

Powering the Evolution of Fermentation Processing

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Wageningen Food & Biobased Research



CLEAN LABEL
CONFERENCE



Outline

- Brief History of Fermentation
- Explanation of fermentation basics
- Examples of fermentation possibilities – including challenges
- An example of a method for HT screening in product matrices

Fermentation – an analogy (Rob Dunn) – big brain



Fermentation – an analogy (Rob Dunn) – adaptive metabolism



Fermentation – a brief History

10,000 BC



Spontaneous dairy
fermentation, North Africa



3,500 BC

Bread leavening, Egypt



500 BC

Moldy soybeans as antibiotic/koji,
China



1856

Fermentations require live
microorganisms, Pasteur, France

7,000 BC

Brewing, China



2,000 BC

Pickled cucumbers, Iraq



200 BC

Fermenting tea leaves, China
'Kombucha'

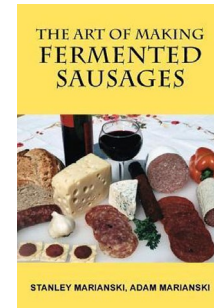


1665

First description microorganisms,
Van Leeuwenhoek,
The Netherlands



Fermented products - examples

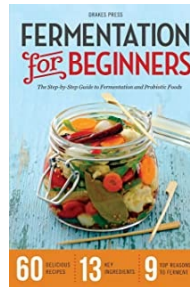
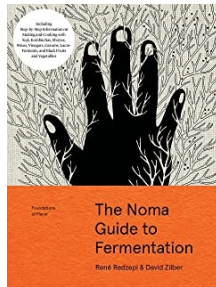


