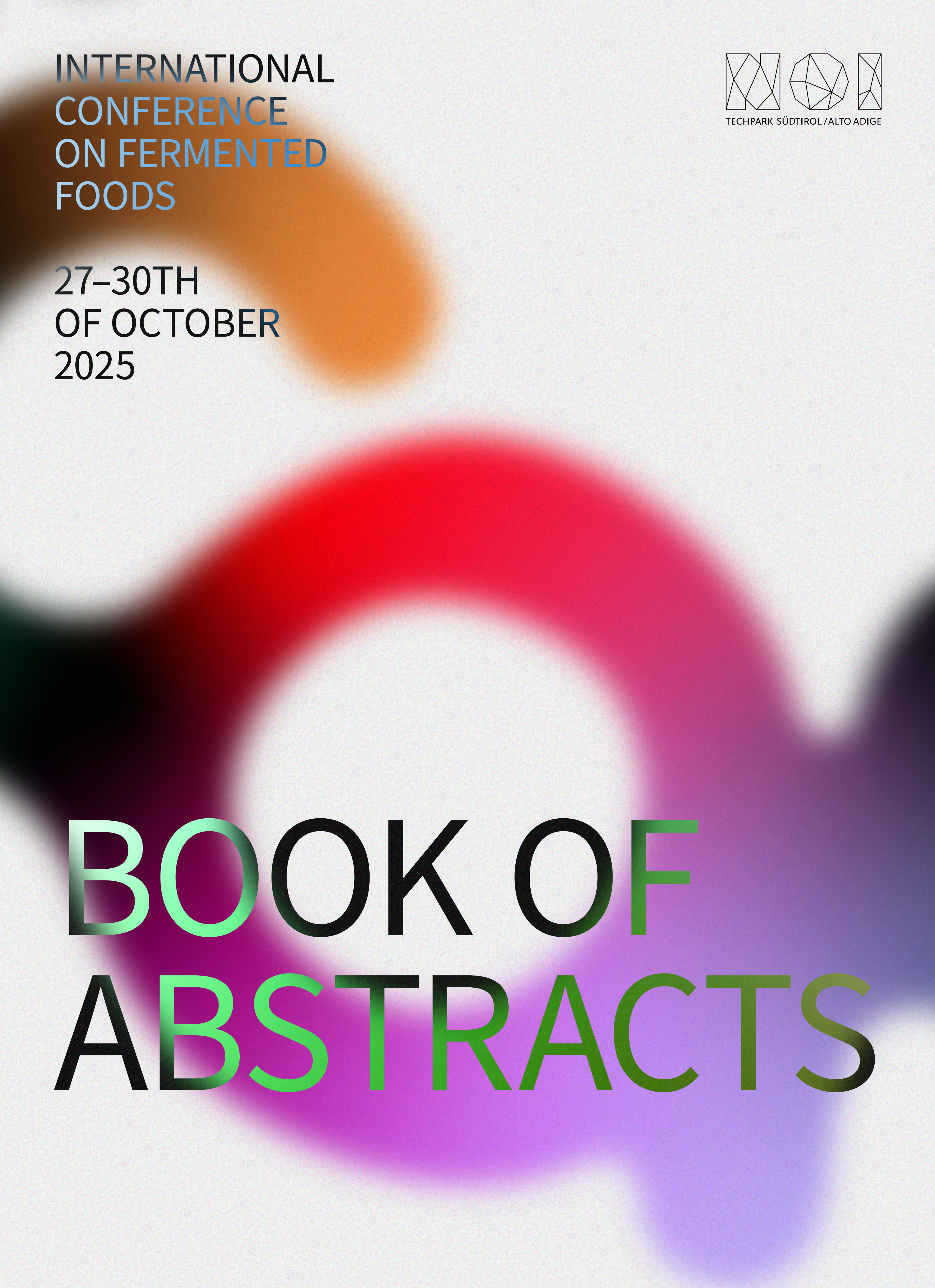
INTERNATIONAL
CONFERENCE
ON FERMENTED
FOODS

27–30TH
OF OCTOBER
2025

TECHPARK SÜDTIROL / ALTO ADIGE



BOOK OF ABSTRACTS

Foreword

● The 1st International Conference on Fermented Foods 2025 (ICFF 2025) is a unique worldwide opportunity that brings together leading experts, researchers, and industry professionals from all continents and many countries to delve into the latest advancements and innovations and future exploitation of the potential. Fermented foods, cornerstones of human nutrition for millennia, have witnessed a great resurgence of interest due to their profound impact on human health, food security and safety, nutrition, industrial business, and sustainability. Because of this importance, fermented foods still face evolving challenges, such as how to advance tradition into future foods with different perspectives; how to steer the processes and guide the microbiomes; how to develop innovative biotechnologies to impact human health, the global food safety, and security and precision; and how to exploit the potential for dignifying food waste and reducing food loss and create the next generation of sustainable foods, also responding to the need of using non-conventional protein sources. Sharing knowledge and best practices globally is vital for accelerating this transformative transition.

This dedicated conference on fermented foods is essential to address these challenges and capitalise on the growing opportunities. By bringing together researchers, industry professionals, and policymakers, we can foster collaboration, advance scientific knowledge, and drive innovation in the production and consumption of fermented foods. We are committed to fostering a vibrant exchange of ideas and knowledge sharing among attendees from diverse backgrounds. Through keynote presentations, oral and poster sessions, and interactive workshops, we aim to create a stimulating environment for sharing knowledge, establishing synergistic collaboration, and supporting networking.

ICFF will become a recurring event held every three years, with its permanent locations at NOI Techpark, aiming to be the unique reference for researchers and professionals in this sector. Because of the great relevance of fermented foods in human tradition, society, wellbeing and economy, ICFF will be the recurrent meeting where scientific achievements will be presented and discussed, and results transferred to industry.

Marco Gobbetti
Chief Scientist, NOI Techpark

Foreword

Dear participants,

Fermentation is one of humanity's oldest biotechnologies. For millennia, it has enabled us to preserve food, nourish communities, and create flavours that define cultures. Across the world – and especially here in South Tyrol – traditional dishes are rich with fermented ingredients, from cheeses and breads to cured meats and fermented vegetables. These foods are not only a testament to our heritage, but also to the ingenuity of generations who harnessed natural processes to ensure sustenance and safety.

Today, fermentation stands at the threshold of new opportunities. Thanks to advances in biotechnology, we can now understand and control fermentation processes with unprecedented precision. This opens the door to foods that are healthier, tastier, longer lasting, and more sustainable. By valorising by-products and optimising microbial activity, we are able to create products that meet the demands of modern consumers while respecting the environment.

Bozen/Bolzano has become renowned for its expertise in food fermentation. Our region’s research centres and companies are at the forefront of innovation, blending tradition with cutting-edge science. It is therefore a great honour for us to host the very first ICFF Conference here in Bozen / Bolzano. With this new initiative, we are bringing together the world’s leading experts in the field of fermented foods to share knowledge, spark new ideas, and build lasting collaborations in our region.

What awaits you at ICFF? Inspiring lectures from leading scientists and practitioners, engaging discussions on the future of fermented foods, and the chance to discover new approaches and solutions. The conference is not only a forum for exchanging expertise, but also a unique opportunity for networking – whether during the sessions, in informal conversations, or on our joint excursion, where you can experience South Tyrol’s landscapes.

We hope you enjoy your time in South Tyrol, find inspiration in the diversity of perspectives, and leave with new connections and ideas that will shape the future of fermented foods.

With best wishes for a successful and memorable conference,

Philipp
Achammer
Provincial Minister
responsible for NOI

Helga
Thaler Ausserhofer
NOI President

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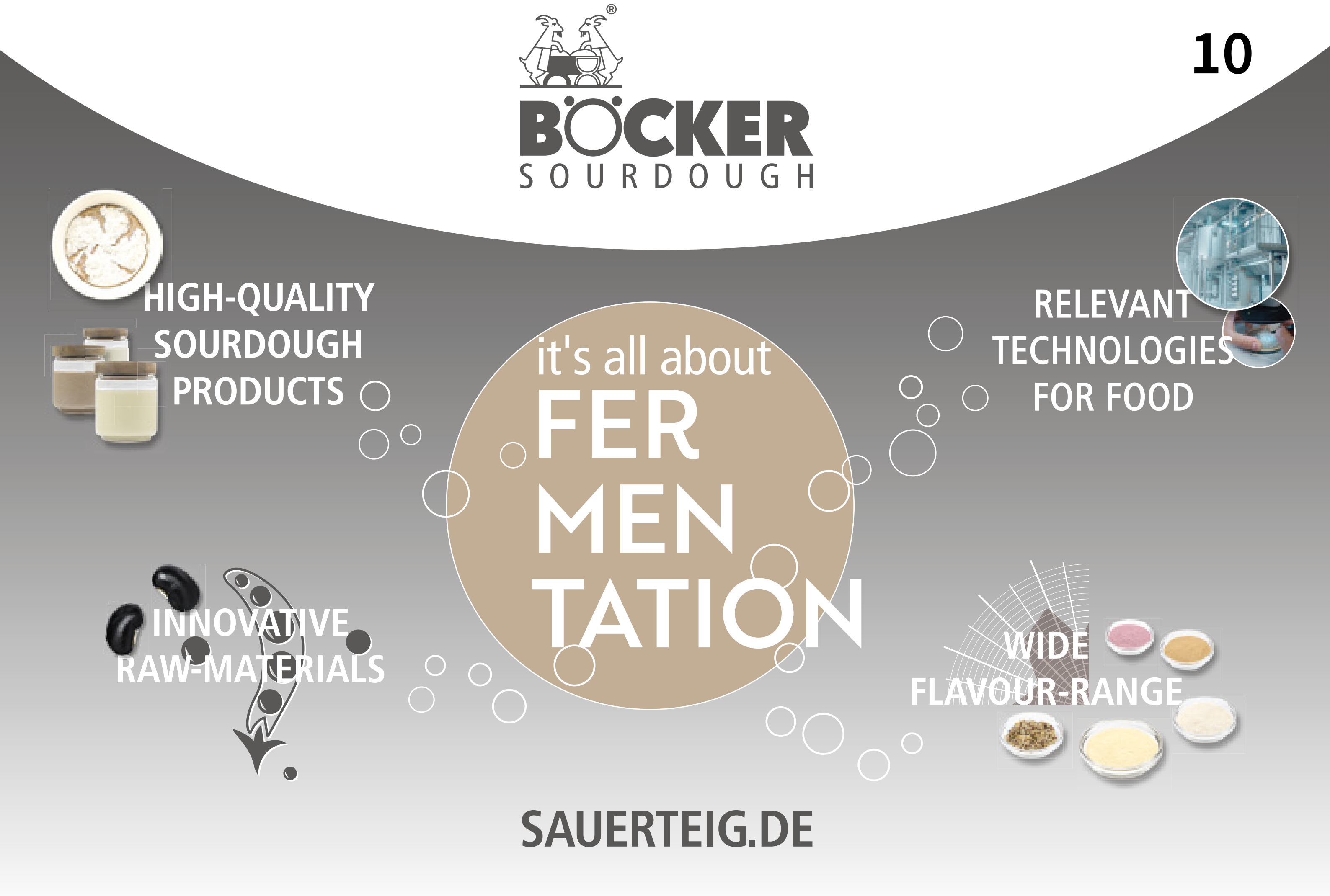
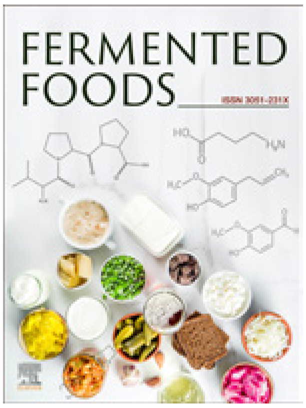
The magic of
Italian pastry art.
Bauli
IL PANDORO DI VERONA
SINCE 1922
MADE IN ITALY
NATURALLY LEAVENED WITH Bauli MOTHER DOUGH
Bauli
CHOCOLATE CROISSANT



Silver

CONSORZIO PER LA TUTELA DEL LIEVITO MADRE DA RINFRESCO



FERMENTED FOODS

GIULIANI INNOVATION

SACCO system

WHAT'S COOKING?

Bronze

Barilla
The Italian Food Company. Since 1877.

Dr Schär

mila SÜDTIROL

NSPulse
Natural Smarter Pulse

Pan

VOG PRODUCTS

ORAL PRESENTATIONS

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Plant Fermented Foods 15–28
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Deconstructing the complexity of health benefits from fermented foods.

[1] **Maria L. Marco**

● Fermented foods are frequently regarded to be healthier than the ingredients used to make them. However, there remain many questions on the unique contributions of fermentation to health and nutrition and the underlying basis for observed beneficial effects. This talk will emphasize current knowledge on the impacts of fermented foods on health, spanning human studies to mechanistic research on individual foods and bioactive compounds. Steps to include fermented foods in dietary guidelines and opportunities to design fermented foods for specific benefits will be discussed.

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Kimchi and its industrial production.

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● Kimchi, a Korean fermented food, is made by fermenting napa cabbage, radish, and vegetables with spices including garlic, ginger, salted seafood, and chili powder. With centuries of history, kimchi is essential to the Korean diet. Its global popularity has grown due to recognized health benefits. Kimchi varies in ingredients and preparation by region and season, with types such as Baechu, Kkakdugi, Gat, and Yeolmu Kimchi. Traditionally homemade, today food manufacturers with mass production systems produce various kimchi types hygienically. The Korean kimchi market size is USD 1.2 billion with major companies like Daesang Jonga, CJ CheilJedang, and Pulmuone leading. In 2024, South Korea’s kimchi exports exceeded USD 163 million, a record high, with Japan, the United States, and the Netherlands as major destinations. Kimchi fermentation involves lactic acid bacteria, mainly Lactobacillus and Leuconostoc. Recent research explores the probiotic functionality of kimchi-derived lactic acid bacteria, reporting anticancer, anti-obesity, and anti-inflammatory effects. Research to develop customized starter cultures for improved and standard kimchi fermentation is ongoing. Rich in fiber, vitamins, minerals, and bioactive compounds from fermentation, kimchi offers health benefits like improved gut health and stronger immunity. The future kimchi industry will likely evolve around standardizing taste and quality, advancing distribution and preservation, and developing new products for diverse consumer demands. Further scientific research on kimchi‘s health benefits will strengthen its global competitiveness. This presentation aims to show the excellence of Korean kimchi to the international fermentation science community and provide a platform for academic exchange for the continuous development of the kimchi industry.

Enhancing techno-functional properties of pea flour through combined enzymatic and lactic acid fermentation for a novel vegan spread.

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● This study aimed to optimize a biotechnological process for developing a pea flour-based ingredient and evaluate its potential for plant-based spread (PBS) production, addressing sustainability challenges in the agri-food sector. The process involved enzymatic hydrolysis using a commercial protease (VERON PS) to release bioactive peptides and enhance protein digestibility.

Fermentation with *Leuconostoc pseudomesenteroides* DSM 20193, combined with in-situ dextran synthesis from sucrose, improved viscosity, texture, and nutritional properties. The optimized ingredient, enzymatically treated and fermented yellow pea flour (YPVF), demonstrated superior functional properties and was incorporated into a PBS formulation. Defatted durum wheat germ and almond flour were added to enhance protein content and lipid profile. The PBS was analyzed for physicochemical, rheological, microbiological, and sensory properties. It exhibited high protein (12.49 g/100 g; 38.5% of total energy) and fiber (11.01 g/100 g) content, meeting EC Regulation 1924/2006 nutritional claims for “high fiber”, “high protein,” and “high unsaturated fatty acid content” (85% of total fatty acids).

Sensory evaluation indicated a balanced flavor with effective masking of legume off-flavors and a stable texture, eliminating the need for additional structuring agents. The PBS maintained microbiological safety and stability for 10 days under refrigerated storage. This study highlights the potential of *in-situ* synthesized dextran (up to 6 g/100 g) as a clean-label thickener, enhancing structure and texture while ensuring product stability. The developed PBS represents a nutritionally valuable and sustainable alternative to dairy-based spreads, supporting the transition toward plant-based food solutions.

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Exploring yeast communities in alcoholic beverages using the FoodMicrobionet database.

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● Since the first paper describing the microbiota of pearl millet slurries in 2010, the number of publications using amplicon targeted metagenomics to describe food microbial communities has been exponentially increasing, but data on fungal communities are far less abundant than those on bacterial communities. We introduced data on fungal communities in the FoodMicrobionet database, whose version 5.1 includes 290 studies, 62 of which containing data on fungi, with abundance data on >6,500 fungal taxa in >2,680 food and environmental samples. Here we illustrate its new features and use it to investigate the distribution of yeasts and their association networks in alcoholic beverages.

Data on fungi were available for wine, beer, cider, peach wine, pulque, palm wine, honey wine, alcoholic beverages produced from rice and their starters, raw materials and food environments. Of the fungal taxa detected, approx. 12% were yeasts. Many genera were shared between environmental and food samples. Given the limitations of cultivation independent approaches targeting DNA, it is hard to infer which actively participated in fermentations. We used inference of microbial association networks to identify co-presence or mutual exclusion patterns using 3 different methods (sparCC, CCREPE and SPIEC-EASI). Many associations were preserved across multiple studies and a consensus network was built. By combining the results of prevalence and abundance and those of conserved associations we were able to select a subset of non-conventional yeasts belonging to the genera *Candida, Debaryomyces, Hanseniaspora, Kazachstania, Lachancea, Pichia, Metschnikowia, Starmerella, Wickerhamomyces,* which are promising candidates for the selection of novel starter cultures.

Fermentation of coffee beans and its impact on the flavour of coffee.

[1] **Jian Zhao**
[1] Hosam Elhalis

● Harvested coffee cherries undergo a natural fermentation process known as primary processing, which can be carried out using the wet, dry, or semi-dry methods. This fermentation serves two critical functions that directly impact coffee quality. First, it facilitates de-mucilage, the removal of the pulpy mucilage adhering to the coffee beans. Second, it leads to the production of secondary metabolites, particularly volatile aromatic compounds that contribute to the sensory characteristics—especially flavour—of the final coffee product. A diverse range of microorganisms participate in coffee fermentation. Bacteria such as *Klebsiella, Erwinia, Bacillus, and Lactobacillus spp.,* along with yeasts including *Pichia, Saccharomyces,* and *Candida* spp., are frequently identified. Many of these microbes exhibit pectinolytic activity, which likely contributes to mucilage degradation. However, the role of specific microbial species in producing flavour enhancing volatile compounds remains less well understood. In our study of microbial communities and metabolite profiles during coffee fermentation, we found that yeasts play an essential role in the process. We isolated six major yeast species and evaluated their abilities in de-mucilage, production of desirable volatile compounds, and tolerance to fermentation stresses. Among these, Pichia kudriavzevii and Hanseniaspora uvarum stood out due to their superior attributes. We then conducted inoculated fermentations using these two yeast species and produced coffee with excellent sensory qualities, as assessed by certified professional coffee graders. These findings suggest that *P. kudriavzevii* and *H. uvarum* have strong potential for development as starter cultures in controlled coffee fermentations aimed at consistently producing high-quality coffee.

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Bacillaceae in ropy bread spoilage: taxonomic diversity, virulence factors, and spoilage dynamics

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● Bread exemplifies the intricate relationship between microorganisms and food, as they influence flavor, texture, and human health. With rising global temperatures, the re-emergence of ropy bread spoilage presents a growing concern. This phenomenon has been linked to several species of aerobic spore-forming (ASF) bacteria. Beyond economic losses, potential health risks may arise, as virulent ASF strains are not uncommon. However, studies addressing the accurate identification of responsible ASF and systematic approaches to quantify their spoilage characteristics are lacking.

To address these gaps, 82 ASF isolates from raw materials, bakery environments, and culture collections were identified using MALDI-TOF MS and confirmed by 16S rRNA, gyrA, or panC gene sequencing. All strains underwent proteotypic and genotypic fingerprinting and were screened for virulence-associated traits, including swarming motility, hemolytic activity and enterotoxin genes. Their rope-inducing potential was evaluated in a model system, and 13 representative strains were further tested in situ by baking endospore-spiked dough.

This study revealed significant inter- and intraspecies variability and added *B. velezensis, B. inaquosorum,* and *B. spizizenii* to the list of species implicated in rope spoilage. Swarming motility, hemolytic activity, and enterotoxin genes were widespread among the tested strains. Additionally, texture analysis, colorimetry and HS-SPME-GC-MS indicated strain-specific spoilage behaviors. Remarkably, specific *B. subtilis* strains were capable of inducing rope formation within 24 hours at contamination levels as low as 10 spores/g.

By introducing a standardized protocol to quantify strain-specific spoilage characteristics, this work significantly advances the understanding of rope spoilage and provides a strong basis for future research.

Beyond fatty acids: new insights into bacterial fatty acid derivatives using avocado fermentation as a model system

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[1] Elisabetta Trossolo
[2] Stefano Tonini
[4] Giuliana Garofalo
[4] Vittorio Farina
[4] Luca Settanni
[1+2] Marco Gobbetti
[1+2] Raffaella Di Cagno

● Despite their relatively week lipolytic activity in comparison to other bacteria and fungi, lactic acid bacteria (LAB) have been already reported to convert fatty acids (FA) into hydroxy or oxo-derivatives with additional functionalities in fermented sausage, fermented milk, cheeses and sourdough. In this study, we investigated the lipid metabolism of three LAB in fermenting two avocado cultivars. Using Liquid Chromatography-High-Resolution Mass Spectrometry, we tracked the fermentation-induced modifications in native lipid profiles. These modifications unveiled an increase in free FA but also the generation of their oxidized derivatives associated with 14 different m/z ratios. A total of 57 species were recognized through the interpretation of tandem mass spectrometry data, corresponding mainly to mono-, di-, and tri-hydroxylated derivatives originating from major unsaturated FA (18:1, 18:2, 18:3), along with oxidized forms of 16:0 and 18:0 FA. Tandem mass spectrometry also suggested oxidized derivatives from minor FA (16:1, 16:2). In certain cases, bacterial-driven hydroxylation processes were confirmed on oleic acid in synthetic media. Overall, bacterial-driven hydroxylation and oxidation enriched avocado with potentially bioactive lipids, offering opportunities for nutraceutical and food applications, with minimal undesirable sensory effects specifically affected by microbial species and avocado cultivar.

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Lactic fermentation as a biovalorization strategy for blue crab *(Callinectes sapidus)*: from garum production to antimicrobial extracts.

