Level 1
How to: Ensure safety + Extend shelf life + Eliminate spoilage?

Plant-based ingredients often contain **unknown & unwanted micro-organisms**

They may **survive processing** or **grow in the finished product**

This has **health** (pathogens), **economic & sustainability** (spoilage) impact

CHALLENGES

NIZO'S APPROACH

3E + 2A

- **E**nsure safety
- **E**xtend shelf life
- **E**liminate spoilage
- **A**void costly recalls
- **A**void brand damage

YOUR BENEFITS

Level 1
NIZO's process approach on Food Safety & Shelf Life

Process

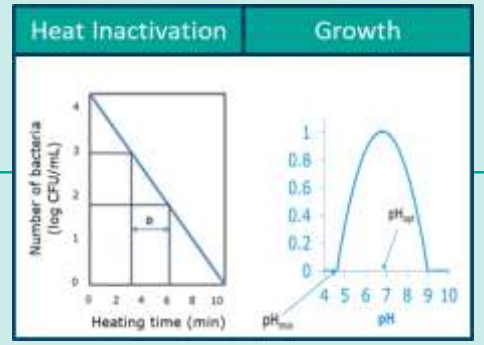
ASSESS
Apply predictive models to assess microbial risks / hazards in the chain

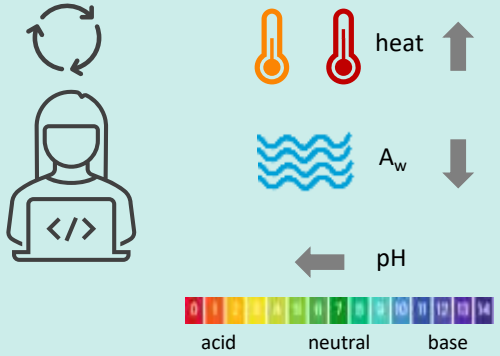
DEFINE
Define risk mitigation strategies based on calculations

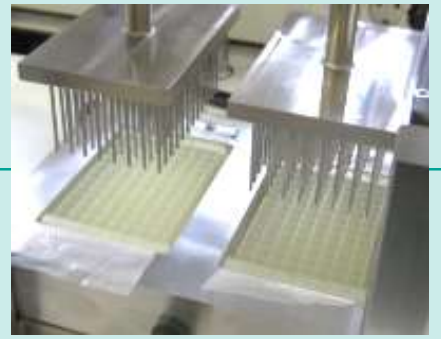
TESTING
Test control of key hazards in process / product variations in high-throughput systems

VERIFY
Verification of key microbes control in final product by challenge testing