A little side-step: Probiotics

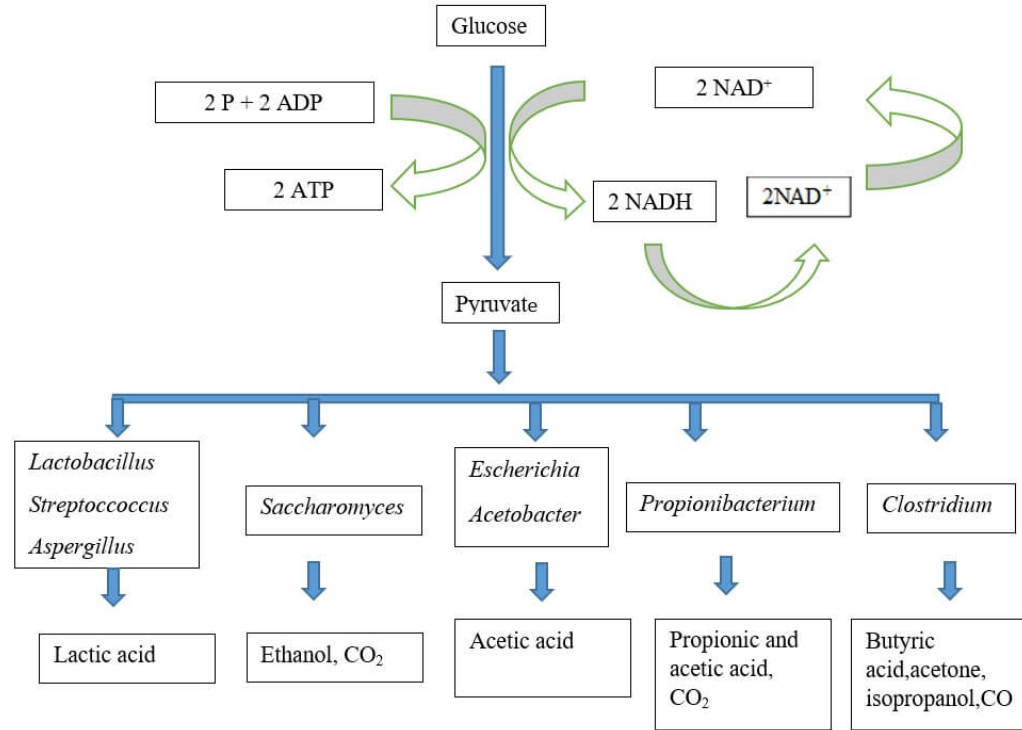
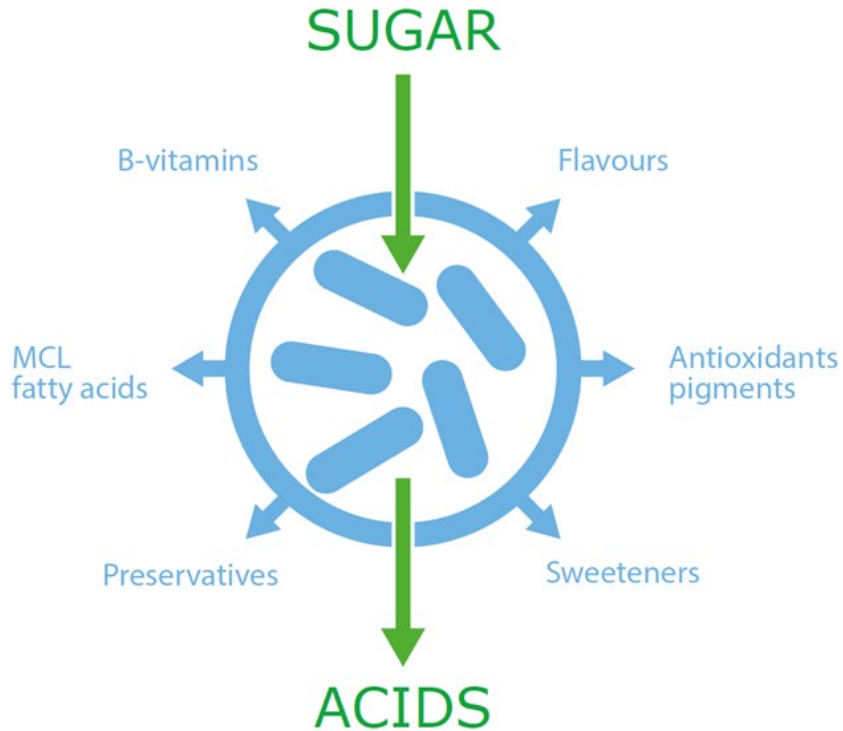
- 'Probiotics are live microorganisms that are intended to have health benefits when consumed or applied to the body.' (National Center for Complementary and Integrative Health)
- They can be found in yogurt and other **fermented foods**, dietary supplements, and beauty products.
- Health-benefits of fermented foods are generally accepted, but are not included here

Strong revival of interest in fermentation

- Never been away, but currently strong interest in fermentation:
 - Consumer interest: perceived as 'natural'
 - Producer interest: interesting as a means to improve products
 - Fast increasing popularity @home fermentations



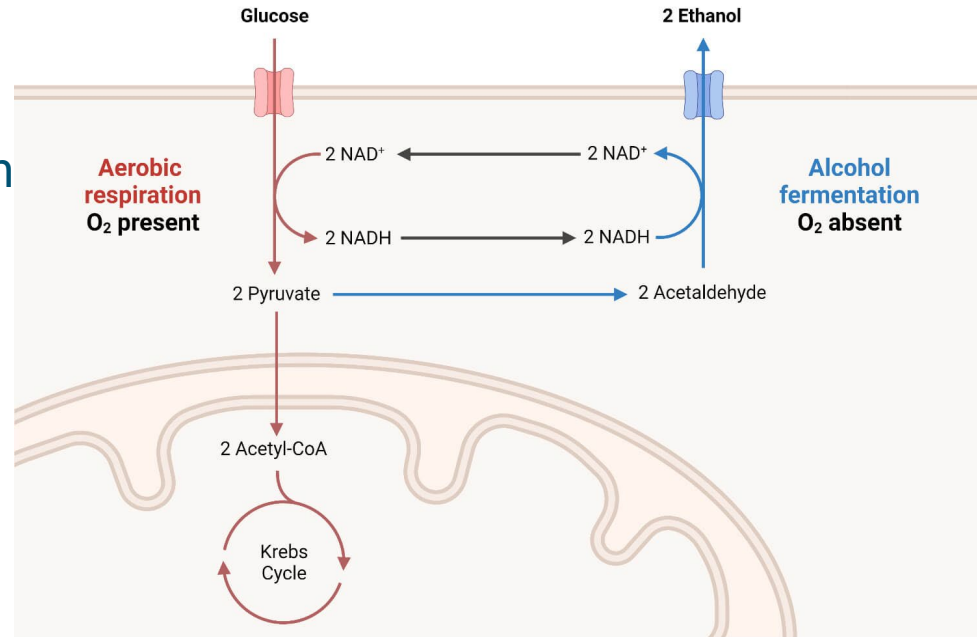
What is Fermentation?