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● The blue crab (*Callinectes sapidus*), an invasive species in the Mediterranean, poses ecological and economic threats but offers promising potential for valorization due to its nutritional richness and bioactive components. This work investigated the applicability of lactic acid fermentation to enhance the value of both its meat and shell. A crab meat-based garum was developed using lactic acid bacteria (LAB) starters (*Lactiplantibacillus plantarum* and *Lactobacillus helveticus*) and fermented for 90 days. However, indigenous microbes ultimately dominated, resulting in the formation of complex aroma profiles with “cheesy” and “sulfuric” notes, as revealed by HS-SPME/GC-MS. Despite encouraging flavor development, high levels of biogenic amines highlighted food safety concerns. On the other hand, fermentation of hydrated, dried crab shells with six LAB strains showed microbial stability and acidification, buffered by inherent calcium carbonate. Ethanol extracts from fermented shells inhibited Salmonella enterica, with the strongest effect observed in *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei* fermented samples. Challenge tests in fish-based products confirmed antimicrobial activity of the extracts, especially under mild thermal abuse. Additionally, fermentation altered the volatile profile of shell extracts, indicating potential for both preservation and flavor enhancement in seafood products. These findings support lactic fermentation as a dual-purpose tool: transforming an ecological threat into a nutritional and functional food ingredient, while promoting sustainable seafood waste utilization. Further studies, including metagenomic and compound-specific analyses, are essential to optimize process control and bioactivity.

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Valorising olive oil mill wastewater as a functional ingredient in traditional Italian bread to mitigate oxidative stress associated with red meat consumption.

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[2] Giuseppe Avellone
[1] Raimondo Gaglio
[2] Luisa Tesoriere
[1] Luca Settanni

● Despite increasing efforts to manage agro-industrial waste, olive oil mill wastewater (OOMW) remains a major environmental and economic concern. This liquid by-product of olive oil production is rich in bioactive compounds, especially polyphenols, but is often underutilized. In this study, OOMW from organically grown olives was repurposed as a functional ingredient in sourdough bread, supporting circular economy principles. Two experimental breads were made by replacing water with 50% (EXP1) and 100% (EXP2) OOMW. Fermentation used a *biga*-like starter with lactic acid bacteria (LAB), including strains from OOMW, and yeasts. Both breads showed improved aroma profiles, with higher levels of alcohols (e.g., ethanol), carbonyls (e.g., benzaldehyde), esters, and terpenes. OOMW also enriched the breads with phenolic compounds like hydroxytyrosol, coumaric acid, and caffeic acid, absent in the control bread (CTR). These compounds boosted antioxidant capacity, measured in Trolox equivalents. Given concerns about oxidative stress from oxidized dietary lipids, the antioxidant potential of the breads was tested using a gastric digestion model with cooked red meat. Preliminary results indicate that the experimental breads can effectively reduce lipid oxidation, suggesting their potential role in mitigating oxidative stress and promoting health. This is particularly relevant given the frequent co-consumption of meat and bread in composite meals such as hamburgers and kebabs. Sensory analysis revealed strong consumer preference for the OOMW breads due to their olive oil-like aroma and flavor, remembering traditional olive bread. This study highlights a sustainable approach to valorise OOMW, turning a waste product into a valuable ingredient for functional food innovation.

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Bio-valorisation of swiss yellow peas through fermentation with functional microorganisms for sustainable food applications.

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● The growing demand for sustainable, plant-based protein sources has brought pulses into focus. Despite their nutritional benefits – high protein content, dietary fiber, and essential micronutrients – they also contain fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs), which can cause gastrointestinal discomfort. Additionally, plant-based diets often lack adequate amount of vitamin B12 and folate. Addressing these nutritional and functional limitations is a key challenge for pulse-based ingredients. The CREATE project aims to develop a holistic bio-valorization strategy for whole Swiss yellow peas using lactic (LAB) and propionic (PAB) acid bacteria combined with yeasts capable of degrading FODMAPs and producing vitamin B12 and folate. A total of ca. 500 strains were screened for their functional potential and selected strains applied in 100-g lab-scale pea-flour fermentations, where up to 100% of galactooligosaccharides (GOS, FODMAPs) were successfully degraded. Moreover, 120 ng vitamin B12 and 700 ng folate/g DM were quantified in the fermented pea flour, corresponding to 5% of the RDA for vitamin B12 (2.4 µg/day) and 0.175% for folate (400 µg/day) per g DM fermented pea flour, respectively. In a proof-of-concept, the processing of fermented pea flour into food prototypes such as meat alternatives, pasta and snacks was tested. While only limited substitution (~10%) of commercial pea protein was feasible in meat alternatives due to texture loss, fermented pea flour showed promising performance in pasta and snack applications, achieving full (100%) flour replacement with desirable textural qualities. These findings suggest that targeted microbial fermentation can enhance the nutritional profile and application range of yellow peas. Future work will focus on process optimization, scale-up, and exploring consumer acceptance to promote circular, health-promoting food systems.

Okra *(Abelmoschus esculentus L.)* flour integration in wheat-based sourdough: effect on nutritional and technological quality of bread

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● Okra, commonly known as gumbo or lady's finger, belongs to the Malvaceae family and is an easily available, renewable and inexpensive natural source. It is consumed in the Mediterranean region and can be considered as a functional food since it is rich in minerals, protein, fat, phenolic compounds and polysaccharides. The aim of this study was to develop an innovative sourdough using dehydrated okra pod flour and to use it in the production of bread. Three different flours (sun-dried S, freeze-dried F, oven-dried O) were individually mixed at 9% with wheat flour (Dough Yield 300) and fermented (N0: 8.0 log10 CFU/g) for 14 h, using *Lactiplantibacillus plantarum* ITM21B, *Weissella cibaria* C43-11 or *Leuconostoc mesenteroides* C43-2M. The results showed that after fermentation, the content of organic acids (lactic, acetic and propionic), exopolysaccharides (EPS), l-glutamic acid and total free amino acids (TFAA) increased and the high molecular weight proteins were converted into smaller proteins. Sourdough based on *Leuc. mesenteroides* and O flour (O_LeuMes) was selected to evaluate its applicability in bread making. It was included in the yeast leavened bread formulation at 20 or 40% (0.6% and 1.21% w/w O flour replacement). Results showed that fermentation limited the negative effects of unfermented O flour on bread quality attributes, mainly the specific volume and firmness. Bread with O_LeuMes at 40% was improved in TFAA, EPS and l-glutamic acid content and showed a higher specific volume and lower moisture and firmness compared to bread with the unfermented O flour.

Innovative food fermentation: case study on fermented tropical mango for fruit fillings

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● At Puratos, we believe that food has the power to nourish, comfort, and bring people together. 60% of consumers are looking for new food experiences according to Puratos Taste Tomorrow global consumer research. At Puratos fermentation is one of the strategic pillars when it comes to generating innovative ingredients for bakery, patisserie and chocolate. Two case studies will be described to show the importance of food fermentation to bring new innovative solutions to consumers worldwide.

A case study related to fruit fermentation, more in particular mango fruit pieces were fermented to generate excellent taste, nice texture and color and techno-functional properties. An in depth screening assay has been setup for 1000 bacteria and 1000 yeast strains to assess their capability to ferment mango fruit pieces. Properties like flavour profile, alcohol content, gas formation, etc were studied. Selection of a specific *Lactiplantibacillus plantarum* LMG S-33688 strain and optimization of the fermentation process resulted in a 100% natural mango fruit filling, able to be used in different patisserie and bakery applications.

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Fermented legume-vegetables as seasoning ingredient.

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● Onions and other vegetables are a characteristic ingredient for seasoning applications such as soups and sauces, especially when roasted or stewed. For industrial processes and as an ingredient in convenience foods, the preferred form is powdered. Such dried powders are often produced from fermented products such as yoghurt or sourdough. Various drying techniques are used. Besides spray drying, lyophilisation is used as a particularly gentle process, while roller drying is regarded as the industrial version of the frying pan.

We have carried out fermentations of red lentils and onions with a consortium of yeast and lactic acid bacteria. After fermentation, the products were freeze-dried or roller-dried. During fermentation, the concentration of asparagine, a precursor of acrylamide, decreased, whereas arginine, glutamic acid and aspartic acid increased. In lyophilised samples, the concentrations of free amino acids and sugars were higher than in roller-dried samples. The reddish colour of red lentils was reduced by roller drying, where the roasted flavour was enhanced.

The composition and flavour profile of vegetables can be modulated by fermentation through the choice of microbiota and processing conditions. This can be further enhanced by the choice of drying technique, considering thermal reactions.

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Sonmat, a citizen-science enabled Kimjang kimchi case study on associations between hand and kimchi microbiota.

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● Vegetable fermentation has been a staple of human culture for the past centuries, with deeply rooted traditions behind them. However, the effect of these traditional practices on the microbes in the final fermented product, and their origin are often not understood. Kimjang kimchi is an important societal tradition in Korea during autumn to preserve harvested vegetables during colder winter times. The origin of the microorganisms that contribute to the flavor and safety during fermentation is still unclear. Although, bacteria present on the raw ingredients are considered to be important colonizers of the fermentation, in Korean culture, the term “Sonmat” is often used, which literally translates into ‘hand flavor’, suggesting a role for hand microbiota in the kimchi fermentation. In this citizen-science project, we explore the impact of the hand microbiome on kimchi fermentation during the Sonmat festival organized in Belgium. The kimchi fermentations contained mainly lactic acid bacteria belonging to the genera *Leuconostoc, Weissella* and *Latilactobacillus*. Associations were found between the relative abundance of *Staphylococcus* on the hand and relative abundance of *Latilactobacillus* and *Leuconostoc* found in kimchi, despite limited overlap between hand and kimchi microbiome. In addition, different microbiota were found to dominate the kimchi made following traditional group Kimjang practices compared to individually prepared kimchi. These findings pave the way for future research into how traditional practices and the skin microbiome influence the unique qualities of kimchi, offering exciting possibilities for enhancing fermentation processes and cultural food heritage through citizen science.

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Technological characterization and safety evaluation of acetic acid bacteria isolated from artisanal kombucha.

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● Kombucha, a traditional fermented beverage originating from Manchuria, is produced by fermenting black or green tea with a symbiotic culture of acetic acid bacteria lactic acid bacteria, and yeasts, embedded within a cellulose-based biofilm. In this study, artisanal kombucha samples were collected from two restaurants in Denmark and Italy. Their chemical composition was analyzed, and microbial groups were enumerated using culture-dependent methods. Microbial isolation in pure culture yielded yeasts from five different species and acetic acid bacteria from three species. No lactic acid bacteria were retrieved. Acetic acid bacteria isolates were investigated for their viability and cultivability under starvation conditions at pH values ranging from 3 to 7 for two months. The viability was assessed using flow cytometry. Among the bacterial isolates, *Komagataeibacter intermedius* TKA5 was the only one capable of producing cellulose in pure culture. Cellulose production and the growth kinetics parameters of *K. intermedius* TKA5 were further tested in kombucha substrate both in monoculture and in co-culture with yeast. Additionally, all bacterial isolates were evaluated for antimicrobial sensitivity. The results showed that the bacterial isolates exhibited a significant higher survival under acidic conditions compared to neutral pH. After seven days of starvation, the bacteria entered a viable but non-culturable (VBNC) state while remaining metabolically active, a state that was not reversible under the tested conditions. Most isolates showed low sensitivity to nalidixic acid and ciprofloxacin, whereas they were sensitive to chloramphenicol, ampicillin, kanamycin, streptomycin, tetracycline, apramycin and gentamicin.

The overall data collected highlighted the viability in acid condition of kombucha isolates, with potential implications for enhancing the microbial stability and functionality of this fermented soft drink.

The challenge of microbiome biobanking and storage: the case of fermented sausages

[1] **Luca Cocolin**

● Microbiome has shown great potential to address food system challenges and there is evidence that microbes with their specific functional activities can have positive impacts. Cataloguing single microorganisms is a well-established procedure and there are international initiatives that overlook the biobanking and exploitation of single microbial strains. On the other hand, very few attempts have been carried out to address the challenge of preserving entire microbiomes. Within a national research project titled SUS-MIRRI (www.sus-mirri.it), funded in the frame of the NextGeneration EU, a specific work package was designed to investigate the preservation of microbiomes from different ecosystems, so to understand the possibility of their valorisation and reuse. In this frame, samples of fermented sausages were prepared following different procedures, and they were stored at -80°C for a period of 12 months. Every 3 months, plate counts and 16S rRNA gene sequencing were performed to determine the viable populations and to profile the microbiota of the sausages after storage. In addition, a propagation in different growth media was also carried to understand the possibility to produce an inoculum to use in the fermented sausage production. The results obtained underlined how the storage procedure was successful to maintain the initial populations in a viable state respecting the original microbial ecology. Regarding the propagation step, a great relevance was given by the population complexity of the initial sample. For samples with simple microbial ecology it was possible the propagation, while an increase in the microbial diversity impacted negatively the population balance.

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The success of cold-smoked salmon biopreservation depends on the food culture and the microbiome signature of the processing plant.

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● Cold-smoked salmon (CSS) industry, a major activity in Europe, is facing microbial problems, notably the possible presence of *Listeria monocytogenes* (Lm) and degradation of sensory quality before the end of shelf-life. In this study, five commercial or private collection Food Cultures (FC) or FC cocktails with different antimicrobial mechanisms have been inoculated alone or with Lm into CSS slices. The strains belong to *Carnobacterium divergens, C. maltaromaticum, Latilactobacillus curvatus* and *Lactococcus piscium* species. The experiments were repeated in CSS from 2 smokehouses with varying endogenous microbiota profiles, biochemical composition and sensory evolution. Cultural microbiology and metabarcoding analysis confirmed the establishment of Carnobacteria in the CSS, while *L. curvatus* and *L. piscium* were less prevalent. Except *L. curvatus*, the FC significantly inhibited Lm with no significant plant effect. With regard to spoilage, none of the FCs acidified the CSS. They all prevented strong unpleasant odor and taste at the end of shelf-life in one batch, but certain strains, including *L. curvatus* and some *C. divergens* and *C. maltaromaticum*, were detected by trained panelists in an unaltered batch, disqualifying them from final selection. The correlation between FC efficacy and the inhibition of the spoiling genera *Brochothrix, Yersinia, Serratia* and *Photobacterium* was unclear. In term of safety, none of the FC produced histamine but some did produce tyramine, posing a risk for sensitive individuals. For the first time, a non-tyramine-producing mutant of *C. divergens* met all the criteria for use in disease and spoilage control applications. Using a cocktail of FC did not improve the protective effect.

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Microbiota associated with commercial dry-aged beef in France.

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● Meat dry aging consists in storing unpackaged meat in a cold room and at a specific and controlled relative humidity (RH), for a period of 1 to 5 weeks or more. We examined the bacterial and fungal microbiota of dry-aged beef at the commercial stage by both culture-dependent and -independent approaches. Fifty-eight samples of dry-aged meat from different producer types (meat processing plants, artisan and supermarket butchers) were studied. Microbial groups were enumerated on various dedicated media. Yeast and mold isolates (n=257) were identified by sequencing of taxonomically relevant genes. Metagenetic analyzes targeting the V3-V4 regions of 16S rDNA and ITS2 were also performed. The aerobic colony count varied between 1.97 and 10.91 log10 CFU/g and was similar to that of Pseudomonas spp., indicating that this bacterial group was dominant. Yeast populations varied between <2 and 9.41 log10 CFU/g, while molds showed abundances between <2 and 7.7 log10 TFU/g, the highest values being found in meats matured with a high RH. Bacterial and mold counts were positively correlated with the dry-aging RH and, to a lesser extent, temperature. The main yeast species were *Candida zeylanoides* and *Yarrowia alimentaria* as well as *Itersonilia pannonica* (identified only in metagenetics). The dominant mold species were psychrophilic or psychrotrophic species, namely *Mucor* complex flavus and *Helycostylum pulchrum/elegans* previously shown to be associated with these products. This study identified the main microorganisms associated with dry-aged meat in France, which raises the question of their role in the organoleptic quality of these higher value products.

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Dairy foods – from old traditions to new challenges.

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● Meat dry aging consists in storing unpackaged meat in a cold room and at a specific and controlled relative humidity (RH), for a period of 1 to 5 weeks or more. We examined the bacterial and fungal microbiota of dry-aged beef at the commercial stage by both culture-dependent and -independent approaches. Fifty-eight samples of dry-aged meat from different producer types (meat processing plants, artisan and supermarket butchers) were studied. Microbial groups were enumerated on various dedicated media. Yeast and mold isolates (n=257) were identified by sequencing of taxonomically relevant genes. Metagenetic analyzes targeting the V3-V4 regions of 16S rDNA and ITS2 were also performed. The aerobic colony count varied between 1.97 and 10.91 log10 CFU/g and was similar to that of Pseudomonas spp., indicating that this bacterial group was dominant. Yeast populations varied between <2 and 9.41 log10 CFU/g, while molds showed abundances between <2 and 7.7 log10 TFU/g, the highest values being found in meats matured with a high RH. Bacterial and mold counts were positively correlated with the dry-aging RH and, to a lesser extent, temperature. The main yeast species were *Candida zeylanoides* and *Yarrowia alimentaria* as well as *Itersonilia pannonica* (identified only in metagenetics). The dominant mold species were psychrophilic or psychrotrophic species, namely *Mucor* complex flavus and *Helycostylum pulchrum/elegans* previously shown to be associated with these products. This study identified the main microorganisms associated with dry-aged meat in France, which raises the question of their role in the organoleptic quality of these higher value products.

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Innovative use of food cultures for meat and other ready to eat products.

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● Microorganisms have played a crucial role in the fermentation of foods throughout human history. Historically, fermentation was an empirical process, neither controlled nor understood. It was driven by microbiological contamination of raw materials and microorganisms from the environment and humans, which cross-contaminated the food during production.

The beneficial role of microorganisms in fermentation and the mechanisms behind it have only been studied in the past 150 years.
The diversity of applications for food cultures and their contribution to food quality is continually expanding. Initially, food cultures were mainly used in traditionally fermented foods such as dairy products (fermented milk, cheese), dough (bread), vegetables (sauerkraut, pickles), meat products (salami, bacon), and fermented beverages (wine, beer). However, their use has now extended to foods not commonly understood to be fermented such as smoked salmon to prevent the outgrowth of *Listeria monocytogenes*, cooked ham to limit photooxidation and maintain stable organoleptic properties during shelf life, and plant-based meat alternatives to improve taste and texture.

The recent use of food cultures has raised questions about their regulatory classification under EU legislation. Food legislation, such as Regulation (EC) 178/2002, exists to ensure that consumers are confident that food is safe, and Regulation (EU) 1169/2011 provides the basis for food information, including presentation and labeling of foodstuffs.

Therefore, the challenge is how to guarantee a high level of food safety and proper consumer information while not delaying the use of biosolutions that can significantly improve shelf life and food safety, thereby reducing food waste.

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Scratching on French PDO cheese surfaces sheds light on an unexplored microbial genomic and metabolic diversity.

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● Cheeses are fermented dairy products consumed worldwide. Their global diversity results from various local variables including technological practices but also from the metabolic activity of diverse microorganisms. In Europe, this typicity can be found in Protected Designation of Origin (PDO) cheeses, from which genetic diversity remains largely unexplored. Combining culturomics (n = 373 bacterial genomes) and metagenomic (n = 146 metagenomes), we performed a national-scale survey of the microbial diversity encompassing 44 French PDO cheeses. Taxonomic (bacteria, fungi and viruses) and functional profiling reveals a high diversity in cheese rind, mainly driven by the cheese technology. We also reconstructed 1,119 bacterial metagenome-assembled genomes (MAGs) encompassing 7 phyla including *Actinomycetota, Bacillota, Pseudomonadota* and *Bacteroidota*. Using GTDB as reference, we found that 221 MAGs encompassing 46 genera as well as 44 bacterial isolate genomes encompassing 8 genera represent potential new species (<95% ANI). These species were particularly numerous among the genera *Halomonas, Psychrobacter* and *Brachybacetrium*, and are currently being formally described in our lab. We combined our genomic and metagenomic datasets into a catalog of 26.2 millions protein clusters, out of which 50% remains unknown. We illustrated the potential of this resource by searching methionine gamma-lyase (MGL), an enzyme playing a significant role in cheese flavor. This protein was predominantly found in *Pseudoalteromonas*, a potentially new MGL-producing genus, *Serratia, Pseudomonas, Proteus* and *Hafnia*, and its prevalence varied with cheese technology. Our study provides a large genomic resource for food microbiologists and cheesemakers to further explore the biotechnological potential of PDO cheese biodiversity.

Monitoring the effect of raw milk refrigeration on milk kefir fermentation: implications for quality and food safety

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● Fermented dairy products are valued for their sensory and functional properties. Kefir is gaining interest as a model for the complex microbiomes involved in fermented dairy beverages. In Italy, a raw milk distribution network targets end consumers, but refrigerated raw milk poses microbial stability challenges. This study, within the OnFoods project, explored how refrigeration affects psychrotrophic growth and the use of such matrices in milk kefir preparation.

Refrigerated raw milk (8°C, 0-48h) and UHT milk were fermented with milk kefir grains (commercial starter, Bionova, Italy). Volatile organic compounds (VOCs) produced during fermentation were sampled automatically every 3h for 96h at 26°C by Proton-transfer-reaction, coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS) and identified via Gas Chromatography Mass Spectrometry (GC-MS). Metabolomic profiling was conducted through Nuclear Magnetic Resonance (NMR), while microbiological analyses assessed psychrophilic flora during storage and fermentation.

Kefir from refrigerated raw milk showed greater compositional diversity than that from UHT milk, with higher levels of fatty acids, carbohydrates, and microbial metabolites. VOCs like acetaldehyde, diacetyl, and acetoin highlighted active fermentation and enhanced sensory properties. Organic acids from lactic acid bacteria dominated, suggesting lower sugar content and improved digestibility. Bioactive compounds like GABA, purines, and butanol were also detected. Microbiological analysis confirmed a richer native microbiota, supporting raw milk kefir's superior fermentative potential.

The metabolomic approach, integrated with microbiological evidence, enabled monitoring of psychrotrophic microflora in cold-stored raw milk and variations in kefir starter dominance across samples with different storage times. Advanced techniques like PTR-ToF-MS and NMR proved effective for quality assessment.

Production and composition of a Caciotta with high ratio of Ca/P for possible use in patients with chronic renal failure.

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● The production of Caciotta with a high ratio of Ca/P cheeses may increase the restricted basket of food available for patients affected by advanced chronic kidney diseases (CKD). These special cheeses proved to be suitable for patients with impaired renal function, because of P trapping caused by Ca enrichment. The aims of this work were: i) to characterize the experimental manufacture and composition of Caciotta cheeses produced by adding 2 g/L of $CaCO_3$ to milk, in comparison to control cheeses without $CaCO_3$; ii) to evaluate the digestibility of Ca and P during an in vitro simulated gastrointestinal digestion of both control and test cheeses. The addition of $CaCO_3$ to the milk in vat modified some physical properties of the test cheese, such as the surface color and the appearance of the cheese, related to the diffused presence of holes, of non-microbiological origin, and carbonate microcrystals. The increased value of the Ca to P ratio did not substantially modify the main chemical characteristics of the cheeses. A small but significant reduction of soluble P in the intestinal digestates (~12%) confirmed previous studies showing a lower phosphoremia in nephropathic patients consuming FriP cheeses. Results indicated that Caciotta with a high Ca/P ratio may be a cheese that may be included in a list of cheese that nephrologists may recommend to patients with CKD. Future research will evaluate both the impact of the addition of $CaCO_3$ to other types of cheeses and their overall sensorial acceptability, also assessed by healthy consumers.