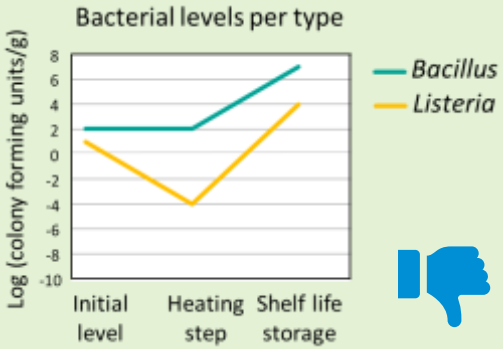
Method

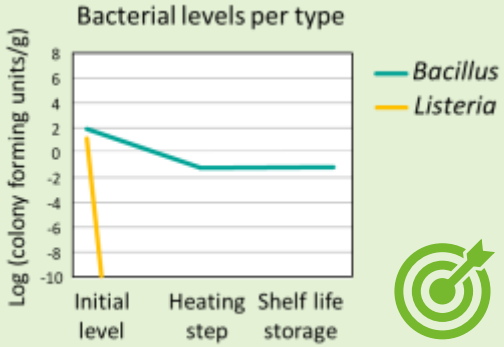






Result

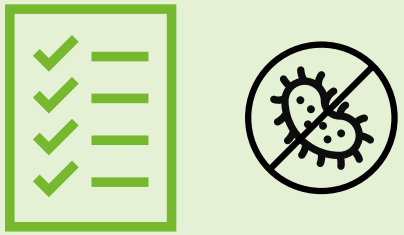




Experimental verification of inactivation and growth inhibition

Bacillus cereus *Listeria monocytogenes*

Demonstrated hazard control of microbes / their toxins



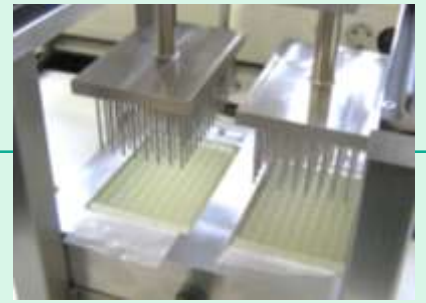
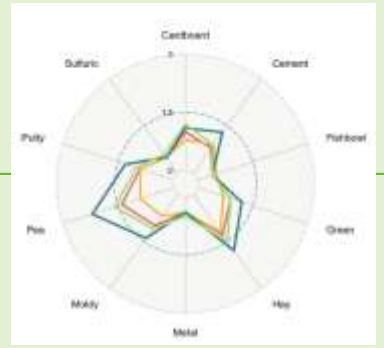
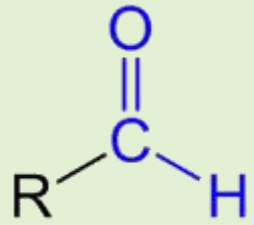
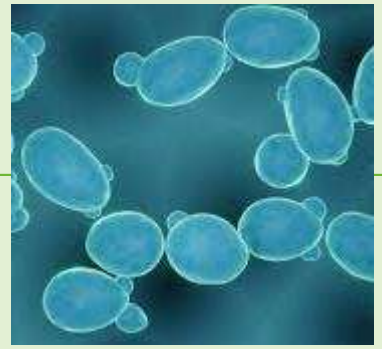
Level 2

How to improve the taste of your plant-based product?

BIOPURIFICATION

- Make plant-protein taste more **neutral**
- Remove off-flavour
- Process is **fast (< 1h)**
- **Applicable** to ingredients and products; with or without fermentation

Level 2
Biopurification to improve taste by reducing off-flavours: the NIZO approach

Process				
	ASSESS Expert sensory panel to assess off-flavours	MEASURE Instrumental measurement of off-flavour compounds	**SELECT** Selection of microbial strains from **NIZO Culture Collection**	TEST Biopurification tests on laboratory and pilot scale
Method				
Result				

Flavour analysis (GC-MS) of pea protein before and after biopurification

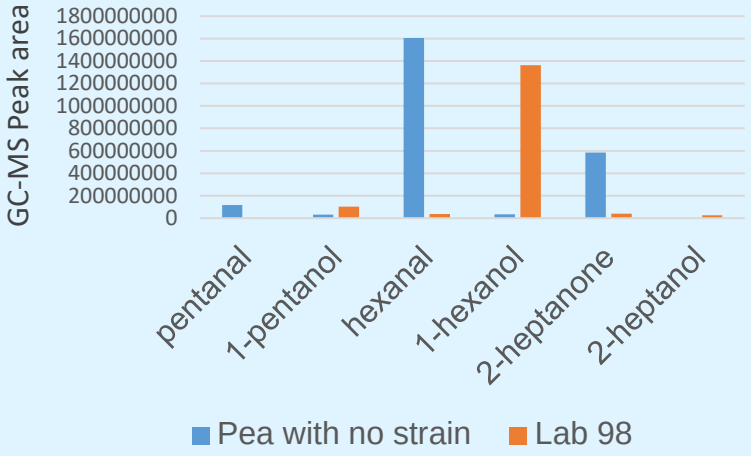
Biopurification of pea protein with strain Lab 98

Beany flavour

Typical Beany flavour = hexanal

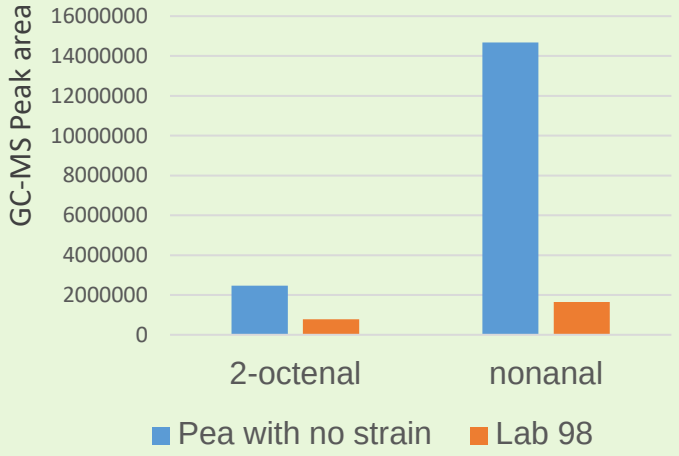
→ **Biopurifaction:**

- Hexanal reduction to hexanol
- Due to higher odor threshold no sensory perception of hexanol