<https://microbenotes.com/fermentation/>

Types of fermentation

1. Lactic acid homofermentation
2. Lactic acid heterofermentation
3. Propionic acid fermentation
4. Diacetyl and 2,3-butylene glycol fermentation
5. Alcoholic fermentation
6. Butyric acid fermentation



Types of Fermentation style

- Submerged or liquid Fermentation



- Solid State Fermentation (SSF)



Products of industrial fermentation

- Production of useful compounds:
 - Ethanol
 - Enzymes
 - Secondary metabolites (*e.g.* penicillin-derivatives, statins, etc.)
 - Organic acids (*e.g.* citric acid)
 - Flavours (vanillin)
 - Amino acids (food, feed, flavour)

Environmental fermentation

- Waste water treatment
- Bioremediation
- Air filtration
- Side-stream stabilization / usage
 - Biogas
 - Methane to methanol → substrate for further fermentation



Applications of fermentation in food

- Flavour: production preferred flavours or masking of off-flavours
- Production of low-caloric sweeteners
- Precision fermentation: production of animal protein by fermentation (*e.g.* casein)
- Microbial biomass and/or microbial protein
- Shelf-life extension
- Production of essential vitamins (B12)

Food fermentation

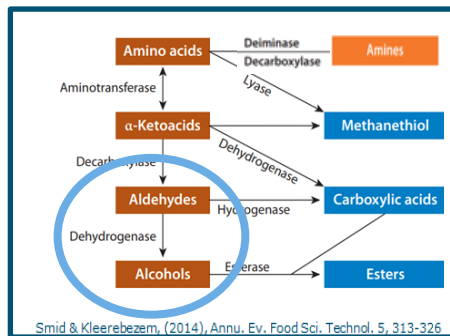


- The current transition from animal to alternative protein sources poses many challenges for maintaining quality
 - Plant-based protein sources:
 - Can lack essential vitamins
 - Often have off-flavours
 - Require many additions to obtain a tasty product
 - Long list of ingredients, a far-cry from a clean label product...

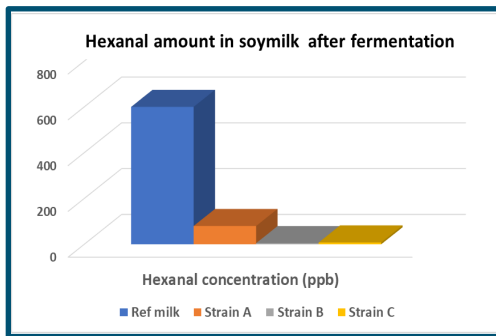
Flavour improvement – reduction off-flavours



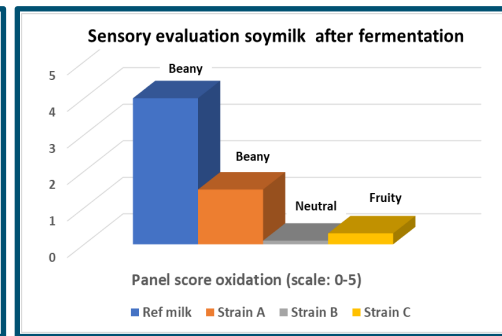
- Plant-based foods and beverages can have a "beany", "oxidation" flavour which is not appreciated by most consumers. This undesired flavour is caused by aldehydes and ketones as a result of oxidation.
- When aldehydes are microbially converted to corresponding alcohols, the off-flavour is nearly gone, because the flavour threshold of alcohols is much higher (50 x) than of the corresponding aldehydes.