The contribution of coagulants to the ripening of cheese.

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● Cheese is one of the oldest fermented foods, and the distinct flavours, textures and character of the vast range of types of cheese around the world today arise from a complex interplay of the actions of bacterial, milk and coagulant enzymes. Chymosin, derived from rennet is the traditional cheese coagulant, but today multiple generations of fermentation-produced chymosin preparations are widely used. In this presentation, recent studies of the suitability of manufacture of Cheddar cheese using a modified fermentation-produced camel chymosin (mCC) are summarised and compared to fermentation-produced bovine chymosin (BC) and camel chymosin (CC). The use of mCC led to reduced proteolysis, and differences in instrumental and sensory hardness and meltability were clearly associated with the different coagulants, while descriptive sensory analysis showed a relationship between coagulant use and flavour. Subsequently, the proteolytic specificity of the different chymosins on caseins made using these coagulants was characterised, and several new peptides were identified, while the heat inactivation kinetics in streams such as whey were shown to differ between enzymes, with broader implications for processing. In conclusion, chymosin plays a key role in the development of cheese character, and selection of different coagulants can enable the tailoring of cheese characteristics best suited to different applications which might require, for example, faster or slower ripening or specific textural and functional properties.

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Raw cow milks form pasture in the pdo massif central cheese region – to what extent origin can be kept during fermentation.

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● The concept of cheese terroir is related to the possibility to sensorially perceive the taste of a place where cheese has been elaborated. Most of studies relate milk and cheese bioindicators to factors that shape cheese terroir. A recent study found that milk and cheese microbial diversity was mainly terroir-driven through their PDO characteristics in which milk-cheese continuum presented a crucial role in revealing cheese's link with its terroir. In order to investigate such continuum, we decide to investigate how lactic acid bacteria (LAB) fermentation shapes the physico-chemical composition of raw cow milks originating from the French Massif central mountain region with several cheese PDOs. The second objective was to discuss the geographical provenance and geological origin of raw cow milks before and after fermentation. The third objective was to evaluate if sensory perception of raw cow milk cheeses within a single PDO would be affected. Our results highlighted that ICP-MS multi-elemental composition and FTIR signatures could distinguish non-fermented raw cow-milks based on terroir parameters. Once LAB fermentation was achieved on those raw cow milks, fermented milks presented homogeneous values in terms of acidity and water holding capacity. Interestingly, FTIR signatures of LAB fermented raw cow milks indicated a more diverse physico-chemical composition compared to non-fermented milks, with a preservation of origin parameters. To deepen such chemical diversity brought by fermentation in the case of cheesemaking, Salers PDO cheeses elaborated from raw cow milks from the same geographical and geological origin presented sensory differences (triangle test) and on specific sensory attributes (descriptive analysis). Our study confirmed the importance of investigating milk cheese continuum to better understand the role of fermentation in cheese terroir expression. Further studies are under investigation to evaluate which cheese aromas could be responsible of sensory differences.

Phage and bacterial host co-evolution in natural whey starter culture over an entire cheese-making season.

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● Phages and bacteria are engaged in a continuous arms race, illustrated by the recent discovery of numerous anti-phage and anti-defense systems. This diversity and prevalence highlight the strong selective pressure of phages on bacteria and vice versa. Yet, in nature, ecosystem functions mostly seem stable. We are largely unaware of how these defense mechanisms are distributed in natural communities and how they adapt. Specifically, do bacteria and their phages persist due to rapid co-evolution (evolutionary response), or is it mainly the selection of preexisting standing diversity (ecological response)? To answer these questions, we analysed natural whey starter cultures that are continuously subcultured over an entire cheese-making season. In these communities, we have the selection for rapidly growing bacterial strains in milk with possible inflows and changes of microbes and phages. Daily metagenome sequencing and extensive metadata collection over several months at three cheese-making sites revealed the persistence of stable multi-strain bacterial communities. On the contrary, we observed hundreds of active lytic phages, prophages, and satellite phages. While many phages were occasional, others persisted without notable effect on the community function, potentially linked to the presence of anti-defense systems in their genome. While the bacterial strain composition remained stable throughout the season, their genomes continuously acquired novel CRISPR spacers and exchanged defense mechanisms. Altogether, these findings suggest that in nature the intricate dance between bacteria and phages is a complex mix of ecological forces (selection of pre-existing diversity) and rapid evolution (de novo) of both the phages and the bacterial hosts.

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Fermented food microbiota: complex interrelationships versus opportunistic invaders

[1] **Luc De Vuyst**

● Fermented foods are foods made through desirable microbial growth and enzymatic conversions of food components. The desirable microbial growth can be achieved through spontaneous fermentation of the raw materials, backslopping of already fermented batches, or the intentional addition of starter cultures at the start of the fermentation process. Several fermentation processes are carried out by the successive growth of different microbial communities, encompassing various species of yeasts, lactic acid bacteria, and/or acetic acid bacteria. Their biochemical and enzymatic activities are often the result of complex interrelationships, which are determined by specific substrates, metabolites, and/or physical process parameters. However, in all these cases, specific species can invade the fermentation batch opportunistically and, whether or not temporarily, interfere with the fermentation process. The survival and growth of these invaders, whether or not desirable, depends on specific fermentation process parameters, such as temperature, pH, and oxygen tension. All this will be illustrated with examples of the production and cold storage of mature sourdoughs, lambic beer production, and cocoa pulp-bean mass fermentation.

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Multi-omics interrogation of fermented food microbiomes.

[1] **Nicholas Bokulich**

● The advent of next-generation sequencing and other omics technologies has revolutionized the exploration of complex microbial ecosystems involved in food fermentation and beyond. This includes the role of environmental and autochthonous organisms in the fermentation of regional foods and beverages, where communities of microbial species and strains appear to contribute to terroir characteristics. In this presentation I will describe how omics technologies may be leveraged to study differences in the complex microbiomes and metabolomes of fermented foods, their precursors, and the natural and built environments in which foods are grown and produced.

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Ecological role-based assembly of synthetic sourdough ecosystems ensures robustness and reproducibility in sourdough fermentation.

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● Sourdough offers a uniquely tractable ecosystem that captures essential ecological principles while maintaining direct applicability to real-world fermentation systems. This study tests whether synthetic microbial communities (SynComs) rationally designed based on ecological roles can replicate the stability and functional output of spontaneous ecosystems. Twenty SynComs were assembled using a curated biobank of sourdough isolates and classified based on ecological function. Communities were cultivated in a wheat flour hydrolysate model and sampled at mid-exponential (M) and early stationary (BS) phases for metatranscriptomics, real time-PCR, and untargeted metabolomics.

Results demonstrated robust stratification of communities at both transcriptional and metabolic levels. Most SynComs clustered closely with the spontaneous reference community (SDG) in functional space, despite compositional perturbations. Seven SynComs representing key substitution types were selected for 30-day propagations (in situ validation), where they maintained stable acidification kinetics, biochemical characteristics, viable cell counts, and species persistence. Low-level *Saccharomyces cerevisiae* re-emergence was detected by RT-PCR in SynComs where it was not inoculated. While initially marginal, *S. cerevisiae* levels increased over time, particularly in SynComs containing alternative yeast species, indicating ecological niches permissive to its enrichment.

Overall, ecological role-based design enabled the construction of SynComs that are functionally resilient, reproducible, and comparable to spontaneous sourdough communities. These findings demonstrate that functional assembly rules can be successfully translated into predictive fermentation strategies, offering a framework for engineering stable, application-relevant microbial ecosystems.

Exploration of the microbial and metabolite diversity of household and artisan bakery sourdoughs through a citizen science approach.

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Sourdough, a fermented flour-water mixture, is produced in a multitude of environments using a large diversity of recipes and is known for its great microbial and metabolite variability. This study leveraged citizen science to explore the largely uncharted diversity of household and small bakery Type 1 sourdoughs from Belgium, The Netherlands, France, and Spain. Type 1 sourdoughs are maintained through backslopping, resulting in stable microbial consortia composed of lactic acid bacteria (LAB), yeasts, and sometimes acetic acid bacteria, which all contribute to flavour and/or leavening. Culture-dependent methods, encompassing selective plating and incubation, and isolate dereplication and identification, were combined with long-read amplicon-based sequencing to profile the bacterial and yeast communities of the 109 sourdoughs collected. Alongside common LAB sourdough species, such as *Levilactobacillus brevis* and *Fructilactobacillus sanfranciscensis*, at least one LAB species previously not reported in sourdough ecosystems, namely *Fructilactobacillus vespula*, was found. The yeast *Saccharomyces cerevisiae* was most frequently found, potentially reflecting prior use of baker's yeast. AAB were present in 38% of the samples collected, mostly belonging to the *Acetobacter* species. Well-known consortia, such as *F. sanfrancisensis* and *Maudiozyma humilis*, appeared to be underrepresented, with other consortia between *Saccharomyces cerevisiae* and several LAB species being more prevalent. Further, a metabolite target analysis and detailed metadata analysis of the sourdough production practices provided insights into the environmental and process-related factors that shape the sourdough microbial compositions. These findings underscored the value of artisan sourdoughs as reservoirs of both interesting microbial species and consortia, hence, offering new perspectives on fermentation and sourdough ecology.

Time course metagenomic analysis defining sustainable production of food flavoring.

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● Gastronomy is of great support in promoting a more sustainable consumption of local products. In searching for the hedonic and sensorial requirements often considerable quantities of secondary products are generated, frequently becoming waste. Microorganisms can be used to preserve food. Over time the usefulness of some microorganisms in adding value to processed foods through the production of useful compounds such as vitamins has also been verified. The synergic activity of diverse microbial communities can lead to products with interesting organoleptic and nutritional characteristics. The exploitation of this virtuous circle in gastronomy allows to increase its sustainability, adding nutraceutical and health elements to innovative food preparations. The process was studied at a gastronomic level for the transformation of protein based secondary products into flavorings, exploiting the Asian tradition producing miso and soy sauce. To evaluate the extent and importance of microorganisms, the aim of this study was to follow the microbial evolution through a metagenomic approach, during the six months of fermentation. Over time the microbial variability decreased and differentiated according to the transformed protein matrix. In this first metagenomic characterization of these food flavorings, the persistence over time of prokaryotic lactic acid bacteria species, and major variation of the relative abundance of the *Weissella, Lactococcus* and *Carnobacterium* genera was observed. Amongst the species identified through ITS, the genera *Aspergillus* and *Millerozyma* stood out. Thanks to this non-culturable approach, we were able to evaluate the microbial species potentially involved in the maturation of this natural condiment.

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Vineyard effect on microbial communities and fermentation dynamics of Nebbiolo grapes: a microbial perspective on terroir

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● The microbial communities present in the vineyard and throughout the vinification process play a key role and contribute to the terroir, which is linked to geographic origin, soil, climate, and local practices. These factors contribute to the development of unique wine characteristics, reflecting the identity and character of the grapes' growing region. Each vineyard's specific conditions lead to distinct expressions of the wine's flavor and quality. This study aimed to explore the regional distribution patterns of fungi and bacteria and their association with the composition of wine. Grapes from thirty-eight Nebbiolo vineyards of seven different geographical zones, divided into two macro areas in Piedmont and one in Lombardy, were collected at harvest time and processed at the experimental winery of the University of Turin (Bonafous, Turin, Italy). Samples were taken for analysis at different time points of the alcoholic fermentation. Fungal and bacterial communities were investigated by high-throughput sequencing on bacterial 16S rRNA and fungal 26S rRNA encoding genes. Chemical composition and volatilome were also determined. Patterns of regional distribution of microbial communities were observed, and associations between microbiota and wine composition were deduced. The importance of this study is to understand the possible use of microbiota as an indicator of origin and, in the meantime, as a predictor of the chemical composition of wine and, therefore, of wine quality.

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Mapping the biogeography of sourdough microbiomes through next-generation citizen science

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● Fermented foods are regaining popularity, yet the processes driving microbial diversity - especially biogeographic variation - remain poorly understood. To address this, the HealthFerm project launched a large-scale, co-designed citizen science initiative involving over 1,000 participants across 33 countries. In total, 671 sourdough samples were collected alongside detailed metadata on fermentation practices and motivations. A central innovation of this “next-generation citizen science” approach was the return of personalized microbiome reports, supported by an AI chatbot to aid interpretation and sustain engagement. This unique dataset enabled multi-omics analyses of home-fermented sourdoughs at an unprecedented scale. We identified biogeographic patterns in microbial diversity, including a pronounced north–south gradient in indoor fermentations, correlating with outdoor climatic and land-use variables. Integration of microbiome profiles with contextual metadata revealed distinct microbial co-occurrence networks, differentiating household from bakery fermentations and highlighting substrate-specific community structures, supported by functional profiling. Our findings demonstrate that both indoor and outdoor environments shape sourdough microbiome composition and functional potential, with geography contributing significantly to diversity patterns. This work provides novel insights into the ecology of food fermentations and exemplifies how co-designed citizen science can generate high-resolution, multi-omics data at continental scale.

The evolving safety landscape of food fermenting microorganism: from traditional assessment to genome editing

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● Bacteria, yeasts, and filamentous fungi have long been utilized in food fermentations, playing a crucial role in ensuring both the quality and safety of the final products. Traditionally, the safety of these microbial cultures has been supported by their “long history of safe use.” However, this conventional approach is increasingly being called into question. Emerging concerns – such as the development and dissemination of acquired antimicrobial resistance, natural microbial evolution, and the creation of novel strains through advanced genomic techniques including genome editing – highlight the limitations of relying solely on historical usage as a guarantee of safety. These developments underscore the need for updated and more rigorous risk assessment strategies to ensure the continued safe use of fermentation microorganisms in the modern food system.

The European Food Safety Authority (EFSA) has recently introduced updated guidance and documentation to support the risk assessment of microorganisms intentionally added to food systems. These include novel frameworks for evaluating microorganisms developed through genome editing technologies, with particular attention to environmental risk assessment in cases of deliberate release. Additionally, EFSA has issued guidance outlining both general criteria and specific methodologies for assessing the safety of microbial cultures. Among the key elements are the utilization of whole genome sequencing (WGS) data to detect acquired antimicrobial resistance and potential virulence factors, reinforcing a more robust and science-based safety evaluation approach.

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Fermented foods and the food system: why a holistic perspective matters

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● Fermented foods are not isolated products of human intervention but are deeply embedded within complex microbial networks that span from soil to table and beyond. A holistic understanding of fermentation requires acknowledging the continuum of microbiomes, beginning with environmental and agricultural ecosystems, which shape the microbiomes of plants, and subsequently influence the microbial dynamics of fermentation processes. These interconnected microbiomes impact not only the sensory and nutritional qualities of fermented foods but potentially also their health-promoting properties. Moreover, anthropogenic pressures such as industrial agriculture, biodiversity loss, and environmental degradation are altering microbial ecosystems, with cascading effects on food system resilience and human health. Recognising the microbiome's flow through the food chain can inform more sustainable agricultural practices, protect microbial diversity, and optimise fermentation outcomes for both ecological and public health benefits.

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Food microbiomes: their importance for food quality and the connection to the gut ecosystem

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● The microorganisms occurring in food and across food systems have a key role in food quality and safety, and also in human health. The study of the complex food microbiomes through metagenomics is fundamental to explore the role of microbes in the food environment and the potential impact for human health also owing to the possible identification of previously uncharacterized microbes. Recent approaches can generate metagenome-assembled genomes (MAGs), very important for functional characterization and strain-level analyses. A recently developed database include >10,000 food MAGs including >300 previously undescribed taxa. Strain-level analyses have recently indicated the potential of food-to-gut transmission and intestinal colonization of some species.

The study of microbiomes in fermented foods and food environments is proving of utmost interest recently, and cheese production is the system where we carried out more studies. Dairy plants were shown to harbour a complex microbiome, characterized by high prevalence of genes potentially involved in flavour development, probiotic activities, and resistance to gastro-intestinal transit. In addition, genes involved in the production of neuroactive molecules have been found in the dairy environment, suggesting potential connections between cheeses consumption and mental health, along with genes related to bacteriocin biosynthesis, possibly enhancing cheese safety and shelf life. Moreover, >70% of all known antimicrobial resistance genes (AMRGs) have been recently shown to circulate across food the food systems. Such findings have broader implications for a better understanding of the interactions between food, the human microbiome, and human health.

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Fermented fruit juices as a source of bioactive organic acids: unveiling their role in adipogenesis and metabolic health

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● The global rise in obesity and metabolic disorders underscores the need for food-based preventive strategies targeting early metabolic dysfunction. Fermentation, beyond its preservative and sensorial roles, stands out for its ability to enhance the bioactivity of food matrices by generating postbiotic compounds with potential metabolic benefits.

This study explores the potential of fermented fruit juices as sources of functional compounds capable of modulating early adipogenesis. Using a derivatization-based analytical method, increased levels of pyruvic and succinic acids – two organic acids involved in key metabolic pathways – were identified as direct outcomes of alcoholic yeast fermentation. To assess their biological relevance, the human SGBS preadipocyte model was used to simulate early adipogenic differentiation in the presence of these acids. Functional and molecular markers related to adipogenesis and lipogenesis (e.g. *PPARɣ, C/EBPα*), inflammation, oxidative balance (ROS, ATP), glucose uptake, and lipid accumulation were evaluated. Results revealed that both pyruvate and succinate improved markers of metabolic function and redox homeostasis, while enhancing glucose metabolism and modulating cellular processes associated with healthier adipose tissue development. Overall, these findings support the relevance of fermented functional beverages as promising modulators of obesity-related pathways, reinforcing the nutritional value of fermentation-derived bioactives in preventive metabolic strategies.

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Assembling microbial consortia for legume-based matrix fermentation.

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● The high demand for animal-based food drives significant global changes. To ensure food security and sustainability, shifting toward more plant-based diets is necessary. High in protein, fibers, and minerals, legumes provide a sustainable alternative but have sensory defaults and anti-nutritional factors that may hinder consumption. Fermentation represents a promising solution to these challenges.

We screened 39 strains of lactic acid bacteria (LAB), ripening bacteria, and yeasts from dairy and plant products for growth on green lentil, coral lentil, and chickpea matrices using *in vitro* methods. Using Genome-scale metabolic modelling (Metage2Metabo software), we proposed microbial consortia based on legume compositions and identified key metabolites. We evaluated consumer acceptance of novel legume-based foods with focus groups.

Out of 39 strains, 33 thrived on all three matrices, with optimal growth on the chickpea matrix, the most popular with consumers. Chickpea-based matrix was then selected for further analysis. LAB grew first and reduced the pH then yeast and ripening bacteria grew later and increased it. Based on the minimal consortia proposed by Metage2Metabo, growth data and our expertise, we designed seven consortia with varying phylogenetic and techno-functional complexity.

Results show promising improvements in the aroma profile. Metabolomic analyses are ongoing to assess the impact of microbial consortia on reducing anti-nutritional factors while enhancing nutritional and aromatic qualities.

This study aims to explore how microbial consortia improve the nutritional and sensory attributes of legume-based foods and establish guidelines for developing and evaluating legume-enriched fermented products that align with consumer expectations.

Microbial species diversity in kaak production: insights into the role of chickpea-soaked water

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● In the Mediterranean, chickpea-soaked water has traditionally been used as a natural leavening agent in baked products, notably in Lebanese kaak. Although this product is of cultural importance, the contribution of chickpea-soaked water to the microbial species diversity, the fermentation kinetics, and the organoleptic quality of the baked product remains unexplored. To address this, a total of 113 kaak samples were collected from 26 bakeries across Lebanon at different production steps. Analysis of fungal diversity, using culture-dependent method and Illumina MiSeq sequencing, identified *Saccharomyces cerevisiae* as the most frequent yeast species, despite the absence of intentionally added commercial strains of *S. cerevisiae*. Bacterial profiling using MiSeq sequencing, revealed an abundance of *Clostridium perfringens* throughout the kaak-making process. To investigate the functional role of chickpea-soaked water, eight *S. cerevisiae* strains, isolated from kaak and from other bread-making sourdoughs, were cultivated in synthetic sourdough liquid media supplemented with chickpea-soaked water that was either filtered to remove microorganisms or kept unfiltered. Interestingly, fermentation onset and maximum fermentation rate were significantly affected by the unfiltered chickpea-soaked water while the filtered chickpea-soaked water had no significant impact, highlighting the influence of the active microbial community. Furthermore, one strain of *S. cerevisiae* behaved differently from the others and fermented more slowly in the unfiltered chickpea-soaked water medium, suggesting competition with the microbial community present. An analysis of volatile aroma profiles using HS-SPME-GC-MS, is currently underway and will shed light on the impact of chickpea-soaked water on the organoleptic characteristics of kaak.

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Has *Lactobacillus helveticus* changed over 50 years? A genomic and technological characterization of recent and old strains.

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● *Lactobacillus helveticus* (LH) is a thermophilic lactic acid bacterium commonly found in natural whey starter (NWS) cultures used for PDO cheese production, such as Parmigiano Reggiano. This study compares LH strains isolated 50 years ago and never revitalized with newly isolated strains from the same niche. After a preliminary genotypic and phenotypic screening, 16 old and 18 new strains were selected for whole-genome sequencing. The results indicate genomic decay over time: older strains exhibited significantly larger genomes (2.09 ± 0.17 Mbp) and higher GC content (37.52% ± 1.19) compared to recent ones (1.95 ± 0.03 Mbp; GC 36.76% ± 0.26). Phenotypic and techno-functional analyses further revealed diversity in dairy-related traits.

Four strains, two old (UNIBO 404, UNIBO 467) and two new (UPCCO 6358, UPCCO 6363), were selected for pilot-scale cheese production, co-inoculated with *Lactobacillus delbrueckii* subsp. *lactis* UPCCO 5365 to mimic traditional NWS cultures. Cheeses (aprox 1 kg) were manufactured in biological duplicates using pasteurized milk and following n experimental protocolsimulating cooked hard cheese production. The samples were ripened for three weeks at 16°C. Microbial dynamics were monitored via plate counts and qPCR and volatile compound profiles were compared through SPME-GC-MS. Findings highlight functional and metabolic differences in terms of microbial development and volatile compound production between the old and new LH strains, shedding light on microbial evolution within dairy fermentation ecosystems.

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This research is supported by EU – NextGenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR)), program Prin 2022 with the project “A 50 years evolutionary history of Lactobacillus helveticus from traditional dairy environments: biodiversity of strains as an opportunity for technological exploitation and new products – Helv4DairHy” (CUP J53D23010690006).

Insights into the development of novel brewing yeast strains through adaptive laboratory evolution.