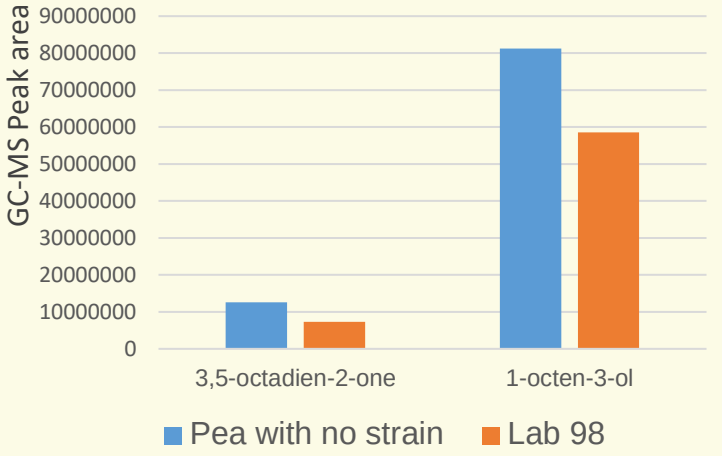
Fatty flavour

Reduction of fatty flavor volatiles



Mushroom flavour

Reduction of mushroom like volatiles



Level 2
Astringency elimination: Is this possible?

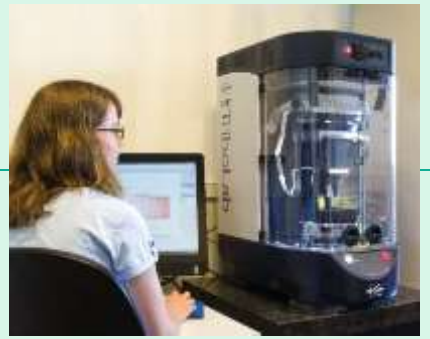
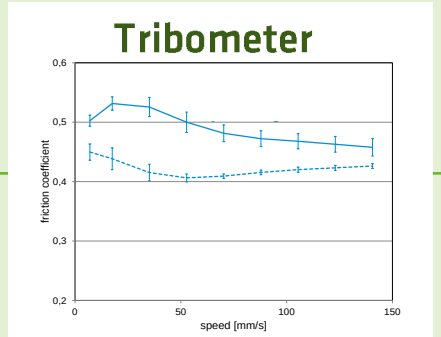
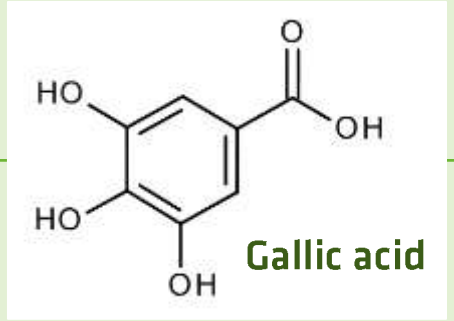
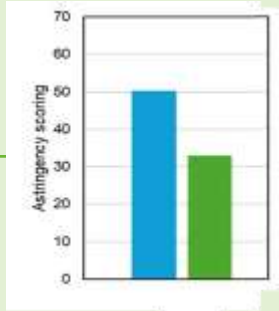
One of the
biggest sensory challenges
of plant proteins

→ **Need for effective mitigation strategies**

- **Knowledge-based** strategy
- A unique & expert sensory panel with **20+ years** of experience in **astringency assessment**
- Supported by our analytical toolbox

- Make plant-based products taste pleasant:
  - ✓ **Smooth**
  - ✓ **Creamy perception**
- Increase **consumer acceptance**

Level 2
NIZO's unique knowledge-based approach to reduce astringency

Process	ASSESS	MEASURE	**DETECT**	REDUCE
	Assess astringency with unique NIZO sensory panel	Instrumental measurement of astringency	Presence and role of astringent components	Reduction of astringency
Method				
Result				

Level 3
Adding value to products by substantiation of health benefits

Plant-based ingredients are often rich in **proteins**, **fibres** and **polyphenols:**

- Association with **beneficial** effects on health.
- **Digestibility and bio-availability** are important features for related health effects.