Metabolic routes forming flavour compounds

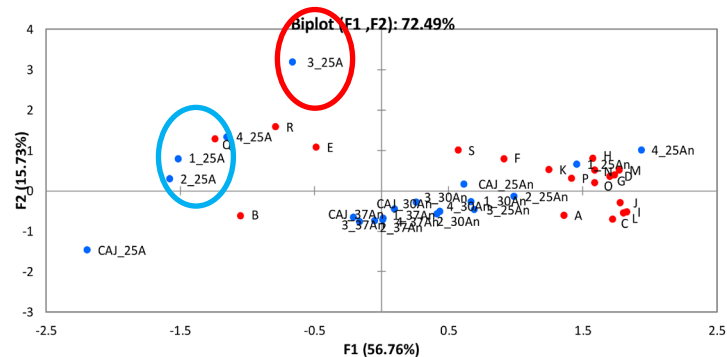
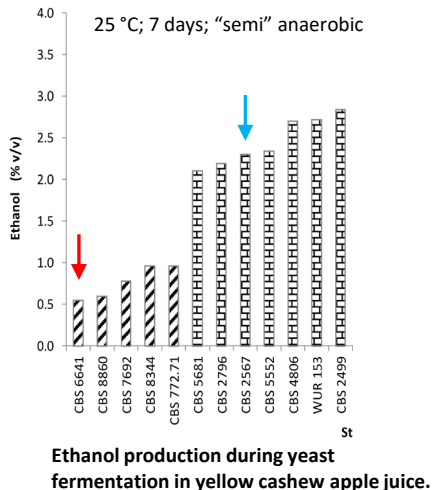
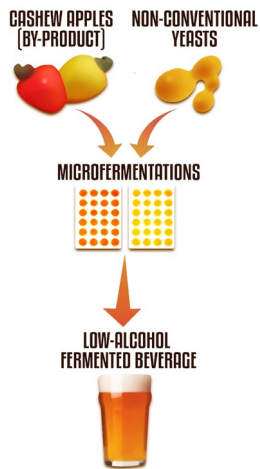


Lower hexanal amount after fermentation



Reduced "beany" flavour after fermentation

Example - Nutritious beverage from by-product



PCA of the aroma profiles of the different fermentations employing four selected strains.

Blue dots: CAJ: 1: CBS 2567; 2: CBS 2734; 3: CBS 6641; 4: WUR 102 under different grow conditions. Red dots: A to S indicate different key aroma compounds (see for more details Fermentation, 2019, 5, 71)

- Several non-conventional yeasts were screened for low-alcohol production and desired flavour profiles (aroma & taste).
- Two interesting yeasts identified: *Torulaspora microellipsoides* CBS 6641 and *Hanseniaspora guilliermondii* CBS 2567

Development of A Low-Alcoholic Fermented Beverage Employing Cashew Apple Juice and Non-Conventional Yeasts.

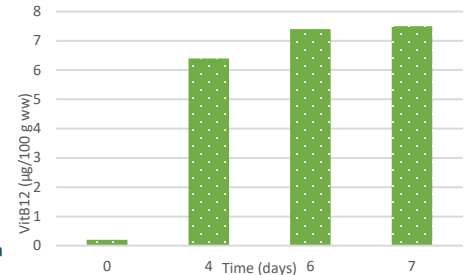
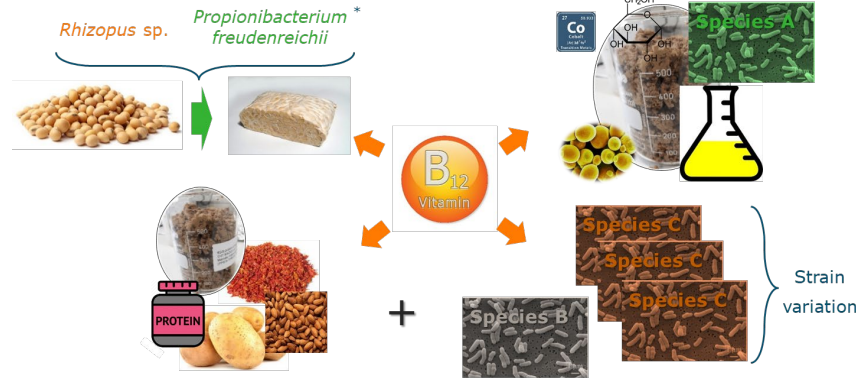
Ampero Gamero, Khas Ren, Yendouzen Landou, Catherine de Jong, Edith J. Smit, André R. Linnemann. Fermentation, 2019, 5, 71