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● The craft beer industry is increasingly exploring alternative raw materials to create novel beer styles. Buckwheat, a gluten-free pseudocereal rich in minerals and bioactive compounds, presents a promising option. However, fermenting buckwheat wort poses challenges, especially regarding yeast performance. Here we report the selection of an autochthonous *Saccharomyces cerevisiae* strain from the complex microbiota of spontaneously fermented traditional cider, and its adaptation to a specific wort prepared from buckwheat – a gluten-free substrate – using adaptive laboratory evolution (ALE). Through 30 successive fermentations in a system of high-pressure fermenters designed to simulate stressful industrial conditions, with weekly re-pitching of biomass into fresh wort, and obtained evolved populations that efficiently consumed maltose. We further characterized the genetic basis of adaptation in clones evolved on buckwheat wort. The evolved clones exhibited genetic changes characteristic of domestication, including loss of heterozygosity, extensive chromosomal or segmental aneuploidy, and rearrangements or loss of mitochondrial DNA. Finally, we evaluated the fitness of these clones in laboratory-scale fermentations, assessed the phenotypic effects of observed genetic changes, and compared the flavour compound profiles of the beers they produced to those of the ancestral strain. These results provide insight into the genetic mechanisms underlying yeast adaptation to different brewing conditions and may inform the development of improved strains for industrial-scale fermentation. Over the past 200 years, a similar process of continuous "back-slopping" under semi-industrial brewing conditions has led to the emergence of novel yeast phenotypes well adapted to the brewing environment.

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Bifidobacteria as beneficial microbes during early life: where, what and how?

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● Bifidobacteria are commonly encountered microbes of the human gastrointestinal tract, particularly during early life when they frequently make up the majority of our gut microbiota. Bifidobacteria have been associated with a wide range of beneficial activities, ranging from immunomodulation to pathogen exclusion, which has prompted their wide-spread commercialization as probiotics. This presentation will outline some of the salient features of this group of high GC content bacteria, how we initially acquire and feed them following birth, and the metabolic activities that make them highly competitive in the human gut before and after weaning. Furthermore, the talk will focus on strain-specific properties of certain bifidobacterial species, emphasizing that not all bifidobacteria do the same thing in terms of their metabolic capabilities or indeed their beneficial activities.

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Advancing gut health in bakery with postbiotic enriched sourdoughs.

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● Gut health has gained significant attention in recent years, with probiotics, prebiotics, and postbiotics contributing to a healthy gut microbiome and overall health. As experts in baked applications, Puratos aims to become the gut health leader in the bakery sector, focusing on the application of postbiotics – a recently recognized category of "biotics" consisting of inanimate and inactive microorganism and their components which makes them ideal for baked goods.

Over 100 bacterial strains were screened for their in vitro postbiotic properties: SCFA production, antioxidant capacity and EPS production. From this extensive screening, 5 strains were selected to develop sourdough breads which were further evaluated in in vitro colonic fermentation experiments to assess their gut microbiome modulation, stimulation of SCFA production, and production of health-related metabolites. Ultimately, one strain, a *Levilactobacillus brevis S-33824,* emerged as the best performer and was used to assess the long-term effects of postbiotic-enriched sourdough bread consumption using the Simulator of Human Intestinal Microbial Ecosystem (SHIME) in vitro model.

The SHIME experiment reinforced the results obtained during the colonic fermentation and demonstrated positive alterations to the gut microbiome, higher production of health-related metabolites (25 – 47% increase in SCFA), and protection of the gut epithelial cell barrier as seen by a 7 – 30% TEER increase in barrier strength and a 3 to 8% decrease in barrier permeability when compared against a baker's yeast leavened bread.

These findings highlight the potential impact of postbiotic-enriched sourdoughs to promote gut health.

The good, the bad and the ugly: story of fermented foods and (global) health

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● Different interactions coexist in the food microbial ecosystem mediated by the environment and human action, which modulates the microbiota present in food throughout the entire food chain. In these interactions, lactic acid bacteria (LAB) play a primary role, and could be considered as a character in a Spaghetti Western: *“the good, the bad, and the ugly.”* For centuries, we have learnt to modulate foods to make more appealing products with greater added value, initially in an empirical way and currently using industrial LAB starter cultures to obtain products that meet consumer expectations while also guaranteeing the uniformity of production batches; *the good*. Similarly, since the implementation of preservation procedures in which the atmosphere where the food is preserved is modified to prevent the growth of harmful microorganisms, conditions have been created for the growth of other microorganisms that can affect the food characteristics, making them less attractive, as in the case of LAB in foods packaged in modified atmospheres; *the ugly*. Finally, in an ecosystem as demanding as the food, it is necessary to find strategies to survive the surrounding microbial competition. To do this, microorganisms have created substances that inhibit the growth of their microbial competitors and even eliminate them. This is the case of bacteriocins produced by LAB. This is a successful strategy that has been implemented at an industrial level to combat foodborne pathogens, *the bad*. In this presentation, we will visit each of the three aspects where LAB are relevant in the amazing food microbial ecology.

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Reduced biocide susceptibility in *Companilactobacillus*: a genome-to-phenotype study with implications for an integrated safety assessment of lactic acid bacteria

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● Resistance to sanitizing agents (SA) is a growing global concern, intensified by the COVID-19 pandemic, which increased disinfectant use whilst exerting a selective pressure on microorganisms. Benzalkonium chloride (BAC), a widely used SA in food production, has been associated with the emergence of reduced susceptibility in both pathogenic and beneficial bacteria, including lactic acid bacteria (LAB).
In this framework, this study investigated changes in susceptibility to BAC in *Companilactobacillus* species, which occur consistently in animal- and plant-based food fermentations, focusing on *Companilactobacillus crustorum*.

A genome-based analysis of 102 high-quality *Companilactobacillus* genomes (available in NCBI) using the nf-core/funcscan pipeline, predicted the presence of sanitizing resistance genes (SARG), specifically *qacJ* and *qacG*, in 20 of 36 *Companilactobacillus* species, including *C. crustorum*. Phenotypic susceptibility was assessed through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests performed on 12 strains – six C. *crustorum* strains and six additional representative *Companilactobacillus* strains. MIC values ranged from 0.3 to 0.6 mg/L, while MBC values ranged from 2.5 mg/L to >5 mg/L, both notably higher than those observed in SARG-negative control strain (*C. tucceti* DSM 20183^T; MIC and MBC below 0.1 mg/L). Although the MIC and MBC values recorded are low relative to typical BAC application concentrations (400 – 2,000 mg/L), these changes in susceptibility are evident across the SARG-positive *Companilactobacillus* species. These results emphasize the necessity for further studies on biocide susceptibility in LAB as part of an integrated safety assessment, especially considering potential cross-resistance to antibiotics and its implications for their applicability.

A functional foods ontology: systematizing bioactive ingredients of foods for specified health uses (FOSHU) in Japan

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● We have developed a functional foods ontology with comprehensive coverage of the bioactive ingredients in Japanese FOSHU (Foods for Specified Health Uses) products, including significant representation of fermented foods and their components. FOSHU represents the gold standard in functional food labelling in Japan: marketing approval requires exhaustive scientific validation including in vitro research, animal studies, and randomized controlled trials. Our Functional Foods ontology encompasses nearly 1,000 bioactive ingredients with multilingual synonyms in both English and Japanese – where possible, mapped to established public ontologies including the Food Ontology, ChEBI, and NCBI Taxon – and organized into logical hierarchies of taxonomic origin and molecular composition, including specialized branches for fermentation products and probiotic bacteria. This systematized knowledge is expected to facilitate literature mining of fermentation-derived ingredients with potential health benefits and support research collaboration across linguistic boundaries. For example, our ontology has already been utilized commercially to extract relationships between functional foods and microbial taxa, microbiome-derived metabolites, and diseases in an AI-supported knowledge graph use case, resulting in the identification of ingredients that modulate dysbiosis in inflammatory bowel disease by promoting butyrate production – a metabolite with therapeutic effects comparable to mesalamine. Future development will include extension to encompass Foods with Functional Claims (FFC – another Japanese regulatory category including fermentation food products – and mapping of fermentation microorganisms to their specific bioactive metabolites.

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Targeted fermentation of chickpea flour enhances nutritional value and gut health-promoting properties.

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● The increasing global demand for sustainable and functional protein sources has driven interest in legumes such as chickpeas (*Cicer arietinum L.*), which are rich in protein and bioactive compounds but limited by the presence of antinutritional factors, raffinose-family oligosaccharides (RFOs) and undesired aroma compounds. This study investigated the targeted fermentation of chickpea flour using microbial strains selected for their ability to reduce RFOs, aldehydes, and the growth of undesirable microorganisms and pathogens, while improving the overall sensory profile. *Lactiplantibacillus plantarum* LP23, alone or in combination with *Debaryomyces hansenii* Y15A, was applied to enhance the nutritional profile and assess its impact in an in vitro gut model. Samples were characterized for their peptide and amino acid content, digestible and damaged starch, antioxidant activity, and volatilome. Fermentation significantly increased soluble peptides and total amino acid (at least doubling their content), reduced damaged starch, enhanced antioxidant activity, and confirmed the reduction in total aldehydes. Moreover, fermented ingredients and their application in a fortified pasta formulation were tested, upon INFOGEST digestion, in a 24 h *in vitro* gut model. The results demonstrated a beneficial modulation of microbial composition and its metabolic activity, with increased production of short-chain fatty acid (SCFA), notably acetate and butyrate. These findings support the application of tailor-made fermentation to develop next-generation plant-based functional ingredients for gut health and sustainable nutrition.

Genome editing of yeast and lactic acid bacteria for safer, tastier, and healthier fermented foods.

[1] **Yong-Su Jin**

● Growing concerns over food insecurity and sustainability demand immediate technological innovations to ensure a reliable and resilient food system. Biotechnology offers a promising solution; however, ensuring the safe application of biotechnology in food production remains paramount. Precision genome editing provides a powerful and targeted approach to enhance food-fermenting microorganisms, improving the safety, quality, and flavor of fermented foods. In this presentation, I will highlight our efforts to develop genome editing tools for yeast (*Saccharomyces cerevisiae*) and lactic acid bacteria (*Lacticaseibacillus rhamnosus* GG), and their application in fermented food production. Specifically, we have edited yeast strains to reduce acrylamide production in potato fries, increase savory amino acid production in rice wine, and improve the freeze-thaw stress tolerance of yeast for bread production from frozen dough. In addition, we corrected mutations in the lactose operon of *L. rhamnosus* GG to enable efficient lactose fermentation, supporting its use in dairy applications. These genome-edited strains do not contain any heterologous DNA and may be used in food production without necessarily being subject to GM regulation. Our work demonstrates how precise, responsible genome editing can drive the next generation of safer, tastier, and more sustainable fermented foods.

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Grain-based food fermentations as a tool to produce health-promoting foods.

[1] **Christophe M. Courtin**

● Food fermentation is performed for a variety of reasons, but the health aspect has clearly been gaining traction. When fermentation leads to the production of essential nutrients like vitamin B12 or a reduction in antinutrients, the health benefits of fermentation seem clear. Also the intake of foods containing (non-pathogenic) live micro-organisms has recently been related to a better health status. Microbial metabolites such as lactic acid and short-chain fatty acids are increasingly confirmed as agents of positive changes in the host, affecting gut, brain and body. When considering grain-based fermentations, other aspects than those just mentioned could play an additional role in enhancing the health-related aspects of resulting foods. Indeed, grain-based foods are rich in starch, reserve proteins (significantly different from animal-based proteins) and fibre, and fermentation could affect their digestion and microbial fermentation in the gastro-intestinal tract, possibly adding to health-related effects. To understand and leverage grain-based food fermentations for healthy diets, the EU-project HealthFerm investigates aspects ranging from microbes for optimal fermentations over fermentations technology to human intervention trials and the gut microbiome, while not losing sight of consumer attitudes and cultural backgrounds. In this presentation, the first results of the citizen science project that was developed in the framework of the HealthFerm project to seek out unique strains for grain-based fermentations from Europe and beyond will be presented. Novel insights into molecular drivers for grain-based fermentation are shared and grain-based fermentation technologies and products discussed. First intervention trial results and consumer attitude data will be shared.

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Probiotics combined with prickly pear seed flour as a mean to fortify fermented milk-based beverages.

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● The application of processing waste/by-products along with probiotics could represent a possible strategy to confer functional aspects to food items. This study aimed to evaluate the impact on yogurt of incorporating prickly pear seed (PPS) flour, rich in fiber, minerals and phenolic compounds, together with individual commercial probiotic strains of lactobacilli (*Lacticaseibacillus casei* BGP93, *Lacticaseibacillus casei* LC4P1, *Lacticaseibacillus rhamnosus* LRB, *Lactiplantibacillus plantarum* LPAL). Preliminarily, we optimized the production protocol by examining various percentages of PPS flour added either before or after fermentation. The best result was achieved adding 5% of PPS flour after fermentation, coinciding with the addition of probiotics. The ability of all probiotic strains to survive in the yogurt matrix and in the presence of the starter cultures was assessed, during 40 days of refrigerated storage. Probiotic lactobacilli and yogurt starters were quantified using both culture-dependent (plate count on MRS and M17 agar) and culture-independent (real-time qPCR) techniques. The inclusion of PPS flour resulted in elevated levels of fiber (more than 3%) and minerals, especially for magnesium, potassium and phosphorus. Furthermore, the antioxidant capacity, measured *in vitro* (via DPPH· and FRAP assays) and in cell line studies, showed an increase in the yogurt added with PPS and *L. casei* BGP93. These findings suggest the suitability of integrating PPS flour and probiotics into yogurt formulations to obtain functional yogurt, leading to a greater availability of phenolic compounds. Further research is needed on consumer appreciation and willingness to pay.

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Does maternal consumption of fermented foods affect neonatal health?

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● Nutrition during pregnancy is significantly associated with foetal development. Inadequate and unbalanced nutrition in pregnancy can negatively affect the growth, metabolism, immune function, brain and cognitive development of the newborn in relation to fetal malnutrition. Today, in addition to the importance of macro- and micronutrients, attention has increasingly turned to the roles of prebiotics and probiotics. However, uncertainties remain regarding the specific strain, appropriate dosage, and optimal duration of use, thereby highlighting the importance of consuming fermented foods. Fermented foods are the most accessible and effective food group to support the healthy development of the microbiota during pregnancy. Maternal consumption of fermented foods has been shown to contribute to healthy neonatal development by altering the composition and function of the gut microbiota, reducing the risk of preterm birth, providing neurodevelopmental benefits, and affecting allergy development and sleep status.

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Sourdough starter culture and breadmaking process impact wholemeal bread characteristics associated with starch digestibility.

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The scientific literature has proposed sourdough breadmaking as a means to modulate starch digestibility and lower the glycaemic index of bread. However, conflicting evidence from postprandial glucose response studies questions this effect. We explored how sourdough affected wholemeal bread's starch digestibility by determining the impact of the sourdough starter culture and breadmaking process on bread characteristics and in vitro starch digestion kinetics, using the static INFOGEST 2.0 protocol. Wholemeal wheat sourdoughs prepared with strains of *Fructilactobacillus sanfranciscensis* and *Maudiozyma humilis, Pediococcus pentosaceus* and *M. humilis*, or *Companilactobacillus crustorum* and *Wickerhamomyces anomalus*, and baker's yeast were used in two breadmaking processes (total fermentation times of 100 and 530 min). The specific volume (1.574–2.90 mL/g), pH (4.14–6.2), total titratable acidity (3.64–11.9 mL of 0.1 M NaOH), and water-extractable arabinoxylan level (0.64–1.23% dm) of different breads were affected by the sourdough used and the process applied. Despite differences in bread characteristics, *in vitro* digestion of starch reached a plateau after 30 min for all bread types. Slightly, but insignificantly, more resistant starch was found in long-fermented bread prepared with *C. crustorum* and *W. anomalus* sourdough (10.9% of total starch) than with baker's yeast (7.1% of total starch). The results showed that wholemeal bread's chemical composition and characteristics were affected by the sourdough consortium and the fermentation time used during breadmaking; however, sourdough addition did not seem to impact the starch's susceptibility in wholemeal bread to small intestinal digestion. These findings enhance our understanding of how sourdough addition and processing influence wholemeal bread's glycaemic index.

Enrichment of fermented olive patè with *Lactiplantibacillus plantarum* as a biological strategy to enhance anti-inflammatory activity and gut modulation.

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● *Lactiplantibacillus (Lpb.) plantarum* strains, widely used as starter cultures in the production of fermented foods, showed the potential to enhance fermented foods functionality, in terms of inflammatory response and modulation of gut microbiota composition. Our studies investigated two selected food-associated *Lpb. plantarum* strains, previously characterized for their ability to restore inflammation *in vitro*, as a tool in a lab scale fermentation process, to enrich the functionality of an innovative biologically debittered olive patè (FDP), recently patented. Thus, microbiomics (qPCR) and metabolomics (SPME GC-MS) analysis have been performed to investigate the impact of FDP samples in modulating human gut microbiota and relative metabolites in an *ex-vivo* colonic fermentation by using a faecal gut fermentation model inoculated with human faeces collected from healthy donors. Results from colonic fermentations showed the ability of FDP to finely modulate gut microbiota with a potential prebiotic, eubiotic and bifidogenic activity on colon, while metabolomic analysis revealed that FDP samples enriched with *Lpb. plantarum* stimulates the production of healthy organic acids and attenuates negative indoles. In addition, the simultaneous oral administration of a diet enriched with fermented olive patè and *Lpb. plantarum* significantly improve the macroscopic and microscopic colitis scores with a significant reduction of inflammatory and pro-fibrotic cytokines in a mouse model of DSS-induced chronic colitis. Overall, our results highlight the beneficial contribution of fermented foods and the application of *Lpb. plantarum* species as a promising tool to enhance the functionality of vegetable-based fermented foods in ameliorating intestinal inflammation and ultimately impact the human gut microbiota.

Solid-state fungal fermentation of plant-based substrates improves the overall protein quality.

[1] **Eddy. J. Smid**

● Animal-based protein production is responsible for nearly half of all food system greenhouse gas emissions. Moreover, livestock farming uses vast amounts of land and water. For those reasons, we need to shift from predominantly animal-based protein sources to more sustainable alternatives like plant protein, fungal biomass, and/or insect proteins. Especially combining plant-sources with novel protein sources, offers new options for high quality protein foods. I will demonstrate how the nutritional and organoleptic properties of various plant-based substrates can be improved to the level of animal-based foods by using fungal and bacterial fermentation. Results will be presented about substantial improvements in protein quality in staple foods using solid-state fungal fermentation. Finally, I will show the power of *in situ* vitamin B12 fortification of lupin-based solid-state fermented food products using tailored dual cultures consisting of a food-grade fungus and a bacterium.

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Biopurification of pulse protein concentrates by lactic acid bacteria.

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● The demand for plant-based, high-protein food products is on the rise. However, their acceptance is limited by the presence of anti-nutritional factors such as Raffinose Family Oligosaccharides (RFOs). Fermentation with lactic acid bacteria (LAB) can be employed as a biopurification method to degrade RFOs. However, the effects of fermentation on RFOs are rarely investigated together with other influencing factors, such as the type of pulse. In this study, we used a full-factorial design of experiments to evaluate the impact of three factors: i) pulse type, faba bean or yellow pea; ii) type of enrichment, starch-rich (SRF) or protein rich fractions (PRF) and iii) LAB species, *Lactiplantibacillus plantarum* WCFS1 (LpWCFS1) and *Leuconostoc mesenteroides* DSM20343 (Lm20343). Doughs were prepared from sterile flours and water and incubated with the bacteria for 48 h. Mono- and oligosaccharides, organic acids, pH and viable cell counts were measured before and after fermentation. While lactic acid concentrations were highest after fermentation of PRFs, sourdoughs made with SRFs had a lower pH, indicating a higher buffering capacity of the PRFs. Initially, RFOs were more abundant in the PRF of both pulses, while mono- and di-saccharides were present in similar concentrations. After fermentation, Lm20343 degraded RFOs to a greater extent, despite LpWCFS1 producing more lactic acid and reaching a higher cell concentration. A PERMANOVA model showed that the LAB species had the strongest effect on RFO degradation. These results show that LAB selection is key to optimise RFOs removal, outweighing the effects of pulse cultivar selection or fractionation strategy.

Lactic acid bacteria fermentation for the valorisation of brewer’s spent yeast: enhancing nutritional, functional, and sensory characteristics

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● With an ever-increasing global population and dwindling natural resources, a shift towards more sustainable food systems is required. Aspects to aid this transition include the reduction of food waste, and a movement towards non-animal proteins. Brewers spent yeast (BSY) is a brewing by-product which is generally regarded as waste, despite its high nutritional value. This study explores the impact of of lactic acid bacteria (LAB) fermentation on the functionality, sensory characteristics, protein quality, and gut microbiome modulation potential of BSY. Fermentation of BSY with *Lactobacillus amylovorus* FST 2.11 resulted in an ingredient (FBSY) that was perceived to be significantly less bitter than the control (CBSY) by a sensory panel, with enhanced intensities of fruity and sour flavours. Fermentation induced protein degradation, resulting in higher protein solubility (+41%) and foam stability (+69%), improved emulsifying stability characteristics, and differences in rheological behavior during heating. *In vitro* digestion (INFOGEST) showed that FBSY had a superior protein quality than CBSY, demonstrating significantly higher *in vitro* bioaccessibility value for almost all amino acids, and a significantly increased *in vitro* digestible indispensable amino acid score (DIAAS) (17% for CBSY; 98% for FBSY). Investigation of the gut microbiome modulation potential of PBSY using an *in vitro* colon model system showed an increase in α-diversity indices and increased abundance of beneficial Mediterranean diet-responsive taxa after 24 h. This study highlights the potential of BSY for the production of a high-quality food ingredient with potential prebiotic effects, contributing to reducing food waste and supporting global food systems.

Bioprocessing food side streams: fermentation as a sustainable tool for functional ingredient development

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● Reducing food waste through the valorization of agro-industrial side streams holds both environmental and economic benefits. Fermentation represents promising, low-impact strategies to unlock bioactive and functional compounds from food by-products. The aim of this work is to show the potential of fermentation as a sustainable strategy to upcycle diverse food by-products into functional ingredients. Various plant-based side streams, including mango peel, tea residue, hazelnut skins, and distilled spent grain, were subjected to microbial fermentation using lactic acid bacteria, and filamentous fungi such as *Aspergillus* and *Monascus* spp. In some cases, fermentation was preceded by enzymatic or physical treatments to improve substrate accessibility. Across matrices, fermentation enhanced the release of bioactive compounds and prebiotic oligosaccharides while also increasing protein solubility. Certain substrates also supported well the microbial growth, indicating a good potential for biomass generation. Overall, these results demonstrate that tailored fermentation strategies can unlock the nutritional and functional value of food by-products, contributing to food system circularity and ingredient innovation.

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Upcycling of agri-food side streams via fermentation: advancing towards “zero-waste” supply chains in plant fermented foods

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● Fermentation currently represents one of the most versatile approaches for the upcycling and valorization of agri-food side streams and surpluses. These by-products can be used as substrates for the cultivation of microorganisms of industrial interest, including food-grade starter cultures, probiotics, and strains capable of contributing to biopreservation and biocontrol. Moreover, fermentation enables the production of microbial biomasses or metabolites with potential applications in the food, agricultural, pharmaceutical, and cosmetic sectors. Entire food waste fractions can thus be transformed into novel ingredients and reintroduced into the production chain, supporting circular economy principles.