OPPORTUNITY

Integrated *in vitro* & *in silico* toolbox:

- INFOGEST
- NIZO MicroColon model
- Cell culture
- Bio-informatics

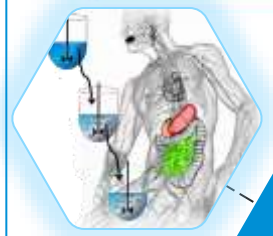
NIZO'S APPROACH

- **Informed decisions** for product development to achieve target health properties
- **Adding value to products** by substantiation of health effects

VALUE

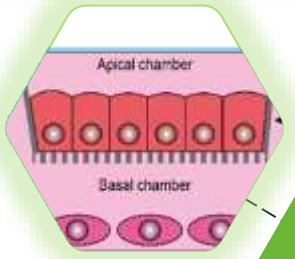
How NIZO can help in defining the health benefits of your ingredient

In vitro toolbox; from digestion to gut health and immune responses



Pre-digestion of food/ingredients INFOGEST

- Releasing active (indigestible) compounds
- Nutrient bioavailability



Filtration of pre-digested material

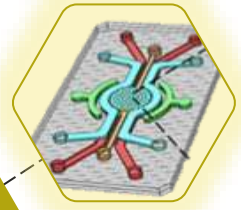
- Mimic absorption of compounds in GI tract
- Remove molecules that normally would not reach the colon

Co-culture transwell models

- Colon epithelium (Caco-2)
- Gut barrier integrity (TEER)
- Macrophage immune (THP1)
- Host response (cytokines and/or RNAseq)

MicroColon high throughput screening model *(adult and infant)*

- Short Chain Fatty Acids
- pH
- Microbiota profiling
- qPCR quantitative assessment
- Neuroactive metabolites
- Bioinformatics



Gut-brain axis on a chip (coming soon)

- Developed at University of Twente (UT)
- Will be offered via NIZO-UT partnership
- Stem cells used to recreate human organs
- Complement cell line models & clinical studies

Ensuring physiological relevance by combining *in vitro* models simulating:

- Upper GI (pre-) digestion, releasing active components from foods/ingredients
- Colonic fermentation by gut microbiota
- Epithelial / immune cell culture to study host responses

Medium/high throughput models mimicking GI conditions in microplate format

Mechanistic understanding of potential health effects and predictive value towards human studies

Fast, cost-effective options to support IP opportunities

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For more information, please contact:

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NIZO High Throughput Protein Extraction Tool

Fi Innovation Awards
Finalist 2024
Future Food Tech Innovation Award

FINALIST
Future Foodtech Innovation Award 2024

Introduction

- Increasing demand for high quality protein ingredients forces seed breeder to develop new cultivars
  → Better smell, taste, functional and nutritional properties

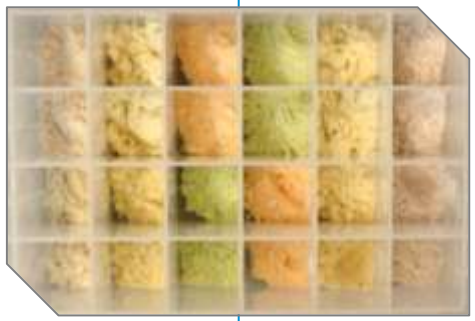
- This requires screening large number of cultivars on composition and functional properties

- NIZO developed the automated **High Throughput Protein Extraction Tool**

- 24 seed varieties can be extracted and analysed simultaneously

How does it work?

Step 1: Dispersion



- Meal or flour is weighted into the 24-well plate.
- Extraction buffer is added.
- Extraction buffer can be varied in composition, pH and amount.

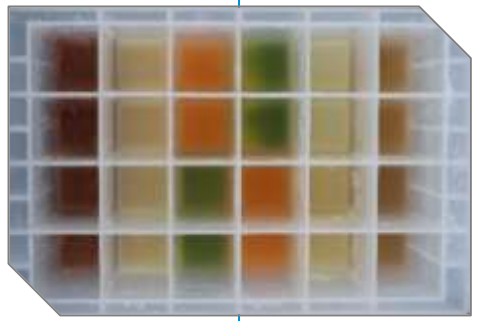
Step 2: Extraction

- The 24-well plate is sealed and installed in the shaker.
- Extraction time, shaking intensity, and temperature can be varied.

Step 3: Separation

- The 24-well plate is centrifuged to separate the solids (mainly starch and fibre).
- The protein solution (supernatant) is automatically transferred to a new plate.

Step 4: Quantification



- Protein extraction yield is automatically determined by BCA analyses.
- Samples can be further processed for analysis of protein composition, volatile and non-volatile compounds.