Vitamin B₁₂ production

- Some essential nutrients like vitamin B₁₂ are by default lacking in plant-based protein-rich products and are therefore valuable targets for introduction via fermentation. Recent work indicated the potential of adding B₁₂ to food products by fermentation.

Vitamin B₁₂

Addition of 2 microorganisms resulted in the production of vitamin B₁₂ in a tempeh like product



akers, J. C. M., Endika, M. F., & Smid, E. J. (2018)

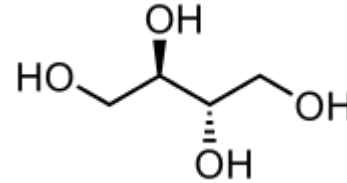


Low caloric sweeteners - stevia



- Derived from *Stevia rebaudiana*
 - Active compounds: steviol glycosides (mainly stevioside and rebaudioside)
 - 50 to 300 times the sweetness of sugar
 - heat-stable
 - pH-stable
 - not fermentable by gut microbiota
- Currently produced via precision fermentation using modified yeast
 - GMO free, labeling as 'stevia glycosides'

Low caloric sweeteners - erythritol



■ Erythritol

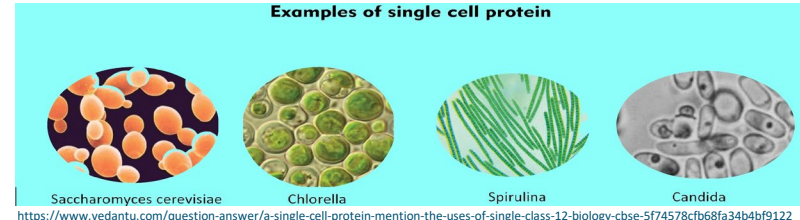
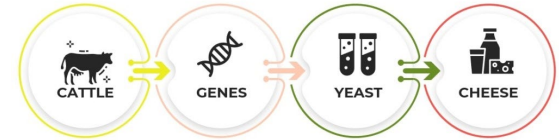
- Sugar alcohol or polyol, (close to) zero-calorie, not affecting blood glucose level or causing tooth-decay, 60-70% sweetness of sucrose
- Osmoprotectant as a cell response to high osmotic pressure of the environment
- Highly osmotic media (up to 40% of glucose) are applied to stimulate erythritol synthesis (*e.g. Moniliella pollinis* or *Yarrowia lipolytica*)

■ Fermentation principle: glucose to erythritol

- Potential application: sugar reduction *in situ*

Microbial protein

- Precision fermentation: production of 'animal' proteins by means of fermentation
 - Casein production in yeast
- Microbial biomass as protein source
 - Single-cell Protein
 - Yeasts, fungi, algae, bacteria
 - Microorganisms could be grown on side streams



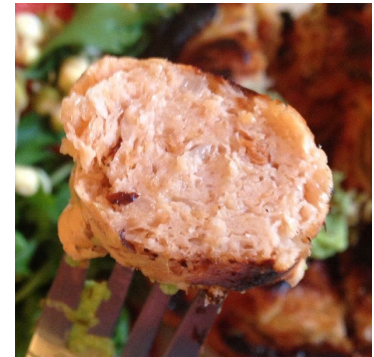
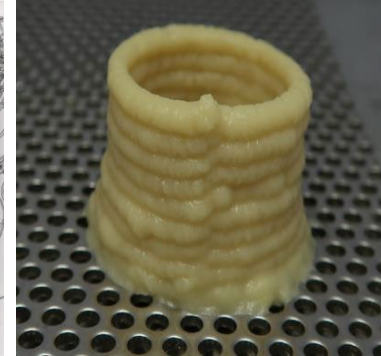
Microbial protein