Beyond food-grade applications, selected fermentation strategies allow for the biotechnological conversion of inedible waste materials into bio-based compounds such as bioplastics or soil amendments. These alternatives extend the potential for waste valorization and contribute to the design of more sustainable, “zero-waste” food systems.

This presentation will explore recent advances in microbial fermentation applied to plant-based agri-food residues, highlighting their potential to reduce environmental impact while generating high-value products. The integration of fermentation into food system redesign offers a strategic tool to close resource loops and support the transition toward more resilient and sustainable food supply chains.

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Crafting future foods: the transformative role of food fermentation

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● FFood fermentation, a fundamental part of human dietary traditions for thousands of years, stands as a strong and flexible biotechnological process that has consistently influenced the nutritional and sensory qualities of foods. Over time, it has adapted to changing ideas of food functionality – from primarily providing energy to serving as a carrier for specific nutritional and therapeutic qualities, including natural, nutraceutical, functional, and health-enhancing benefits. In light of modern global challenges, food fermentation is called upon to create sustainable dietary solutions that consider nutrition, health, environmental sustainability, accessibility, and cultural significance throughout the entire food supply chain, from production to waste disposal.

Emerging protein sources, including plant-based materials, insect meal, mycoprotein, and microalgae, are increasingly recognised for their potential to enhance dietary health, safety, and sustainability. However, their widespread adoption is constrained by factors such as consumer neophobia, cultural preferences, and sensory acceptability. Food fermentation biotechnology offers a scientifically grounded and consumer-accepted strategy to address these challenges. By leveraging the techno-functional capabilities of lactic acid bacteria, fermentation can precisely modulate the texture, flavour, aroma, colour, and nutritional profiles of novel food matrices, thereby facilitating their integration into mainstream diets.

This keynote will critically examine the role of tailored fermentation processes in overcoming barriers to the adoption of novel protein sources, emphasising their capacity to enhance consumer acceptance while advancing sustainable food systems. Through a rigorous exploration of current research and innovative applications, we will elucidate how food fermentation can bridge traditional practices with cutting-edge biotechnological advancements, contributing to the development of resilient and equitable food systems for the future.

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Flavor-driven fermentation: unlocking okara's potential

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● Each year, approximately 14 million tons of okara, the solid by-product from soymilk production, are generated worldwide. Despite its promising physicochemical and nutritional properties, okara faces challenges in culinary applications due to its limited organoleptic qualities, including a fibrous, grainy texture and low solubility. Solid-state fermentation (SSF) has emerged as a promising approach to valorize agri-food by-products; however, developing sensorially appealing fermented okara products remains underexplored.

This study presents a practical framework for upcycling okara via SSF to enhance its organoleptic properties and expand gastronomic applications. Filamentous fungi from the *Aspergillus*, *Rhizopus*, and *Neurospora* genera were evaluated *in vitro*, using okara as the primary nutrient source, and in vivo through SSF on okara substrate. Key sensory attributes including flavor, aroma, and texture were assessed for each species. *Rhizopus oryzae* and *Rhizopus oligosporus* produced milder flavors and firmer textures, while *Aspergillus oryzae* and *Aspergillus sojae* offered a more complex flavor profile but with a less compact mycelial structure. *Neurospora sitophila* exhibited a strong fermented aroma, which was generally rejected by consumers, although its texture was comparable to *Rhizopus* species.

Based on these findings, several culinary applications such as cheese analogs, meat substitutes, beverages, and flavor enhancers were explored. *Rhizopus spp.* and *Neurospora sitophila* showed the greatest potential for creating meaty textures and co-inoculations with *Lactobacillus spp.* and *A. sojae* were used to enhance flavor.

Uncovering the stable microbial core of pulque: Insights from a traditional Mexican fermentation

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● Pulque is a traditional Mexican alcoholic beverage produced by the spontaneous fermentation of *aguamiel*, the sap extracted from various *Agave* species. Pulque has been produced since pre-Hispanic times and is considered the most essential and studied traditional non-distilled fermented beverage in Mexico. In the present study, we evaluated the temporal stability of the core microbiota responsible for pulque fermentation across different sample types, fermentation stages, and seasons. Microbial diversity was characterized in *metzal* (the residual scraped maguey pine), while aguamiel, overnight-fermented pulque, and laboratory-controlled six-hour fermentations were analyzed for microbial composition and metabolite profiles. Samples were collected from three *A. salmiana* plants across four seasons (2021–2022) in the traditional pulque producing location of Huitzilac, Morelos, Mexico. Bacterial and fungal communities were profiled using 16S rRNA (V3–V4) and ITS1 amplicon sequencing, and sugar, ethanol, and organic acid concentrations were quantified by HPLC/UPLC. A stable core microbiota dominated all analyzed samples included *Zymomonas, Lactobacillus, Leuconostoc, Lactococcus, Acetobacter, Kazachstania, Kluyveromyces*, and *Saccharomyces*. Positive correlations were observed between several identified OTUs and sugar consumption and metabolite production. Despite seasonal shifts in diversity, functional core dominance remained stable. The core microbiota identified here substantially overlapped with a previously reported core determined in a single sampling season in November 2019, confirming the persistence of key taxa despite environmental and temporal variation. These findings highlight the stability of the pulque core microbiota and support its potential use in microbial quality control and standardized starter cultures.

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Microalgae as alternative protein-rich microbial biomass.

[1] **Shao Quan Liu**

● Microalga has emerged as one of the most efficient food biomass and protein producers to address the issues of climate changes, environmental protection, and food production sustainability owing to its land-efficiency and high productivity relative to traditional crops such as soybeans. Conventional cultivation of microalgae (biomass fermentation) under photosynthetic conditions requires large areas of land, which is not suitable for a city-state like Singapore. Indoor cultivation of heterotrophic microalgae under non-photosynthetic conditions offers a promising alternative in an urban environment. Food processing side streams (by-products) contain rich nutrients that can be released upon hydrolysis and serve as the medium for cultivating heterotrophic microalgae. This presentation discusses the replacement of conventional medium with soy whey and brewer's spent grain hydrolysates for microalgae growth (*Auxenochlorella protothecoides*) with comparable maximum biomass production and productivity to that of conventional medium being achieved but at much lower costs. This approach provides a novel way of cultivating microalgae as protein-rich microbial food biomass to contribute to the circular bioeconomy and sustainable food production.

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Fermented hybrid cheese: sustainable, tasty, and nutritious

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● Driven by global challenges linked to food production, there is growing interest for sustainable dairy alternatives. Although many plant-based dairy alternatives exists, they often lack taste and nutritional value. In particular, this is the case for existing cheese analogues, which generally lack proteins and are not fermented. This study explores the potential of fermentation of hybrid matrices, consisting of dairy and plant-based ingredients, to create more sustainable, tasty and nutritious cheese alternatives. Fermentation was chosen to improve the taste and nutritional value by reducing the plant-related off-flavours and anti-nutritional factors (ANFs) as well as produce desired flavours and improve digestibility. Hybrid Gouda and Camembert cheese analogues were developed by varying ratios of milk and pea protein as well as processing conditions aiming for optimal curd formation. Suitable starter cultures were selected based on acidification, microbial growth, flavour formation and off-flavour reduction. Interestingly, acidification by lactic acid bacteria (LAB) was improved in the dairy-pea hybrids compared to pure dairy and plant-based matrices. Moreover, fermentation by LAB and fungi reduced beany off-flavours and generated cheese-related flavours in particular during the ripening process. Notably, addition of pea protein significantly increased cheese yields and prevented moisture loss during ripening due to its high water-binding capacity. Furthermore, the protein content and digestibility significantly increased in the hybrid cheese compared to both dairy cheese and plant-based cheese analogues, and ripening contributing to this. Overall, this study demonstrates that fermentation and ripening of hybrid cheese matrices is a promising strategy to produce sustainable high-quality cheeses analogues.

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Sensory quality and safety of plant-based and hybrid foods: the role of fermentation, microbial growth and substrate availability

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● To improve the sustainability of food production a shift away from animal proteins is desired. While the availability of plant-based products grows, sensory and safety issues are still significant challenges. Fermentation offers a promising avenue to improve the final product quality. We here show that the use of microbes under different conditions allows to improve flavor profiles of fermented plant-proteins. This can be achieved through the removal of off-flavors as well as through the production of desired flavors. Interestingly some of the metabolic conversions can occur in carbon limited conditions without microbial growth or product acidification, and they can take place in 10–20 minutes. Such a step allows to make food ingredients more neutral tasting without a classical fermentation, a process we call bio-purification. Besides the removal of specific off-flavors the formation of desired flavor volatiles in plant-based dairy analogs like cheese is a challenge. We hypothesize that this is in part due to a shortage in essential precursors for flavor formation. Using a plant-dairy hybrid system we investigated volatile development after incubation with various lactic acid bacteria. The results show that in hybrid products flavor active compounds from carbon and nitrogen metabolism are formed in a strain- and substrate dependent manner. Since plant-based substrates often contain high levels of spoilage organisms, we use a broad collection of strains for selecting functional starters, combining the desired volatile alterations with acidification in fermented products. Gene clusters predicting observed phenotypes were identified, demonstrating future directions for strain selection.

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Boosters and hurdles towards innovation in fermented foods.

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● PIMENTO, the European COST Action 20218 for Promoting Innovation of ferMENTed fOods, engaged the EU Fermented Foods (FF) industry to examine its perception of boosters and hurdles to innovation. A 60 questions survey was made available on the Conjointly® platform and disseminated throughout COST countries. Important feedback from a large population of 141 industry representatives located in 25 countries helped painting a qualitative picture of the foreseen field and types of innovation, the impact of regulatory limitations, and related technical and safety considerations.

For the next 5 years, they focus their innovation by exploring all possible options, in terms of new matrixes, cultures, processes and formulation indicating strong needs in fermentation science.
Regulatory speaking, interestingly, industry showed common agreement on proposed definitions (fermented foods, dietary microbes, microbial cultures) and shared the need for dedicated science-grounded regulatory definitions in the FF sector. However, when considering a wider microbial diversity/raw materials/ingredients/formulations innovation, they tend to avoid novel foods classification if possible.

Technically, only operative issues reached the top five, highlighting difficulties with industrialization and scale up. Safety of FF issued from new matrixes/ingredients/formulation is seen as relevant foreseeable bottleneck, yet do not impede respondents' will to innovate.

Considering these results, even if the FF sector is very fragmented, there are common issues that could be more efficiently addressed by a European-wide organization supporting all stakeholders to promote innovation. A FF cluster could work at identifying needs, connecting complementary academic and industry partners, while catalysing a dialogue with policy-makers and consumers.

Development of functional fermented foods to support human health.

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● Fermented foods offer numerous health benefits that are not only attributed to the microorganisms they contain, but also to their metabolic products – known as microbial metabolites.

In an in-silico study we compared the genomes of 600 strains derived from the Liebefeld Culture Collection, comprising >15,000 isolates mainly originated from the dairy industry, with the metagenome of the human gut microbiome. We found that 24 strains from this collection were sufficient to cover 89% of all annotated enzymatic reactions of an average human microbiome. We therefore hypothesized that fermented foods could be specifically produced to functionally complement the metabolic activity of a dysbiotic gut microbiota.

Consequently, we enhanced the production of certain bioactive substances (e.g. vitamins, amino acid derivatives) in fermented milk, by selecting strains based on the presence of genes encoding enzymes in the targeted metabolic pathways. The functionality of the fermented milk was subsequently tested in mouse models. In particular, we showed that the immune regulatory activity of the gut microbiota could be rescued by feeding germ-free mice with a fermented milk enriched in microbial tryptophan catabolites.

Finally, we developed Scoary2, an ultra-fast microbial genome-wide association studies (mGWAS) software, to link the production of specific metabolites (e.g., carnitines) to the genetic content of the bacterial strains used for the fermentation.

Our approach shows that the combined analysis of bacterial genomes and food metabolomes using modern bioinformatic tools offers promising potential for the targeted development of functional fermented foods.

Modulating the aroma and taste profile of soybean using novel strains for fermentation.

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● Soybean is a significant source of protein globally and is widely used in various culinary traditions. A key factor in consumer acceptance of soybean products is the aroma and taste profile, which can be modulated and improved through fermentation with the use of unique microbial strains. Fermentation can modulate of the aroma and taste profile of soybean products through the introduction of unique strains in the fermentation process. This study identifies and characterizes novel microbial strains with the potential to enhance flavour profiles, including umami, while reducing undesirable notes such as beany aromas. Results show an increase in free amino acids, fatty acids, and the generation of unique aroma compounds, such as “smoky”, “cheesy” and “floral” notes. In conclusion, this study shows that the innovative use of novel microbial strains in soybean fermentation is a potential strategy to modulate the aroma and taste profile of soybean products. Further research is required to optimize fermentation processes, such as using a consortium of strains, with the goal of enhancing the palatability of fermented soybean and improve the acceptability of soybean products as an alternative protein source.

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Wheat by-product valorisation: microbial fermentation as a powerful tool for the production of interesting ingredients from wheat bran

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● The cereal industry is the sector with the highest percentage of food waste at the primary production level, with wheat bran accounting for approximately 90 million tonnes of waste per year. However, this side-stream is particularly rich in dietary fibers (37–60%), minerals, and vitamins, making it a particularly attractive substrate for its exploitation. In addition, it is also rich in carbohydrates, such as hemicellulose, cellulose and starch, which require sample pretreatments (i.e. enzymatic hydrolysis, heat treatment) to enhance their bioavailability. Recent studies have proposed fermentation as a “green”, low-cost, and sustainable biotechnology to enable wheat bran recycling while also improving nutrient bioavailability. In this scenario, bioprocesses such as enzymatic treatment (i.e. amylases, proteases, cellulase, etc.) and/or fermentation represent new promising solutions. Aiming at wheat bran valorisation for the production of ingredients with high technological value, growth and acidification kinetics of various strains of lactic acid bacteria (LAB) and yeasts were individually tested for their ability to ferment wheat bran substrate. LAB and yeast strains in co-culture and/or in combination with enzymes were also used to assess the effect of combined starters and enzymatic treatment on wheat bran substrate. Analytical techniques were applied for the characterization of the fermented wheat bran (i.e. sugars, organic acids, short chain fatty acids). Furthermore, to assess their functional properties, proteolytic and phytase activities, and exopolysaccharides (EPS) production were investigated. After optimizing fermentation process and enzymatic treatment, the final ingredient will be further characterized and adapted to industrial contexts for possible future application in food products.

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Comparative analysis of sourdough fermentation: traditional starter vs. probiotic strains for type II and type IV fermentations in bread making

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● Sourdough fermentation, an ancient method for fermenting cereal flours, improves the nutritional, texture, and sensory qualities of leavened baked goods through microbial metabolic activity. This study aims to compare type II and type IV sourdough fermentation with a standardized and reproducible starter against a multi-strain starter of probiotic strains. In the first phase, the protocol for the development of a mature type-IV sourdough was optimized. The fermentation conditions were standardized based on the persistence of inoculated probiotic starters, as confirmed by RAPD-PCR analysis.

Additionally, a control type-II sourdough fermented with *Lactoplantibacillus plantarum, Furfurilactobacillus rossiae,* and *Saccharomyces cerevisiae* for 24 h was made for comparative assessment. All sourdoughs were subjected to proteolytic digestion by using commercial protease enzymes to evaluate the synergistic effect of gluten degradation by starters and enzyme. The sourdoughs (inoculum size of 30%) were then used in the preparation of sourdough breads along with baker's yeast bread as the control. The resulting breads were characterized for nutritional (residual gluten content, in vitro protein digestibility (IVPD), predictive glycemic index (pGI), and free amino acids), and texture profile (hardness, gumminess, adhesiveness, and color) parameters. Results revealed that type-IV sourdough, fermented with functional probiotic strains with or without enzyme, had lower gluten content compared to type-II sourdough and baker's yeast bread. Additionally, it exhibited higher IVPD and lower pGI than baker's yeast bread and comparable to type-II sourdough bread. These findings indicated that incorporating functional probiotic strains in sourdough fermentation can enhance the functionality of leavened baked goods by reducing gluten content, improving digestibility, and lowering pGI.

[Keywords] Sourdough fermentation, probiotics, lactic acid bacteria, protein digestibility

Advances in understanding wholemeal sourdough breadmaking: molecular mechanisms by which organic acids influence dough strength and bread volume

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● Driven by consumer demand for healthiness and sustainability, the food industry faces the challenge of creating high-quality wholemeal bread within the constraints of clean-label formulations. Sourdough offers a promising strategy, however, contradictory scientific evidence regarding its functionality in wholemeal wheat breadmaking highlights the need for a more comprehensive understanding. This study aimed to elucidate the molecular mechanisms by which sourdough addition influences the breadmaking process and the bread volume. Sourdough addition lowered the amount of water and the mixing time required to maximise the specific volume of wholemeal bread. In-depth analyses across multiple length scales showed that dough acidification down to a pH of 4.5, 5.0, and 5.5 with acetic acid, lactic acid, and succinic acid increased wholemeal bread dough extensional viscosity and bread volume (+10, +15, and +22% respectively) compared to an unacidified control. This resulted from more interactions between flour constituents, evidenced by a reduced water-binding capacity of the bread dough and increased interconnectivity in the protein network. Analysis of the secondary structure of proteins using Attenuated total reflectance Fourier-Transformed Infrared spectrometry showed that more intermolecular β-sheets were formed upon acidification (pH 4.5), at the expense of intramolecular β-sheets. The findings suggest that succinic acid and acetic acid form more stable interactions with dough constituents, enhancing protein interconnectivity to a greater extent than lactic acid. These novel insights will prove useful in selecting sourdough consortia for sourdough production, optimising breadmaking processes, and strengthening the emerging position of wholemeal bread in the global bakery market.

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Understanding the key enzymatic conversions for successful lactic acid fermentation of kilned oat wholemeal.

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● Fermentation of heat-treated raw materials, such as oat wholemeal from kilned groats, is often hindered by low endogenous enzyme activities limiting nutrient availability for lactic acid bacteria (LAB). Building on our previous finding that acidification during lactic acid fermentation (LAF) was slower for kilned compared to non-kilned oat wholemeal (<pH 4.5 within 14 h and 6 h of fermentation, respectively), this study investigated the key enzymatic conversions influencing successful LAF with *Lactiplantibacillus plantarum* of kilned oat wholemeal. Kilned oat wholemeal was fermented with *L. plantarum*, and the impact of the addition of proteases, amylases, and a combination of both was monitored (i.e. acidification rate, microbial growth, lactic acid production and sugar consumption) over time. Final pH values after 24 h of fermentation reached 4.2 (no added enzymes), 4.0 (amylases), 3.7 (protease), and 3.4 (both enzymes). Protease addition accelerated acidification and overall fermentation metabolism over time. After 24 h, lactic acid content increased from 1.6% dm (control) to 2.4% dm (protease) and 4.2% dm (amylases and protease). These findings led to the evaluation of two strategies: using LAB starter cultures with amylase and peptidase activity, and supplementing *L. plantarum* with an oat malt aqueous extract as a natural source of endogenous oat enzymes. Only the latter approach effectively enhanced acidification during LAF of kilned oat wholemeal and matched the results observed upon exogenous amylase and protease addition. The insights gained from this study have the potential to advance the application of oats in innovative food fermentations.

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Innovative food fermentation: case study on fermented tropical mango for fruit fillings

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● At Puratos, we believe that food has the power to nourish, comfort, and bring people together. 60% of consumers are looking for new food experiences according to Puratos Taste Tomorrow global consumer research. At Puratos fermentation is one of the strategic pillars when it comes to generating innovative ingredients for bakery, patisserie and chocolate. Two case studies will be described to show the importance of food fermentation to bring new innovative solutions to consumers worldwide.

A case study related to fruit fermentation, more in particular mango fruit pieces were fermented to generate excellent taste, nice texture and color and techno-functional properties. An in depth screening assay has been setup for 1,000 bacteria and 1,000 yeast strains to assess their capability to ferment mango fruit pieces. Properties like flavour profile, alcohol content, gas formation, etc were studied. Selection of a specific *Lactiplantibacillus plantarum* LMG S-33688 strain and optimization of the fermentation process resulted in a 100% natural mango fruit filling, able to be used in different patisserie and bakery applications.

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Innovative food fermentation: case study on cocoa fermentation based on single-origin varieties from the Philippines

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A case study on cocoa fermentation and the importance of single-origin varieties in the Philippines will be presented. Different single-origin varieties were used for cocoa fermentation. Depending on the single-origin variety used and the microbial population fine flavours like floral flavours can be generated in chocolate. This study demonstrated that chocolate flavour can be fine tuned not only by having the right microbial population but also by selecting specific mixes of single-origin varieties of cocoa beans.

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Energy-efficient organic french fries through lacto-fermentation.

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● French fries are among the most popular convenience foods worldwide, but their production is highly energy-intensive due to blanching, pre-frying, shock-freezing, and long-term frozen storage. Even post-distribution, maintaining the cold chain remains essential until final preparation. In light of growing pressure to develop more sustainable food systems, this project explores fermentation at harvest as an alternative preservation method, inspired by traditional sauerkraut-making. By eliminating energy-demanding steps like blanching and freezing, this approach could drastically reduce energy consumption while maintaining product quality. To test this hypothesis, various parameters were evaluated to achieve a pH reduction in the brine. Changes in inoculum concentration and anaerobic/aerobic conditions did not lead to the expected pH drop associated with the development of lactic acid bacteria and fermentation. However, adding sucrose resulted in a significant pH reduction within 2 days. To avoid adding sucrose to the product, the cold-induced sweetening of potatoes was investigated. During this process, potato tubers accumulate reducing sugars, increasing sugar availability for bacteria, which led to a pH drop to 4. Consumer tests revealed that blanching was a key step for achieving a soft interior texture in the fries. However, 75% of participants found the fries still insufficiently crispy. Reducing the salt concentration in the brine to 1% was well-received, with most participants finding the saltiness well-balanced. Acidity remains an area that needs improvement; while 42% of participants found the acidity “just right,” 48% described the fries as “slightly too acidic.” Adjustments, such as reducing the fermentation time, could help address this issue. Ultimately, the test highlighted strong consumer interest, with the majority willing to recommend the product for future restaurant orders. These results suggest that fermentation could be a promising alternative for more sustainable French fry production and preservation.

Regenerative recycling of agro food waste for enhancing the Mediterranean food system's circularity.

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● Agriculture-based industries generate a large amount of waste each year, which is often released into the environment without proper disposal. Several Mediterranean agri-food side streams have a unique structure and nutritional composition, with inherent potential functional and bioactive properties such as antioxidant, antifungal, and/or gut-modulatory effects.

This project aims to dignify nutritional complementing agri-food wastes and by-products by developing smart and effective biotechnological solutions through two steps: solid-state fermentation (SSF) and liquid-state fermentation (LSF).

Several overall results will be generated: developing biotransformation technologies can lead to the production of valuable enzymes and bioactive compounds useful for food or beverage fortification. Specifically, two-stage fermentation strategies will be explored by employing edible fungus *Pleurotus ostreatus* (SSF), followed by characterised QPS-yeast (LSF) to improve the efficiency of functional ingredient production. The pretreatment with *P.ostreatus* will support the subsequent fermentation by yeast by enhancing the breakdown of complex polymers, which can lead to better growth yields and a more efficient overall process.