How does it work?

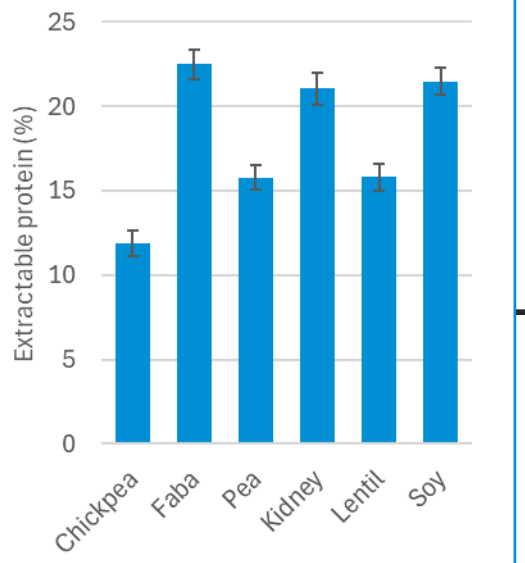
Step 1: **Dispersion**

Step 2: **Extraction**

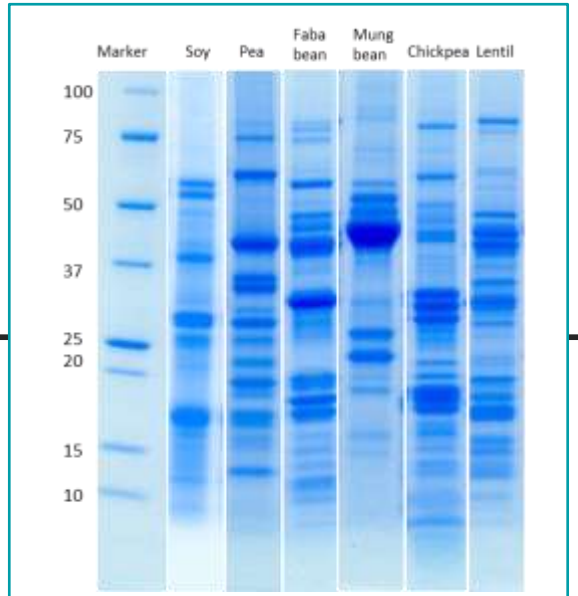
Step 3: **Separation**

Step 4: **Quantification**

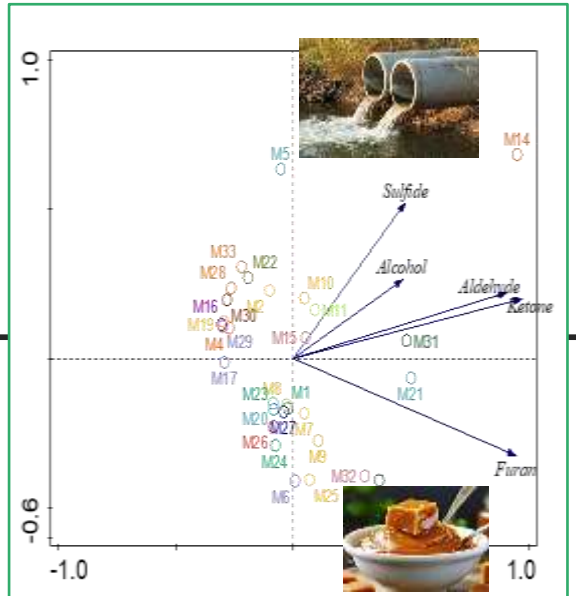
Step 5: **Characterisation**



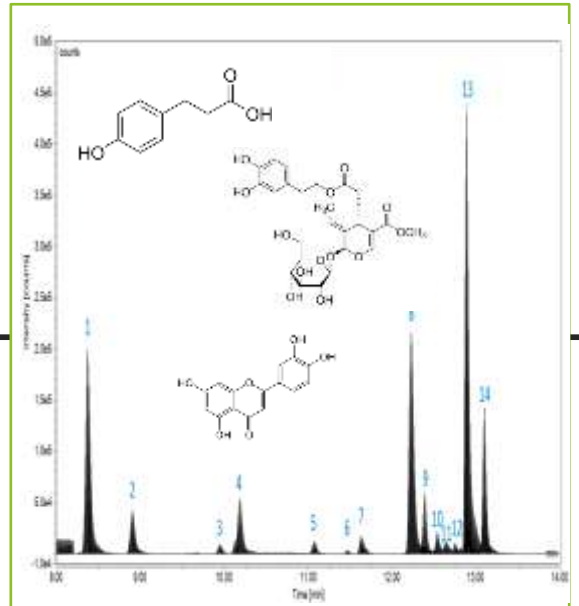
- Extractable protein



- Protein composition



- Aroma → Volatiles



- Taste → Non-volatiles

Protein transition: current and future vegan protein sources

NIZO has hands on experience with these proteins

ESTABLISHED


Soybean


Pea


Lupine


Potato


Rice


Corn


Faba bean


Chickpea


Lentils


Oat


Almond


Wheat

EMERGING

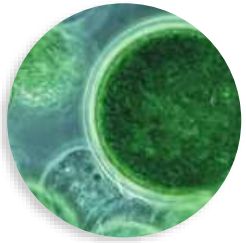

Sunflower


Mung bean


Rapeseed


Green leaves


Quinoa


Microalgae

UP COMING


Nuts


Single cell/ Fermentation


Duckweed


Flaxseed


Hemp


Seaweed

Photos by Sanjay Acharya, Shihmei Barger, Kristina D.C. Hoeppner, K.R. Harsha, Luis Molinero, Stefan Malmesjö, Mirjam van de Velde and others.

NIZO has been conquering this ‘protein-berg’ for over 75 years

10 years
Global
plant-based trend

25 years
NIZO’s
plant-protein research

> *Which ingredient to use in my application?*
> *How to process my raw material?*
> *Can I use fermentation?*

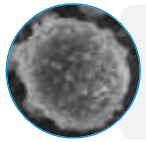
75 years
NIZO’s
protein knowledge

> *How does the protein interact with other ingredients?*
> *How is the food safety/microbial quality affected?*
> *What is the source/origin of off flavour and taste?*
> *Which functionality can be delivered by alternative proteins?*
> *What is the best micro-organism and fermentation conditions?*

INNOVATING
TOGETHER

NIZO
FOR BETTER FOOD & HEALTH

NIZO's 75 years of protein knowledge



Development of **Casein Micelle Models**

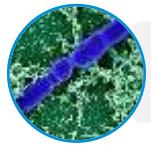
First Flavour Analysis

Development of **Spray Drying Models**

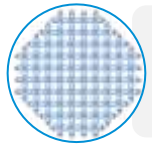