- Microbial biomass as protein source
 - Mushrooms
- Microbial fabric
 - Fungal or bacterial



Microbial protein

- Microbial biomass as protein source
 - Mushrooms
 - Mycoprotein
 - 'Whole fungal biomass'
 - Extracted protein from fungal biomass



Challenges in Fermentation



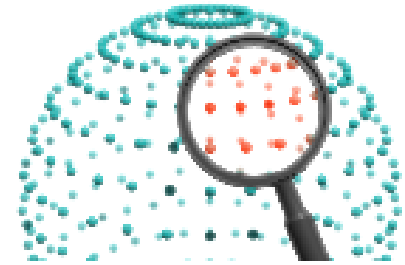
- Novel substrates and/or products pose unknown challenges
- Well-known what microorganisms can do in dairy-derived substrates
 - Yogurt, butter, cheese

→ Alternative protein sources? Unlikely that one will end up with similar tasting end-products

- **How to achieve similar tasting products?**



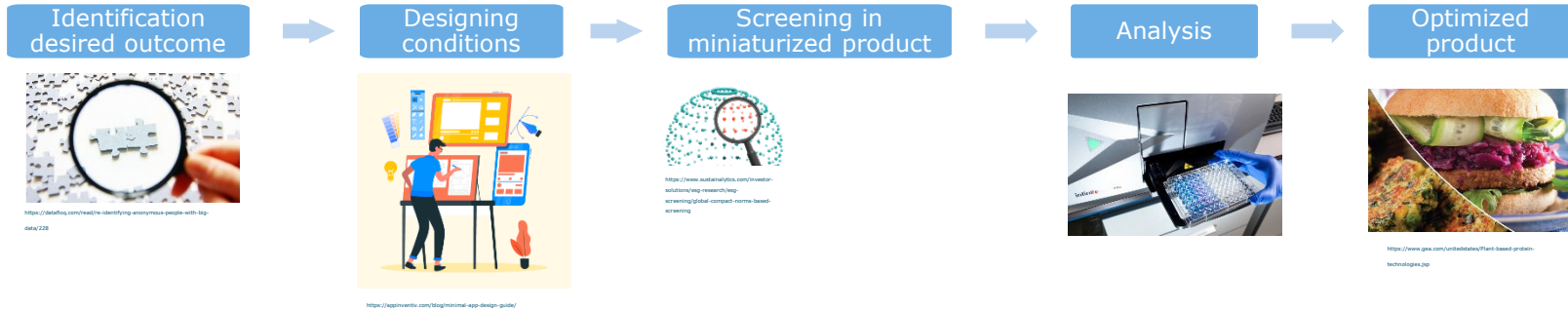
The importance of screening



- Difficult predicting the outcomes of novel fermentations
→ The answer lies in screening combinations of substrate with different microorganisms
- Pre-selection of most likely combinations
- Fermentation in relevant matrices and screening for functionality

MINIScreen platform

MINIScreen platform



- A fast and efficient screening platform allowing to screen for **fermentative improvements** of their products by **screening directly in the miniaturized version of the relevant product matrix of interest**.

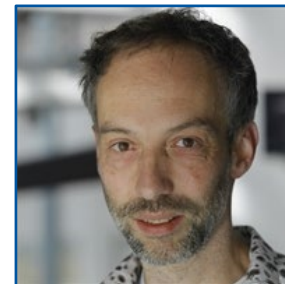
Overall goal MINIScreen:

Predict, design, and develop natural solutions to improve food products through the application of fermentation – Powering the Evolution!

Acknowledgements

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Thank you for your
attention!



To explore
the potential
of nature to
improve the
quality of life