In the second part of the project, new snacks and/or beverage formulations will be designed, exploiting different fermented repurposed by-products. Food prototypes will be characterised based on their key nutritional value (vitamins A, B12, and D, antinutrients, such as phytate, tannins, and oxalates, and minerals, such as Zn, Fe, Ca, and Mg) and bio-accessibility, and their health traits (oxidative stress and inflammatory process, gut microbiome homeostasis).

Thus, biotransformation strategies through fermentation would represent a cost-effective strategy to upcycle agro-food waste and to boost the food system's circularity.

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Innovative fermentation strategies for extending quality and shelf life of ready-to-(H) eat meals.

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● Nowadays, the shift towards more sustainable food systems represents a critical global challenge for stakeholders and industries. Pasta, a mainstay of many people's diets worldwide, generates approximately 1.98 kg of loss and waste throughout its lifecycle for each kg of pasta produced. Therefore, aiming at a "zero waste economy", the study focuses on the development of ready-to-eat pasta by innovatively recycling pasta scraps (industrial leftovers of the lasagna making process) through sourdough biotechnology. In order to facilitate the growth and subsequent isolation of the autochthonous lactic acid bacteria (LAB), a type I sourdough was prepared by mixing pasta scraps and water and allowing it to ferment spontaneously. LAB strains were further characterized for their growth and acidification kinetics, carbohydrates and organic acids metabolism, proteolytic activity, and exopolysaccharide (EPS) secretion on selected food-like media. The best performing LAB strains in terms of acidification capacity and proteolytic activity were used as starter cultures for pasta scraps-based sourdoughs (PSS) making. Sourdoughs production processes were optimized and the new formulated PSS were characterized for their technological, nutritional, and sensory properties, and subsequently used for new pasta production. Textural parameters and nutritional and sensory aspects of newly formulated pasta prototypes, along with the study of their shelf-life, will also be analysed. In conclusion, our findings will shed light not only on the development of possible new fermentation strategies for recycling food waste to reduces the environmental impact, but also on the possibility of improving the nutritional profile of a common food such as pasta.

Investigation of sourdough stability originated from artisanal bakeries and maintained in the sourdough library under different maintenance protocols.

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● Sourdough is characterized by complex microbial communities that influence bread quality and shelf-life. This study examined the microbial stability and dynamics of seventeen artisanal sourdoughs preserved at the Puratos Sourdough Library compared to their original bakery conditions. Chemical, biochemical, and microbial compositions were analyzed using both culture-dependent and culture-independent methods across four maintenance protocols: library-maintained, bakery-refreshed at 15-day and 30-day intervals, and continuously bakery-maintained. Variations were evident in biochemical parameters such as acidification, organic acids, sugars, and ethanol content, with library-maintained samples generally exhibiting more pronounced acidification compared to bakery-maintained ones. Lactic acid was predominant, accompanied by diverse fermentation profiles across batches. Sugars and ethanol content also showed notable variability, reflecting dynamic microbial metabolic activities. *Lactiplantibacillus plantarum* and *Fructilactobacillus sanfranciscensis* were consistently the dominant lactic acid bacteria, with yeast communities mainly comprising *Saccharomyces cerevisiae* and *Kazachstania* humilis, along with occasional occurrences of rare yeast species such as *Naumovozyma castellii*. Comparative analysis indicated that continuous bakery maintenance frequently reduced initial microbial diversity, whereas intermediate bakery refreshment facilitated the restoration of microbial diversity in most of the samples, highlighting notable microbial resilience and adaptability. This research underscores the dynamic nature of sourdough microbial ecosystems and emphasizes the crucial influence of maintenance practices on microbial stability and diversity.

Insights into the unpredictability of lambic beer production processes.

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● Belgian lambic beers are beers from a spontaneous origin, made in Brussels and the Southwest area of Brussels. The production of these lambic beers is characterized by unique features, such as the use of unmalted wheat in addition to malted barley, an open metal coolship to cool down the boiled wort, and horizontal wooden barrels for the fermentation and maturation process that takes up to three years. Each of these features play a role in the outcome of the final lambic beer by influencing both the microbiology and biochemistry of the fermenting lambic beer wort and maturing lambic beer. The present study aimed to get more insights into the identification of the key inoculation sources of the lambic beer wort. Therefore, samples were taken from the raw materials, swab samples of the brewery room and equipment, and air samples during brewing and of the environment for a culture-dependent and culture-independent microbiological analysis. Further, several lambic beer productions were monitored both microbiologically and biochemically, the latter with the use of liquid and gas chromatography systems combined with the most suitable detectors. Also, the physicochemical parameters pH, temperature and dissolved oxygen were measured. The results revealed that the spontaneous inoculation and process parameters had a great impact on the final lambic beers. The wooden barrels and house microbiota were the most important inoculation sources of the lambic beer wort. Furthermore, the temperature determined which *Saccharomyces* species were present during the alcoholic fermentation phase, whereas the pH determined which *Brettanomyces* species were present during the maturation phase. The dissolved oxygen concentrations as a result of micro-oxygenation in the wooden barrels determined the cell density of the acetic acid bacteria. To conclude, the use of wooden barrels and monitoring of the temperature are key for the quality of lambic beers.

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Unlocking the potential of brewer’s spent grain: solid-state fermentation for culinary applications

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● Brewer’s Spent Grain (BSG), the main by-product of beer production, represents 85% of the total by-products generated in the industry. Annually, 39 million tons of BSG are produced, with 20 kg generated per 100 L of beer. Often considered low-value waste, BSG is disposed of in landfills or used as fertilizer or animal feed. Its high moisture content (70–80%) makes it prone to microbial spoilage, posing environmental risks such as soil degradation.

Additional processing of BSG should be considered to enhance its structural and textural properties for food applications. The aim of this study was to develop a new ingredient from BSG through koji-like solid-state fermentation, for further application in culinary uses.

Fermentations with *Aspergillus oryzae* and *A. luchuensis* were studied, characterizing the process using classical microbiological, physicochemical, and sensory analysis techniques to understand the production process. An Asian-inspired product, amino paste with 4% salt, was developed. Additionally, a full factorial design was used to create different formulations of a cake to replace wheat flour with spent grain koji flour at various percentages. The final products were analyzed for moisture, protein content, texture properties, and sensory analysis.

Both *A. oryzae* and *A. luchuensis* grew well during the fermentation process. The physicochemical characterization will be performed, but so far, successful prototypes have been developed and will also be characterized. Brewer’s spent grain could be successfully transformed via solid-state fermentation to obtain both amino pastes and flour for bakery products.

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From waste to functional ingredients: application of fermentative processes for the valorisation of Tuscan agricultural by-products

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● The economic value of agri-food by-products in Italy has been estimated at €8.5 billion/year. The interest in using by-products as functional ingredients is strongly increasing. Despite the growing value of the economic sector of by-products in Italy, most of them are still treated as waste and disposed of in landfills. Their disposal represents a cost for agri-food companies, environmental damage, and a loss of bioactive compounds. Fermentation is a suitable tool that can contribute to the valorisation and reuse of by-products. Therefore, the project aimed to obtain dried fermented food-grade products from Tuscan agricultural by-products and waste from fruit and vegetable processing, with enhanced nutritional potential and peculiar properties. The agricultural by-products were 13, including olive and grape pomace and different fruits and vegetables. The fermentation process for each by-product was optimised using up to 4 consortia of microorganisms (yeasts and lactic acid bacteria), testing different times and temperatures of fermentation and drying. All substrates supported microbial growth, and a general increase in the antioxidant activities was observed. For instance, a fermentation of 48 h at 30°C increased the total phenolic compounds of olive pomace, especially tyrosol and hydroxytyrosol. The results allowed the selection of the best conditions for each by-product to be used during the scale-up process. The scale-up enabled the production of 13 food-grade functional fermented powders with high added value and innovative features, demonstrating the suitable application of fermentation for the valorisation of Tuscan agricultural by-products.

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Microbial fermentation and high-pressure homogenization as integrated strategies for the valorization of brewer's spent grains into functional food ingredients.

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● Every year, around a third of the food intended for human consumption is lost, including significant quantities of cereals, fruit and vegetables. The PROACTIVE project (PRIN 2020, Prot. 2020CNRB84) focuses on the valorisation of food by-products to obtain bioactive peptides to be used in health-oriented food applications. Among the approaches investigated, microbial fermentation and high pressure homogenisation (HPH) could potentially improve the increase of bioactive peptides in food by-products such as brewer's spent grains (BSG). In our study, after substrate optimisation, BSG was fermented with selected yeast and lactic acid bacteria with or without prior HPH treatment on the strains (500–1000 bar). The resulting samples were analysed for their peptide content and the most promising fermented samples were further characterised. Microbial growth and the absence of cytotoxic effects were confirmed in the intestinal cell lines Caco-2 and HT-29 using the MTT assay. In addition, the protective role of these samples against SDS-induced inflammatory stress and their anti-inflammatory potential in Caco-2, HT-29 and RAW264.7 macrophage cells were evaluated by measuring nitric oxide (NO) production. The results showed that the fermentation process, which was influenced by both microbial strain selection and HPH treatment, resulted in the production of peptide-rich samples with different functional properties. These results support the potential application of fermented BSG as a source of functional ingredients.

Sub-standard peas fermented by *Lactiplantibacillus plantarum* as a biologically active ingredient to fortify bread: a sustainable approach to food waste valorization

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This study investigated the application of fermentation by *Lactiplantibacillus plantarum* strain ITM21B as a sustainable approach for valorizing legume waste. Sub-standard peas (sP), an abundant waste generated after the harvesting of peas during the spring season in Italy, were grinded, mixed with water (40:60 ratio) and fermented (log10 5.0 CFU/g, 14 hours at 37°C) to obtain a functional bioingredient for bread fortification. After fermentation (BioP) a significant production of lactic acid, glutamate and TFAA was observed. The bioingredient BioP was evaluated for its biological properties showing higher antioxidant activity and total phenol content than unfermented sP sample (CTR). BioP and CTR extracts were tested on HepG2 liver cells at 0.01, 0.1 and 1 mg/mL, following treatment with oleate/palmitate (OP) to mimic hepatic steatosis demonstrating a significant reduction of OP-induced lipid droplet accumulation, particularly at lower concentrations. Freeze-dried BioP was also used to partially replace wheat flour (2.5%, 7.4%, 16.6% and 25.4%) in bread formulation to produce fortified products in comparison to control bread (CB). The increased polyphenols in BioP led to a significant lowering in predicted glycemic index and starch hydrolysis in fortified bread compared to CB, positively correlated to the amount of BioP added. The protein content significantly increased already at the lower BioP replacement. Texture analyses revealed a significantly positive impact of BioP on hardness, cohesiveness and chewiness of bread at 2.5% and 7.4% replacement. Thus, fermentation of sP improved the biological properties of legume waste and allowed the production of a bread-fortifying ingredient, while also reducing waste.

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Comparison of three gas monitoring devices: Risograph, RF Gas Production System & YeastForce

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● Bread volume is a crucial quality aspect in industrial baking. After mixing where gas bubbles are incorporated in dough, yeast activity mainly generates CO_2, responsible for expanding gas cells and so to dough expansion during proofing and baking. Monitoring gas production in yeasted dough during proofing is therefore a key factor in breadmaking. Traditionally, this monitoring has been carried out empirically by the evolution of height gain in a graduated pot put in a proofing cabinet, or using Risograph (National Manufacturing Co, USA) according to the AACC Method 89-01. After discontinuation of National Manufacturing activities and the unavailability of Risograph on the market, there is now a need to find alternative solutions to fill this gap. The objective of this study is determining whether alternative gas monitoring devices, such as YeastForce (BlueSens, Germany) and RF Gas Production System (Ankom, USA), could provide reliable gas production measurements comparable to Risograph.

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Optimization of aeration rate and aeration timing for ethanol production from date byproducts by *Saccharomyces cerevisiae* using response surface methodology.

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● The substrate is the main cost component for industrial ethanol production and it is essential that ethanol production should be carried out with cheap substrates. In Algeria, the conditioning units of dates generate significant quantities of waste arising from sorting deviations. This biomass, until then considered as a waste with high impact on the environment can be transformed into high value added product such as ethanol. Dates fruit are suitable resource for ethanol production. They contain considerable amounts of sugars (glucose, fructose and sucrose) about 73 to 83%. Aeration is one of the significant factors affecting yeast physiology and impacts ethanol production. The aim of this study was to optimize the aeration rate and aeration time of ethanol production by *Saccharomyces cerevisiae* using a factorial design based on response surface methodology (RSM). The aeration rate (0.5, 1.5 and 2.5 vvm) and the aeration timing (2, 4 and 6 h) (agitation rate 120 rpm) were applied. The ethanol production medium was date juice containing 150 g.L–1 of total sugar and 1 g.L–1 of urea. The fermentation was carried out at 30°C in a 2-L fermenter. Response surface methodology analysis showed that the optimal aeration for ethanol production were an aeration rate and aeration time at 1.5 vvm and 4 h, respectively. Under the optimal conditions, the distilled and rectified date juice generated the highest ethanol 90°C. Under an unaerated condition (control treatment), the ethanol production after distillation was 78°C. The results clearly indicated that optimal aeration can improve ethanol production.

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[Keywords] aeration, ethanol production, date byproducts, Saccharomyces cerevisiae, response surface methodology

Data-driven design of microbial communities to enhance the sensory and nutritional quality of faba bean protein through fermentation

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● Plant-based protein sources such as faba bean are promising alternatives to animal-derived ingredients but are hindered by undesirable off-flavors and antinutritional compounds, requiring innovative and sustainable processing approaches. Fermentation offers a natural solution, yet its broader application is limited by the absence of tailored microbial cultures optimized for diverse plant matrices. As part of the Novo Nordisk founded MiCoP project, this study aimed to investigate the fermentation potential of selected lactic acid bacteria (LAB) and Bacillus spp. to improve the sensory and nutritional quality of faba bean protein and to generate foundational data for a machine learning (ML) AI-assisted design of microbial communities. Fermentations were performed using single cultures of *Limosilactobacillus fermentum*, *Lactiplantibacillus argentoratensis*, *Bacillus velezensis*, and *Bacillus subtilis*. Strains were identified and characterized through whole-genome sequencing with Oxford Nanopore Technology. Metabolic outputs, including volatile compounds, organic acids, free amino acids, ɣ-glutamyl peptides, and the antinutrients vicine and convicine, were assessed as microbial Key Performance Indicators (KPIs). All strains reduced aldehydes associated with beany off-flavors. Species-specific differences were evident: LAB strains promoted alcohol and lactic acid formation, *B. velezensis* yielded diacetyl and acetoin, and *B. subtilis* produced highest amounts of esters. Furthermore, *Bacillus* spp. exhibited strong proteolytic activity, enhancing nutritional quality and kokumi attributes through essential amino acid and ɣ-glutamyl peptide release. This dataset will serve to train an ML model for AI-assisted design of fermentation cultures. The project will deliver open-access data, tools for microbial selection, and insights into optimizing plant-based fermentation for improved taste, nutrition, and sustainability.

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Real-Time impedometric evaluation of lactic acid bacteria and yeast growth behaviour in different plant-based matrices.

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● Fermentation is one of the most widely used strategies for food preservation moreover, the use of microorganisms can have beneficial effects on food matrices by enhancing their sensory, nutritional, and safety properties. This study aimed to evaluate the fermentative capacity of 25 lactic acid bacteria (LAB) strains, 5 LAB co-cultures, and 7 yeast strains-each characterized by distinct phenotypic test in different plant-based matrices.

A total of 259 strain-matrix combinations were tested in duplicate across 7 plant-derived substrates, including soy, chickpea, oat, almond and coconut. All the strains and matrices were provided by the company Sacco srl.

Fermentation was monitored using impedometric analysis, a real-time monitoring, replicable, and cost-effective technique. After 48 hours of fermentation, the pH was measured to determine the final acidification and the impedometric data were used to determine the Lag values (time required for strains adaptation to each matrix). The strains showed different growth behaviours depending on the species and the matrices, highlighting how the metabolic activity depends on strain-specific characteristics.

Based on these results, the strains which showed the best growth performances were identified as key candidates for further studies on biological activity, such as their ability to decontaminate plant-based matrices or their impact on aromatic profile.

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Valorization of vegetable side streams into high-quality and low-processed fermented foods for human consumption using solid-state fermentation.

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● One-third of the food produced for human consumption is lost across the food value chain, amounting to 1.3 billion tons per year. The vegetable processing industry generates substantial food-grade side streams that are commonly discarded despite their nutritional value, primarily due to safety concerns and high consumer expectations. This study explores the potential of solid-state fermentation (SSF) to valorize two of such streams – white button mushroom and mung bean sprout production residues – into safe, palatable, and protein-rich food prototypes for human consumption. Inspired by traditional tempeh production, we developed a two-step fermentation approach adapted for vegetable streams. First, selected lactic acid bacteria (LAB) strains were screened for their ability to rapidly acidify low-moisture substrates, enhancing safety by suppressing pathogens. Subsequently, various filamentous fungi, including *Rhizopus* spp. and *Aspergillus* spp., were evaluated for their ability to generate a compact, protein-rich texture through mycelial growth. The resulting prototypes were then assessed for a range of possible industrial applications and post-processed to yield scalable food products. Our results demonstrate that SSF can effectively transform vegetable side streams into nutritious fermented foods with potential consumer appeal. This approach presents a scalable, circular solution that aligns with sustainable food production goals and paves the way for next-generation plant-based food products.

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Maintaining whole microbial consortia over extended periods: insights from sourdough as a model ecosystem

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● A shift toward an ecosystem-based approach is currently emerging in fermentation research. It is well known that microbial consortia (MC) form the basis of fermentation processes. Therefore, controlling food fermentation using pure (axenic) cultures is ecologically simplistic and fails to exploit the process's full biological potential. Within this framework, the Project Micro4ever (PRIN-PNRR 2022, n. P2022RJYCN) investigated the impact of cryopreservation (CrP) and freeze-drying (FD) on the functionality of whole MC following storage and reactivation. Sourdough was chosen as a model system because its complex MC harbors high microbial diversity and generates consistent biotic pressure, which is constantly under threat. To maintain the viability and functionality of five natural sourdough MC during medium-term (four months) and long-term (eight months) storage, we used dimethyl sulfoxide (DMSO) or glycerol as a cryoprotectant for CrP, while skimmed milk or sucrose was used for FD. The post-preservation characteristics of the MC were assessed using a combination of culture-dependent methods, fluorescence microscopy, and both metataxonomic and meta-phenomic analyses. This integrated analytical approach allowed for a nuanced understanding of how storage conditions and cryo-/lyo-protectants impact the composition and functional traits of preserved MC.

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Formulation of a functional cucumber pickle using *Salicornia* spp. as salt substitute.

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● This research investigates the potential of *Salicornia* spp. as a natural substitute for sodium chloride (NaCl) in cucumber pickle fermentation. Six experimental groups (T1–T6), each with different proportions of NaCl and *Salicornia* spp., were analyzed over a 21-day period. Various physicochemical, microbiological, and sensory aspects were monitored. Findings showed that partial NaCl replacement – especially in T3 and T4 (3%–2% NaCl with 20–30 g *Salicornia* spp.) – successfully reduced sodium levels while preserving fermentation and flavor. Lactic acid bacteria remained dominant throughout, ensuring safety. While total replacement worked, partial substitution yielded the best texture and flavor. *Salicornia* spp. improved nutritional value and presented a promising approach for reducing sodium in fermented items. These results endorse using halophytic plants in health-focused food development.

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[Keywords] Salicornia europaea, salt substitute, lactic acid fermentation

Fermentation of coffee cherries by selected yeast strains and its impact on the final aromatic quality of the coffee.

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● Coffee bean fermentation is a critical stage in coffee processing that significantly impacts the final product's quality and flavor. The use of selected microorganisms, particularly yeasts, can help produce coffee with unique flavor profiles that cannot be achieved through spontaneous fermentation with naturally occurring yeasts. This allows for more consistent flavor profiles across different batches of coffee, which is essential for coffee producers who aim to maintain high-quality standards.

The primary objective of this study was to screen a panel of yeasts and select the most suitable strains for coffee bean fermentation. The selection was based on the yeasts' ability to tolerate the stressful environmental conditions encountered during the fermentation process, as well as the complexity of their aromatic profiles.

We first evaluated the growth of different yeast strains in synthetic culture media designed to mimic the challenging conditions of coffee fermentation. From this initial screening, the 12 most robust yeast strains were then tested in fermentation trials on Marsellesa Arabica coffee at a coffee farm in Nicaragua, using a natural processing method.

The fermented coffee beans were dried, roasted, and subjected to sensory analysis. The results revealed that while some yeast strains had great potential in enhancing the flavor and aroma of the coffee, others demonstrated a more neutral profile. Interestingly, certain strains produced a complex cup with very pleasant aromas.

In summary, this study aimed to identify the most suitable yeast strains for coffee bean fermentation, with the goal of improving the quality and consistency of the final coffee product.

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Understanding the survival mechanisms of yeast during breadmaking via transcriptomic analysis.

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● Yeast activity is an important factor in a good oven rise during baking, which leads to optimal bread loaf volume. We have recently demonstrated a strong positive correlation ($R^2 = 0.95$) between the viability of yeast strains at the end of the oven rise and the increase in dough height. Notably, the top-performing yeast strain exhibited a relative viability of 42.1% at the end of the oven rise, when the dough temperature reached 77°C, while the worst-performing strain could only retain a viability of 5.9%. This data highlights the importance that yeast might still play during the oven rise through continued fermentation, and suggests the potential for survival pathways to be upregulated.

To further investigate, we conducted a transcriptomics experiment to understand the physiological changes yeast strains undergo throughout the breadmaking process, aiming to identify characteristics that enhance yeast survival during baking. The findings show that yeast strains are conditioned for the intense heat of baking due to the hyperosmotic stress experienced during fermentation. This osmotic stress triggers the production of the osmolyte glycerol and activates other stress pathways such as the cell integrity pathway. As anticipated, yeast cells attempt to adapt their physiology during the heat stress of baking, however, due to the rapidly changing and lethal environment at the end of oven rise, only limited changes in gene expression can be picked up.

These results provide insights into the key characteristics of high-performing yeast strains in the breadmaking process, offering potential for further yeast optimisation in the breadmaking process.

Understanding yeast's behaviour and survival in a liquid and a semi-solid dough fermentation system: a transcriptomics approach

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● Targeted yeast selection for breadmaking is challenging due to the contrasting conditions yeast cells encounter in semi-solid dough fermentation compared to laboratory-controlled liquid media. Using stress-tolerant yeast strains to study their effect on oven rise, we previously found a strong positive correlation ($R^2=0.95$) between yeast viability during the baking phase and dough rise performance. Notably, all yeast strains remained 100% viable at high dough temperatures (59°C), whereas in a liquid system with the same heat rate, yeast viability dropped below 50% at 50°C.