Development of **Cheese Proosdij**

Development of **The first low fat, low salt cheese**

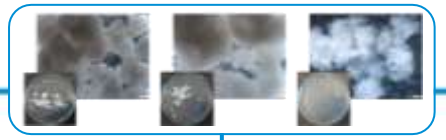
Plant Protein knowledge



Strict anaerobes HTS and upscaling



Advancement of **Casein Micelle Model**



***In Vitro* Gastric Digestion Tools** to characterise dairy and plant protein gastric behaviour

SPRINT project expansion of the food-grade pilot plant to **promote protein transition** and **valorization of by-products**

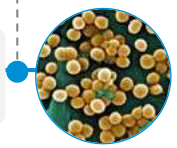
1948 1960 1970 1980 1990 1996 2004 2009 2014 2017 2019 2020 2023

Development of **NIZO Butter Process**

First heterologous enzyme expression in bacteria & yeast

Development of **Maasdammer Cheese** - a Gouda style specialty cheese

NIZO Starter Culture Collection



Complete **genome sequence** of *L.plantarum*

MicroCheese and other HTS platforms

Development of **0% FAT soft ice cream**

Development of **plant protein fibers** used in **meat replacement products**

Dairy-knowledge based development of **plant-based dairy alternatives**

Wheat Soy Potato RuBisCO Pea Faba bean Oil seeds Single cell

Our customers & partners

Integrated approach

Confidentiality

Connected in FoodValley and other eco-systems

Higher chance of success

Excellent track record

Excellent project management

Speed

Tailor-made solutions for your next step to market by combining science, technology & practice

75-year experience

Leading experts

Knowledge and innovations through consortia

5m€ recent investment on food-grade scaling up facilities for dairy and meat analogues

Expansion for Biotechnology Fermentation upscaling facility, including Precision Fermentation up to 10,000 l – Coming soon

Expertise in sustainable processing & upscaling, microbiology & fermentation, protein functionality, sensory and health.

The largest open-access food grade pilot plant in Europe & food application center

80+ specialized labs