To investigate the effect of the matrix on yeast survival and behaviour, a transcriptomics experiment was conducted tracking the expression profile of standard baker's yeast during the breadmaking process and a simulated liquid process. The hyperosmotic, anaerobic conditions of semi-solid dough fermentation force yeast to adapt from the beginning of fermentation, likely providing a preparatory advantage during baking. In contrast, the aerated liquid medium provides a more stable environment for the yeast, leaving them less prepared for the heat stress of baking. Understanding the impact of the food matrix on yeast is a crucial step towards more targeted selection processes for future strains in breadmaking applications.

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The quest for an optimal starter culture mixture for cocoa fermentation.

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● Cocoa fermentation is the first step in transforming raw cocoa beans into chocolate. The fermentation process is important for removing the mucilaginous cocoa pulp and the development of flavour and colour precursors in the cocoa beans. Cocoa fermentation is still a spontaneous process that results in cured cocoa beans of varying quality and flavour profiles. Starter culture mixtures have been increasingly studied during the last decades as to assess their capacity to steer the microbial community dynamics during fermentation and their influence on the quality of the end-products. Whereas a well-performing starter culture mixture has been composed in the past, containing strains of *Saccharomyces cerevisiae*, *Limosilactobacillus fermentum*, and *Acetobacter pasteurianus*, follow-up research was conducted to investigate whether additional yeast strains could improve the final cocoa flavour. Also, the impact of using the starter culture mixture on different cocoa genotypes was assessed. The results showed that the combination of the previously selected *S. cerevisiae* strain with a representative *Pichia kudriavzevii* strain had a beneficial effect on the final flavour, whereas the combination of the *S. cerevisiae* strain with a representative *Hanseniaspora opuntiae* strain did not result in that beneficial effect. Although there was not a large influence of the cocoa genotype, except for the presence of plant-related metabolites, diffusion of volatile organic compounds produced during fermentation in the pulp had to diffuse into the beans to have an impact on their flavour.

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Utilization of *Ziziphus jujuba* fruits in kombucha fermentation: effects on microbial ecology and polyphenol transformation

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● This study focuses on the molecular characterization of *Ziziphus jujuba* fruits and their application in kombucha fermentation. Despite its traditional medicinal use, *Z. jujuba* remains poorly explored phytochemically. A preliminary multi-analytical investigation revealed the presence of bioactive molecules such as flavonoids, anthocyanins and tannins, along with high antioxidant capacity. Based on these results, we evaluated how the addition of *Z. jujuba* fruits affects the microbial ecology of the kombucha starter (*Symbiotic Culture Of Bacteria* and *Yeast,* SCOBY). Fermentations were conducted in black tea, with the addition of the dried fruits, for 11 days at 30°C using the microbial consortium in the SCOBY consisting of *Clavispora lusitaniae, Zygosaccharomyces bailii, Saccharomyces cerevisiae,* and *Komagataeibacter rhaeticus*. In addition, since *C. lusitaniae*, *Z. bailii*, and *S. cerevisiae* were expected to enzymatically modify phenolic molecules, increasing their release, we determined whether the bioavailability of fruit polyphenols increases during fermentation; thus, increasing the bioavailability of polyphenols. Our results aim to define the potential of a kombucha beverage enriched with more readily bioavailable bioactive compounds.

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Selection of functional lactic acid bacteria strains through millet bran fermentation.

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● Fermentation represents a sustainable approach to exploiting agro-industrial by-products. Millet bran, the by-product of the millet processing industry, contains an abundance of nutritious components, however, it remains underused. Hence, the research aimed to select functional lactic acid bacteria (LAB) strains to valorise millet bran. Millet bran was mixed with water and subjected to daily refreshments for seven days. The acidification was very limited (pH over 6), and LAB were detected, but they were not the dominant population, ranging from 104 CFU/g to 106 CFU/g. Among the isolates, thirteen LAB strains were characterised by RAPD-PCR, and identified through 16S rRNA gene sequencing. *Weissella paramesenteroides* was the dominant species. Several technological and functional properties of the strains were assessed. Acidification and growth capacity were determined by inoculating the strains in millet bran dough and millet bran enriched with glucose. The addition of glucose enhanced LAB activity, decreasing the final pH below 4 in most samples. Chemical analyses highlighted efficient glucose depletion and organic acid production of *W. paramesenteroides* MIL10 and *Lactococcus lactis* MIL42. Exopolysaccharide production was detected exclusively in *W. paramesenteroides* MIL46 strain. Concerning functional properties, the screening revealed strains capable of increasing the phenolic content and antioxidant capacity in doughs obtained with millet bran. The ɣ-Aminobutyric acid production was quantified through *in vitro* test, identifying strains with promising biofunctional properties. Millet bran fermentation represents an effective tool for isolating LAB strains with health-promoting potential, supporting future applications in producing fermented foods from plant by-products.

Ancient brews in new bags: historical techniques in fermentation-driven beverage processing

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● *Fuqqāʿ* ("foamy beer") is a medieval fermented beverage first described in the 10th century, commonly drunk in the Arabic states. The exact composition of the beverage is still debated, maybe due to the many different varieties that existed. Commonalities among these varieties include a short fermentation period (1–2 days), low-alcohol content (0–2%), and self-carbonation. Mentioned ingredients include different grains and/or bread as the source of carbohydrates as well as many others, of which the most common ones were dried fruits, spices, sugar, honey, and herbs. The cereal most commonly used initially was barley, while later on wheat or sorghum were used, depending on the regions and the agricultural context. Two different variants of fuqqāʿ were recreated in triplicate through a spontaneous fermentation of ten days. Two recipes, including raisins or dried pomegranate seeds and Arabic gums, were formulated. An additional experimental set-up was implemented using dates, which was conducted in sealed bottles. This last fermentation process was followed during two days. Microbiological parameters, including growth, species diversity, and metabolite dynamics, were determined. After 24 h of fermentation, lactic acid bacteria (LAB) such as *Leuconostoc citreum* and *Lactococcus lactis* drove the fermentation process, while yeasts (e.g., *Saccharomyces cerevisiae*) appeared only after 5-8 days of fermentation, depending on the conditions. The presence of yeasts was reflected by the ethanol content (0–1%), which was found in these later stages of the fermentation process. In conclusion, spontaneous fermentation following different fuqqāʿ recipes led to similar microbiological profiles, which were driven by LAB.

Sourdough fermentation as a tool to improve the bioactive properties of spelt.

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● Spelt (*Triticum aestivum* ssp. spelta L.) is a low-input plant that has gained attention due to its adaptation to harsh ecological conditions and high nutritional value. In baked products, the nutritional value of spelt flour may be further enhanced by sourdough fermentation, which contributes to protein degradation, leading to low-molecular-weight compounds such as bioactive peptides with nutraceutical potential. This study aimed to investigate the bioactive properties of the low molecular weight fraction (LMW) containing peptides extracted from sourdough spelt bread (SDS) prepared with previously selected lactic acid bacteria. Three other breads were prepared: sourdough bread with wheat flour (SDW), bread with bakery yeast and spelt flour (BYS) and wheat flour (BYW). LMW were tested on different cell lines: murine macrophages to evaluate the effects on oxidative stress after lipopolysaccharide treatment, and the Caco-2 cell line to assess their impact on intestinal barrier integrity and functionality. Both protein and free amino acid content were higher in sourdough breads compared to the respective bakery yeast formulations. LMW of SDS, SDW and BYS showed a higher antioxidant activity, reducing reactive oxygen species to a higher extent compared to BYW. All samples were able to contrast the barrier damage caused by treatment with lipopolysaccharide, inducing a recovery in epithelium integrity; SDS, SDW and BYS showed the strongest ability to enhance barrier integrity. Results highlighted the positive combined effect of spelt flour and sourdough fermentation, which can be exploited to manufacture bakery products with beneficial effects on oxidative and inflammatory status.

Compositional variation in phenolic compounds during lactic acid fermentation of Nordic berry juices.

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● Berries have been commonly applied in the food industry due to the health beneficial effects of phenolic compounds. Yet, during food production, the berry-derived phenolics degrade and form new compounds. The variation in phenolic profile can affect their health benefits, as well as the sensory properties of the products. It is essential to monitor the changes in phenolic composition during berry processing. However, previous studies focus only on the variation in total phenolic content with colorimetric methods or the changes in major compounds using liquid chromatography. Current research lacks a thorough investigation of the effects of food processing on berry phenolics.

Therefore, we started a sturdy to systematically reveal the changes of various phenolic compounds in berries during the juice production. Crowberry (*Empetrum nigrum*), a commercially underutilized Finnish berry, was selected and fermented by using two strains of lactic acid bacteria (*Lactiplantibacillus argentoratensis* and *Lactiplantibacillus plantarum*, respectively) for 5 days. A total of 65 phenolics were identified from crowberry juices with liquid chromatographic and mass spectrometric methods, including anthocyanins (10 compounds), flavonols (30), flavan-3-ols (2), proanthocyanins (9), hydroxycinnamic acids (10), and hydroxybenzoic acids (4). For each identified compound, the variation in its content from pasteurization to the end of fermentation was discovered. Employing *L. argentoratensis* and *L. plantarum* strains significantly reduced the degradation of anthocyanins. This protective effect of the strains on anthocyanins and other phenolics was enhanced by adjusting pH of crowberry juice before fermentation.

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[Keywords] Empetrum nigrum, Berries, Lactic acid fermentation, Phenolic compounds, pH adjustment

High-throughput screening of lactic acid bacteria with biotechnological potential in leavened baked goods.

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● Lactic acid bacteria (LAB), widely recognized for their pivotal role in food fermentation, can enhance the sensory, nutritional and functional properties of fermented foods through their portfolio of enzymes driving diverse metabolic activities. The selection of robust and high-performing strains is crucial for driving and optimizing food fermentations. In this study, approximately fifty LAB strains from various species such as *Lactiplantibacillus, Leuconostoc, Fructilactobacillus, Levilactobacillus,* and *Pediococcus* were screened by using a high-throughput approach. Four distinct growth media, simulating food-related environments with cereals represented with wheat flour hydrolysate (WFH) and vegetables with tomato juice media, were proposed to evaluate their growth capability and adaptability to different nutrient sources. Growth kinetics modelling revealed that most strains exhibited effective growth capability in wheat flour hydrolysate medium supplemented with additional substrates such as yeast extract, peptone, and sugars. Moreover, species such as *Lactiplantibacillus plantarum, Levilactobacillus brevis, Leuconostoc citreum*, and *Pediococcus pentosaceus* showed the best growth in tomato juice-based medium. The screening platform included the acidification kinetics, proteolytic activity, carbon source utilization, enzymatic activities, reduction of anti-nutritional factors, and antimicrobial properties. These efforts aim to develop a high-throughput method for identifying LAB strains with enhanced biotechnological potential for application as baked goods starters.

[Keywords] Lactic acid bacteria, High-throughput screening, Growth kinetics, Tailored fermentation, Starter selection

Use of preserved tempeh in back-slopping: the effect on the aminoacidic composition and qualitative aspects

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● Back-slopping consists of using a portion of a previoulsy successfully fermented product as an inoculum to start a new fermentation batch. This practice can affect the microbial community and, consequently, the biochemical properties of the final product, including sensory aspects and amino acid profile.

The use of preserved tempeh as a starter can help maintain a stable and beneficial microbial community, which is crucial for achieving consistent fermentation results and amino acid profiles. However, if preservation methods are inadequate, there is a risk of introducing indesirable microbes that might degrade proteins and alter the amino acid spectrum.

The aim of this research was to preserve tempeh with different long-term preservation methods, such as drying, freeze-drying and freezing and to reuse the stored product as a starter for later back-slopping. After 3 and 6 months, the stored samples were employed as starter for subsequent back-slopping fermentation. The effect of the different preservation techniques was evaluated through physicochemical analysis, the assessment of the microbial composition, and the evaluation of the volatile organic compounds (VOCs) by HS-SPME-GC/MS. Proteolysis was further investigated by TNBS assay and by quantifying the free amino acid (FAA) content through HPLC analysis.

The results highlighted that the typical mold growth in tempeh was consistently obtained in all cases, confirming the essential role of the beneficial microbes occurred in the stored temeph. However, back-slopping may also introduce microbial variability depending on the storage conditions of the starter culture affecting both the flavour (mainly alcohols, aldehydes, ketones) and the aminoacidic profile.

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Functional lactic acid bacteria strains and field horsetail in fresh cheeses to enhance health and aroma.

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● Fermented dairy products, such as cheese, are gaining renewed attention not only for their nutritional value but also for their potential as vehicles of bioactive compounds. In this study, an innovative fresh cheese ("Primo sale") was developed by incorporating *Equisetum arvense* (field horsetail), alongside selected lactic acid bacteria (LAB) strains (*Lactiplantibacillus plantarum, Pediococcus pentosaceus* and *Enterococcus faecalis*) with potential probiotic traits in addition to the commercial starters (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*). The plant addition was chosen for its antimicrobial, prebiotic, and antioxidant properties, contributing to product safety, functionality, and sensory profile.

Microbiological analyses and the whole genome sequencing revealed that the Equisetum sustained LAB viability (>8 Log CFU/g) during refrigerated storage of over a month while inhibiting spoilage microbiota i.e. *Pseudomonas* spp. and *Enterobacteriaceae*. Volatile compound profiling indicated that the presence of the LAB strains enriched the aroma profiles compared to the controls prepared with only the commercial starters, particularly in organic and short-chain fatty acids, enhancing sensory quality. Notably, the enhanced production of volatile acids and modulation of the microbiota suggest a synergistic effect between the bioactive plant compounds and the probiotic LAB strains. Furthermore, the presence of both LAB mixture and *Equisetum* significantly increased the total phenolic content and antioxidant activity ($p<0.05$) compared to control in particular during storage.

These findings support the potential of functional by-products in improving both the health-promoting properties and the sensory appeal of fresh cheeses, offering promising avenues for sustainable innovation in the dairy sector.

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Raw cow milks from pasture in the pdo massif central cheese region – to what extent origin can be kept during fermentation?

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● The concept of cheese terroir is related to the possibility to sensorially perceive the taste of a place where cheese has been elaborated. Most of studies relate milk and cheese bioindicators to factors that shape cheese terroir. A recent study found that milk and cheese microbial diversity was mainly terroir-driven through their PDO characteristics in which milk-cheese continuum presented a crucial role in revealing cheese's link with its terroir. In order to investigate such continuum, we decide to investigate how lactic acid bacteria (LAB) fermentation shapes the physico-chemical composition of raw cow milks originating from the French Massif central mountain region with several cheese PDOs. The second objective was to discuss the geographical provenance and geological origin of raw cow milks before and after fermentation. The third objective was to evaluate if sensory perception of raw cow milk cheeses within a single PDO would be affected. Our results highlighted that ICP-MS multi-elemental composition and FTIR signatures could distinguish non-fermented raw cow-milks based on terroir parameters. Once LAB fermentation was achieved on those raw cow milks, fermented milks presented homogeneous values in terms of acidity and water holding capacity. Interestingly, FTIR signatures of LAB fermented raw cow milks indicated a more diverse physico-chemical composition compared to non-fermented milks, with a preservation of origin parameters. To deepen such chemical diversity brought by fermentation in the case of cheesemaking, Salers PDO cheeses elaborated from raw cow milks from the same geographical and geological origin presented sensory differences (triangle test) and on specific sensory attributes (descriptive analysis). Our study confirmed the importance of investigating milk cheese continuum to better understand the role of fermentation in cheese terroir expression. Further studies are under investigation to evaluate which cheese aromas could be responsible of sensory differences.

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The hidden hue: investigating granadaene-producing acidipropionibacteria isolated from raw cheese milk

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● *Propionibacterium freudenreichii* is the best-known of four dairy propionic acid bacteria (dPAB), widely used as a starter in Swiss-type cheeses. However, when propionic acid fermentation is undesired – as in selected raw milk cheeses – dPAB can be spoilage agents, causing cheese defects such as discoloration or spotting. Both have been linked to pigment production in *Acidipropionibacterium jensenii* and *A. thoenii*, which produce granadaene, a pigment also found in group B streptococci with hemolytic, cytotoxic, and antioxidant properties.
Despite its implications, little is known about pigmented dPAB. This study examined pigmentation and hemolysis in 48 acidipropionibacteria isolates from raw milk and antimicrobial resistance in 12 of them, investigating resistance to 27 antibiotics by disc testing. Strains were incubated for seven days at 30°C under anaerobic, microaerophilic, and aerobic conditions on YELA and Columbia sheep blood agar (anaerobic). Pigment production was measured colorimetrically; hemolysis was assessed visually. The presence of the *cyl*-like gene cluster, associated with pigmentation, was investigated by PCR using newly designed primers targeting the *cylB* gene.

Pigmentation varied depending on atmospheric conditions, but was observed in up to 55% of isolates, all of which were hemolytic and carried the *cyl*-like gene cluster. Notably, *cylB* was also present in non-pigmented strains. All strains were resistant to at least 3/27 antibiotics, and though general trends emerged, antimicrobial resistance varied by strain.

While no direct health risks have been associated with pigmented acidipropionibacteria, the limited understanding of granadaene's role and impact in these strains underscores the need for ongoing research and monitoring.

Monascus purpureus biomass as a new high-quality and protein-rich food ingredient.

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● *Monascus purpureus*, a filamentous fungus traditionally employed in the production of red yeast rice, produces secondary metabolites with recognized bioactivities, including alkaloids, phenolics, and flavonoids with antioxidant, anti-inflammatory, and cytoprotective effects [1]. However, the used as a protein source remains limited despite its GRAS status.

The optimized biomass obtained through solid-state fermentation (SSF) and submerged fermentation (SmF), reached yields of 3.95 g/L and 6.00 g/L (dry weight), respectively. Protein content was quantified at 21% (SSF) and 35% (SmF), with an amino acid profiling with six essential amino acids, lysine predominating. Volatile metabolite profiling by GC–MS revealed 17 compounds in SSF and 15 in SmF, including furfural derivatives, fatty acids, and nitrogenous volatiles. Antioxidant exceeds that of several commonly consumed foods an it is similar between both matrices (3.65 ± 0.14 mmol vs. 3.33 ± 0.10 mmol TE/100 g). Functional property assessment showed high emulsifying activity, though limited gelling and foaming capacities.

These results highlight the feasibility of *M. purpureus* biomass as a high-quality and protein-rich ingredient. Its acid-tolerant fermentation profile, nutritional value, and techno-functional properties support its integration into novel food formulations and reinforce its potential contribution as a new sustainable protein alternative.

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Characterization of autochthonous lactic acid bacteria in Njeguški cheese: study for improving quality and safety

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● Njeguški cheese, one of Montenegro's most renowned traditional cheeses, belongs to the category of semi-hard cheeses and is primarily made from ewe's milk, though cow's milk is also commonly used. This study aimed to enhance the quality and safety of Njeguški cheese by exploring and characterizing the diversity of its autochthonous microbiota for potential applications in the food industry. Fourty-two autochthonous lactic acid bacteria (LAB) were isolated, identified, and characterized through biochemical analyses, including acidification activity, antimicrobial potential, exopolysaccharide (EPS) production, pH and salt tolerance, and esterase activity. Acidification tests revealed that isolates NC4 (*Lacticaseibacillus paracasei* subsp. *tolerans*) and NA10 (*Lactococcus lactis* subsp. *hordniae*) exhibited superior acidifying capabilities. In vitro EPS production was limited, with only *Leuconostoc mesenteroides* NC1 and *L. mesenteroides* subsp. *jonggajibkimchii* NC13 demonstrating the ability to produce homopolysaccharides, resulting in mucoid colony formation. None of the isolates exhibited heteropolysaccharide production, antimicrobial activity, or esterase activity. However, 35% of the isolates were inhibited by pH 4.00. Regarding salt tolerance, all isolates grew on MRS agar with up to 2% NaCl, but *Lentilactobacillus otakiensis* NA6, *Streptococcus thermophilus* NA9, *Lc. lactis* NB3, and *Lc. lactis* NB10 failed at 5% NaCl. Among isolates, 45% of them did not grow at 7% NaCl. No isolate grew above 10% NaCl. This work was supported in part by the Italian Ministry of Foreign Affairs and International Cooperation, grant number ME23GR01 within the bilateral project entitled "Valorization and innovation of Montenegro traditional fermented foods" (FOODVALUE).

Untargeted screening based on UHPLC-HRMS of total folates produced by lactic acid bacteria in fermented milk and during yogurt shelf life.

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● Folate is an essential vitamin involved in critical physiological processes, including DNA synthesis, methylation, and amino acid metabolism. As humans can't synthesize this micronutrient, it must be obtained from dietary sources, making deficiency a public health concern. Fermented dairy products represent a promising strategy for folate biofortification using lactic acid bacteria (LAB). This study aimed to develop a method to identify high folate-producing strains suitable for functional food applications.

Initially, 36 strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* from traditional fermented milks were screened with a microbiological assay (MA). To overcome MA's limitations, an untargeted and semi-quantitative method combining ultra-high-performance liquid chromatography (UHPLC) with high-resolution mass spectrometry (HRMS) was developed. Nine representative strains were further analyzed with this approach that allowed the identification of six key folate metabolites: dihydrofolate, tetrahydrofolate (THF), 10-formyl-THF, 5-methyl-THF, 5,10-methenyl-THF, and 5,10-methylene-THF. Based on UHPLC-HRMS results, top-performing strains were selected for lab-scale yogurt production and folate levels were monitored over a 14-day shelf life.

The UHPLC HRMS analysis revealed that all nine selected LAB strains produced multiple folate derivatives. Notably, lab-scale yogurts exhibited a steady increase in folate content throughout shelf life, unlike the commercial control.

These findings demonstrated that advanced folate screening through UHPLC-HRMS is a reliable approach to identify high-folate producer strains. Nevertheless, the development of a targeted UHPLC-MS/MS method remains necessary to enhance the sensitivity, specificity, and overall efficiency of folate quantification in complex dairy matrices.

Physical chemical and microbial dynamics during Kaminwurz processing.

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● Kaminwurz is a traditional dry-fermented and smoked sausage from South Tyrol still produced with both old and industrial ways of processing.

Historical methods of meat preservation differ from the industrial procedures for some aspects, including the use of smoking by combustion instead of pyrolysis and the use of ripening rooms in uncontrolled conditions. This study investigated the physicochemical and microbiological parameters of Kaminwurz experimentally obtained through an old processing method and pH, water activity (aw), moisture content and weigh loss were analysed at different processing steps. The microbial analysis focused both on lactic acid bacteria (LAB) and species of *Micrococcus/Staphylococcus*, well known associated with ripening processes of fermented foods, and on some spoilage and pathogenic bacteria of relevance for regulation.

Results showed a considerable batch-to-batch variability owing to raw material intrinsic variability, unstandardized environmental conditions and different smoking locations, making standardization of product challenging. Despite this considerations, Kaminwurz resulted to be a low-acid sausage and *Clostridium botulinum* growth can be hindered also without nitrites, provided that appropriate salt concentration and temperatures are maintained. At these conditions, the microbial growth of useful bacteria was similar to that observed for most of Italian salami and the ripening process took at least three weeks in order to achieve a water activity (a_w) value of 0.92, which is required by the product specification. Samples resulted sometimes positive to *L. monocytogenes* and this aspect still remains of concern as for the ComBase prediction, even if nitrites allow a meaningful reduction of the pathogen growth.

Less waste by using autoaggregating streptococci starters in quark and cheese production.

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● The present study relates to a streptococci dairy starter able to aggregate. Aggregation in lactic acid bacteria (LAB) is considered a beneficial probiotic trait present in less than 2% of the isolates[3]. In *S. thermophilus* CC40-4S, a dairy strain isolated form artisanal Serbian cheese we have identified and characterized a novel gene involved in aggregation, AggS.

AggS is a large SFCBAF-type aggregation factor (237 kDa) containing a repeat region, but surprisingly it does not contain collagen-binding domains like others. Cloning and heterologous expression of AggS in *Lactococcus cremoris* MG1363 confirmed a role in aggregation.

We show that the autoaggregating phenotype of *S. thermophilus* CC40S is particularly useful for cheese and quark production, leading to an increased product yield. Furthermore, we determined a significant decrease in cell retention in the whey after separation in quark or pasta filata cheese. In line, pH stabilization of pH in the whey was observed compared to when using non-aggregating streptococci starters.

During cheese manufacturing, approximately 90% of the starter culture is entrapped in the curd fraction, while the rest ends in the whey[2]. The starter culture in the whey can continue producing lactic acid decreasing the pH of the whey, negatively impacting its quality. Although unexpected, we found a link between the aggregation ability of a dairy starter with that of pH development, thereby increasing the quality of the whey.

This study elucidates a novel "aggregation gene" in LAB, providing a tool around aggregating starters cultures and in elucidating potential benefits for food applications.

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Technological characterization of yeast and lactic acid bacteria strains for the production of fermented goat drinks.

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● Consumption of fermented goat milk products has recently become popular due to some attractive features compared to cow milk, e.g. lower allergenicity, higher digestibility and content of short/medium-chain fatty acids. However, from a technological point of view the production of fermented goat milk is critical, and fermented drinks often have low viscosity and water holding capacity, poor textural properties.

In this work, 22 strains of lactic acid bacteria (LAB) and 11 yeasts were evaluated for their technological potential to ferment goat milk. The most promising strains were used in co-culture, and the fermented products were characterized for their exopolysaccharides (EPS) content, viscosity and volatile profiles. The screening resulted in the selection of 4 strains which led to fermented drinks with firmness and viscosity comparable to some commercial products due to the accumulation of EPS (up to 500 ppm). Overall, the fermented drinks were appreciated by 20 untrained panellists for their pleasant aroma profiles which was strongly affected by the combination of strains used as starter cultures. This study showed that proper strain selection is a promising strategy to obtain fermented goat beverages with improved antioxidant properties, tailored texture and sensory features to improve consumers' acceptability.

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Polyphasic characterisation of lactic acid bacteria diversity associated with Sub-appennino Dauno sheep milk from “Gentile di Puglia” breed, for application in Pecorino cheese and the design of a cereal-based fermented beverage.

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● Preserving and valorising Apulian food biodiversity is a key strategy to promote the innovation of agro-food systems associated with regional Marginal Areas, including a diverse range of animals, plants, and micro-organisms (especially those of interest for food fermentation). “Gentile di Puglia”, is an autochthonous sheep breed of millenary origins typical of the South of Italy, particularly associated with Foggia province (i.e. *Subappennino Dauno* and *Tavoliere* areas), also recognised for traditional cheese production. Here, we report a case study related to the marginal area of the *Subappennino Dauno/Monti Dauni*, in the north of Apulian Region, i) isolating lactic acid bacteria (LAB) from sheep milk (breed “Gentile di Puglia”) and ii) performing a polyphasic characterisation of LAB for the design of multi-strains starter culture for Pecorino cheese and the design of a cross-over cereal-based fermented beverage (“Senatore Cappelli” durum wheat variety). The evaluations concerned protechnological properties, antimicrobial activity, sensorial aspects, and the study of volatile organic compounds. MF, HC, GS and VC are supported by the funding of the European Union Next-Generation EU [PNRR – Mission 4 Component 2, Investment 1.4 – D.D. 1032 17/06/2022, CN00000022] within the Agritech National Research Centre for Agricultural Technologies. FB and MP received funding PNRR, Investment 1.3-Award Number: Project code PE00000003, Project title: “ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods”.

Autochthonous lactic acid bacteria as starter or adjunct culture of Feta cheese and multi-omics interrogation of the microbial community during ripening.

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● Feta, a Protected Designation of Origin (PDO), white, brined cheese from Greece, is one of the most important local and international market-share products. The addition of lactic acid bacteria (LAB) as starter or adjunct culture is important because they contribute, among others, to the organoleptic properties of the product. The aim was a) to compare Feta made with autochthonous LAB to this of the industrially made cheese and b) to apply a multiple meta-omics approach, along with data integration analysis, to shed light in the microbiota dynamics and evolution during cheese ripening. In the first part, two different batches of cheese were manufactured by the addition of commercial cultures (control) and the addition of five LAB strains, isolated from artisanal Greek cheeses and raw sheep milk, in different combinations (8 trials including control). At specific time intervals (2d, 12d, 30d, and 60d) samples were subjected to microbiological, physicochemical, molecular (16S amplicon sequencing) and sensory analyses. Based on the results, the best trial was chosen for the second part of the experiment. Three different batches of cheese were manufactured (control and best trial). In addition to the previous analyses, shotgun metagenomics, metatranscriptomics, and metabolomics were also performed. The bacterial genomes added as starter or adjunct culture were successfully reconstructed. Genome annotation revealed the presence of several flavor-related pathways. Metatranscriptomic data showed which genes were downregulated or upregulated during ripening. Integration of DNA and RNA sequencing data with metabolomics showed which metabolic pathways shape flavor formation of the final product.

A genomics and phenomics approach to screen lactic acid bacteria for their qualification as starter or adjunct culture in cheese manufacturing.

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● The members of the cheese microbial community and their interplay is of paramount importance for the development of the characteristic organoleptic properties of the fermented cheeses. This community consists of starter lactic acid bacteria (LAB) and non-starter LAB. Although LAB features associated with flavor are important, other phenotypic properties need to be taken into account for their use as starter or adjunct culture, such as the capability to overcome technological stresses met during cheesemaking. The aim of this work concentrated on the molecular (Whole-Genome Sequencing) and phenotypic (impedance microbiology-IM and phenotype assays-PA) characterization of seven LAB strains isolated from artisanal Greek cheeses and raw sheep milk. The strains were sequenced and molecularly characterized by the application of advanced computational and bioinformatics tools. LAB isolates were examined for their growth performance in milk in combination with the presence of industry-related technological stresses, and for their ability to ferment different nutrient sources and resist chemicals. This strategy qualified some strains as the best candidates to be used as starter and/or adjunct culture in the production of cheeses. IM and PA are two techniques that quantify microbial growth by monitoring the changes in electrical conductivity and color, respectively. With this work a quantitative way to determine the microbial metabolic signatures was made and therefore, these methods can be recommended as a valid approach to assess the industry-related performances of LAB to be used in cheesemaking. Finally, computational tools were applied to investigate gene-phenotype correlation useful for the reconstruction of metabolic pathways.

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Microbial ecology and ripening dynamics of semi-hard South-Tyrolean cheese: influence of bio farming systems

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● This study explores microbial dynamics in semi-hard South Tyrolean cheese during maturation, comparing microbiota from cheeses produced under conventional indoor conditions and grazing-based biofarming. Conducted on a biofarm with 13 dairy cows of mixed breeds, cows were fed diets based on either pasture grazing or indoor hay. Cheese production in Varna (BZ), Italy, involved heating milk to 20°C, adding rennet (25 mL/100 L), and coagulating at 33°C in 25 minutes. The curd was cut, cooked to 40°C, rested at 42°C, molded, and pressed. Molds were rested in a steel bath and brined (4 hours/kg, 20% brine) to a pH of 5.2. Ripening lasted three months. Culture based analyses involved total counts and isolation of presumable *Bifidobacteria*, mesophilic cocci and lactobacilli, and thermophilic cocci. Cheese from grazing systems showed higher bacterial diversity and total counts (8.6 and 8.3 CFU/g in two grazing batches) than indoor cheese. Bifidobacteria levels remained stable (7.2–7.7 CFU/g), mesophilic lactobacilli ranged from 7.3 to 7.9 CFU/g, and thermophilic cocci showed a slight decline in grazing samples (from 6.5 to 7.5 CFU/g). These findings highlight the influence of bio farming and starter cultures on cheese microbiomes: indoor milk and cheese generally exhibited higher *bifidobacteria* and mesophilic lactobacilli counts, likely due to dietary and environmental differences. Future analyses involve the complete biochemical characterization of cheeses during ripening as long as culture independent analysis (shotgun metagenomics) to further elucidate microbial dynamics and their influence on ripening, contributing to sustainable, high-quality cheese production while preserving traditional dairy practices.

Fermented and non-fermented second cheese whey as sustainable ingredients in bread-making: preliminary technological insights

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● Second cheese whey (SCW) is a liquid by-product generated in large quantities during whey cheese production (Fancello et al., 2024). Fermentation of SCW with selected strains of *Propionibacterium freudenreichii* led to its enrichment with vitamin B9 vitamers, cobalamin derivatives (vitamin B12), and propionic and acetic acids (Coronas et al., 2023). These are known for their shelf-life-extending properties in the food industry. The objective of the present study is to evaluate the potential of SCW as a sustainable alternative to water in bread formulations by assessing its impact on dough rheological properties and bread quality. Fermented SCW (F-SCW), obtained with one selected *P. freudenreichii* strain (Coronas et al., 2023), and non-fermented SCW (NF-SCW) were used to replace water at 50% and 100% substitution levels. Preliminary results showed that doughs prepared with 100% and 50% F-SCW exhibited enhanced dough development and fermentative activity, respectively. This was evidenced by their significantly higher ($p<0.05$) fermenting parameters in comparison to the control (100% water) and to the other samples. Viscoelastic analysis revealed that all SCW-containing doughs showed significantly ($p<0.05$) higher storage (G′) and loss (G″) moduli relative to the control, indicating an improved viscoelastic behavior. On a microbiological level, F-SCW enhanced the microbial stability of bread by delaying mold growth and reducing mesophilic plate counts in a dose-dependent manner. This finding highlights its potential as a natural preservative. Bread quality evaluation is currently ongoing, including the determination of specific volume, textural profile, and color parameters. Furthermore, the characterization of bread volatile profile, shelf-life modelling using the Avrami equation, and thermal transition analysis during starch retrogradation are in progress.

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Designing fermented milk with targeted probiotics: a step toward female-focused functional foods

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● Fermented milk is a well-established vehicle for delivering probiotic bacteria, with growing interest in its potential as a functional food to support human health. As part of the FUNPRO-L project, this study aimed to identify the most suitable probiotic strain among five of vaginal origin (*Lactobacillus crispatus* BC1, BC4, BC5 and *Lactobacillus gasseri* BC9, BC12) for inclusion in a functional fermented milk made with skimmed milk and the exopolysaccharide-producing starter culture ST-440 (*Streptococcus thermophilus*, Sacco). Initial screening assessed the survival of the strains in the post-fermentation and after 28 days at 5°C. BC1, BC4, and BC5 were selected for further testing at a concentration of 8 log CFU/ml. Among them, BC1 showed the best viability (0.5 log reduction) followed by BC5, while BC4 had the largest decrease. Technologically, BC5 outperformed others, maintaining the highest water-holding capacity (23.3%) and superior rheological properties. Probiotic addition enhanced the release of free amino acids, notably in BC5 after fermentation and BC4 during storage. Each strain influenced volatile compound profiles differently, with increases in acetic acid and various fatty acids observed. Antimicrobial assays demonstrated strong and sustained activity from BC1 and BC5 against *E. coli* (ETEC), *Yersinia enterocolitica*, and *Salmonella Typhimurium* throughout storage. Overall, BC1 and BC5 emerged as the most promising candidates for developing functional fermented milk aimed at improving women's health, underlining the need to balance probiotic viability, technological traits, and sensory attributes in formulation.

Genome-centric analysis reveals key bacterial and fungal players for iron acquisition in cheese.

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● Microbial communities on the surface of cheeses are composed of a wide variety of interacting microorganisms. The structure of these communities, composed of inoculated and non-inoculated microbial strains, remains to be fully understood. Several features indicate that iron acquisition mechanisms play a significant role in microbial growth on the cheese surface and in the structuring of these communities.

This study aimed to investigate the diversity and distribution of iron acquisition systems in cheese microbiomes, in order to understand how these systems contribute to abiotic and biotic selection processes in cheeses. For this purpose, we explored a dataset composed of 135 metagenomes and 1,400 genomes/MAGs (metagenomes assembled genomes) from 44 French PDO cheeses, using an updated set of Hidden Markov Models to detect genes involved in the production and transport of siderophores (chelators able to capture iron from the environment) or in direct iron transport.

A wide variety of iron acquisition systems were identified. Among them, siderophores appear to be key elements for surface-associated microorganisms. We detected about twenty different siderophores biosynthetic pathways, including enterobactin and desferrioxamine. Genomic analyses revealed the main bacterial and fungal siderophore producers, including *Glutamicibacter, Corynebacterium, Staphylococcus,* and *Penicillium* species. Siderophore producers represented only about a quarter of the MAGs. However, we detected siderophore importers in a higher proportion of MAGs, suggesting widespread interactions mediated by siderophore/iron complex uptake. Investigating these interactions may help clarify how microbial communities assemble on cheese surfaces. Ultimately, these findings may also contribute to designing ripening cultures with improved colonization capacities.

Effect of fermentation on selected techno-functional properties of mealworm *(Tenebrio molitor)* and cricket *(Acheta domesticus)* powders.

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Investigating the teat skin microbiome: spotlight on cheese spoiling risk caused by *Clostridium* spp.

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● Raw milk quality has a critical influence on cheese quality. A major concern for cheesemakers is late blowing in hard and semi-hard cheeses, primarily caused by butyric acid-producing clostridia (BAPC), as clostridial growth can severely compromise flavour and texture. *Clostridium* spp. may enter raw milk during milking via teat skin. As raw milk contamination is typically assessed by culture-dependent methods, the impact of cleaning on the skin microbiome – and consequently on milk quality – remains unclear.

This study analysed the microbiome of 192 teat skin swabs from eight Austrian dairy farms across three seasons (summer, winter, and spring), before and after cleaning to assess the presence of BAPC, and compare results to culture-dependent assessment. Seasonal, farm-, and cleaning-related differences on the microbiome were evaluated and confirmed with species-specific qPCR targeting *Clostridium tyrobutyricum*. The microbiome was analysed by 16S amplicon sequencing (NGS).

This study provides a comprehensive analysis of the teat skin microbiome, revealing pronounced differences between farms, seasons and cleaning practices. NGS indicated a high microbial diversity, a factor known to influence raw milk quality. Correlations between culture-dependent and culture-independent methods were demonstrated through comparison with collected microbial data. *Clostridium* spp. were identified as dominant members of the teat skin microbiome across all farms, and the presence of *C. tyrobutyricum* was confirmed. These findings demonstrate that the farm environment and teat skin hygiene significantly influence raw milk quality, with effective cleaning practices markedly reducing both clostridial contamination and overall bacterial counts, thus preventing food waste and safeguarding high cheese quality.

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Sustainable packaging for meat products: fermentation can help!

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● Oxygen is together with light the most important factor affecting the color stability of sliced meat products packed under modified atmosphere. With the increasing use of L.E.D at retails, the reduction of nitrite concentration in cooked meat products and the use of (more) sustainable packages (recyclable monolayer and/or packaging more permeable to oxygen) both set-up by the European Commission, avoiding greying and oxidation during the shelf life is a challenge.

The purpose of this work was to evaluate if a specific *Lactococcus lactis ssp. lactis strain* could consume enough oxygen from meat packages to delay oxidation and greying of cooked meat products. The aim was also to evaluate the minimal requirement (temperature, time…) needed to observe the specified effect.

Cooked products (cooked ham, mortadella) were sprayed with *Lactococcus lactis ssp. lactis strain* during slicing, MAP packed and stored between 4 and 7°C during their shelf life. Samples were first store in the darkness to mimic storage at meat producers and then exposed to the light to mimic storage at retails.

Residual oxygen, pH, red color intensity and sensory assessment (hedonic test) were carried out all over the shelf life of the meat products.

It was demonstrated that *Lactococcus lactis ssp. lactis strain* significantly reduces the residual oxygen level in MAP packages to reach a level significantly lower than in the control. The a-value is more stable and no greying is measured. From a taste point of view, no negative impact of the culture was noticed by the panelists.

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Acidification behaviour of dairy isolates in various protein matrices: impact of microbial concentration and nutrient supplementation

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● Developing fermented foods from both dairy and plant-based matrices requires a clear understanding of microbial growth and acidification dynamics. Lactic acid bacteria (LAB) play a central role in fermentation, driven by their metabolic activity. This study evaluated the acidification behaviour of dairy-derived LAB strains that produce bioactive compounds, based on pH reduction during milk fermentations with various supplementation strategies. Reconstituted skim milk (RSM), with or without 1% glucose, 1% yeast extract, or both, was used with 1% and 5% inoculation levels. In the control RSM, none of the strains reduced the pH below 6.0. Yeast extract enhanced acidification by *Lactiplantibacillus plantarum* D2, D5, D7, and C37 (final pH 4.62–5.19), while *Latilactobacillus curvatus* D8 and *Levilactobacillus brevis* strains remained unresponsive. L. plantarum D4 showed stronger acidification with glucose (pH 4.85) than with yeast extract (pH 5.72). At 5% inoculum, combined supplementation decreased pH to 3.70–4.55 in *L. plantarum* and 3.80 in *L. curvatus*. In oat milk, pH dropped from 5.13 to 4.62, 4.51, and 4.44 with *L. plantarum* D2, D5, and C37, respectively. In hazelnut milk, L. curvatus D8 and the same L. plantarum strains reduced pH below 4.60. In contrast, *L. brevis* strains and *L. plantarum* D4 and D6 showed poor growth. Based on these findings, ongoing research focuses on exploring the adaptability of specific LAB strains that achieved stable acidification in pea, rice, and wheat protein matrices, aiming to enhance strain adaptability and improve the sensory and textural properties of plant-based fermented products.

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Microencapsulation as a protection strategy for biotic and abiotic stressors in natural starter cultures.

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● The maintenance and viability of starter cultures remain critical challenges in the production of traditional raw milk dairy products, particularly when fermentation is impaired by biotic and abiotic stressors. Encapsulation of starter cultures has been shown to offer several advantages over conventional methods, notably by enhancing bacterial tolerance to bacteriophages, contaminant bacteria, high temperatures, and low/high pH conditions. In this study, ten *Lactococcus lactis* strains isolated from traditional Trentino dairy products were selected to produce experimental microcapsules (EMI), coated with whey proteins and caseins at three different percentages each. A total of 60 EMI formulations were produced and analyzed at 1, 7, 14, and 30 days for encapsulation efficiency (EE%), and thermal stability. The average EE% was 68.26%, with casein coatings slightly outperforming whey protein coatings. Throughout storage, *Lc. lactis* strains maintained high viability (9.20 log CFU/g at day 1 and 8.3 log CFU/g at day 30 as average) and showed good thermal stability at 44°C (9.02 log CFU/g as average) and 55°C (6.01 log CFU/g as average). Based on these results, twenty promising EMI formulations using whey protein and caseinate were selected for further studies under biotic stress conditions. Milk fermentation assays of co-inoculation with an E. coli strain for 24 hours, demonstrated that encapsulated strains have maintained acidification capacity (average pH, 4.5) compared to unencapsulated ones (average pH, 5.5). Following these trials, six EMI formulations were selected for scale-up experiments to further investigate acidification performance in experimental cheeses.

Non-GMO strategies for yeast improvement: integrating classical and modern approaches to enhance brewing performance

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● Yeasts play a crucial role in beer production, influencing not only fermentation performance but also aroma complexity and product stability. In response to increasing consumer demand for natural and GMO-free products, there is a growing interest in developing novel brewing strains through non-GMO strategies.

In this context, two parallel approaches were undertaken to generate new yeast strains using non-GMO techniques, emphasizing not only the individual effectiveness of each method but also the synergistic benefits of combining them to achieve desired phenotypes.

The first strategy involved the creation of an interspecific hybrid between a cryotolerant *Saccharomyces bayanus* x *Saccharomyces eubayanus* strain (NBRC1948) and a wild S. cerevisiae strain (Y15) isolated from unconventional ecological niches such as sourdough. Although Y15 exhibited promising traits, it carried a phenolic off-flavor (POF+) phenotype. Random mutagenesis was successfully applied to reduce this undesirable trait, leading to the selection of an improved POF−hybrid with potential industrial applicability.

The second strategy also relied on interspecific hybridization, combining the same cryotolerant NBRC1948 strain with a *S. cerevisiae* brewing strain (JB735), previously selected for its high aromatic compound production. To further enhance volatile aroma production, the resulting hybrid underwent adaptive evolution under selective pressure.

This work highlights the value of non-GMO strain improvement strategies – both traditional (hybridization, random mutagenesis) and modern (adaptive evolution – and underscores the advantages of integrating multiple approaches to maximize the likelihood of success in developing high-performance brewing yeasts.

Microbiological and biochemical characterization of sourdoughs for sweet leavened baked goods production.

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● Sourdough is a mixture of flour and water spontaneously fermented by lactic acid bacteria (LAB) and yeasts with leavening and acidifying capacities used to produce baked goods. The microbial species in sourdough play a crucial role in the development of flavor, texture, nutritional availability and shelf life of baked goods.

This study explores three different production protocols for the utilization of sourdough to produce Pandoro, an Italian sweet leavened baked good, coming from three different industrial production sites at 3 different stages of their protocol. A combination of culturedependent and culture independent methods (e.g., shotgun metagenomics), as well as biochemical characterization will be used to identify the sourdough's microbial composition, create a biobank of sourdough strains and explore its stability-resilience after fermentation based on its production protocol and biochemical indicators. To improve stability-resilience of resulting sourdoughs, diverse fermentation protocols will be tested altering temperature and time.

From each sourdough at in the different stages of the fermentation protocol, have been isolated twenty presumptive yeasts, thirty presumptive LAB in aerobic conditions and thirty presumptive LAB in anaerobic conditions. Preliminary results showed variation in terms of microbial cell density along the sourdough fermentation protocol, considering LAB/yeasts ratio and biochemical parameters. Therefore, future research will focus on the management of the fermentation protocol to improve sourdough stability. The outcomes of this study will support the development of customized sourdough protocols for leavened sweet baked goods.

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High-throughput screening of culture collection to select suitable candidates for plant-based and fruit matrices fermentation.

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● In recent years the consumption of new food products as alternatives to those derived from animals has grown exponentially worldwide. The reasons for this phenomenon include dietary preferences, ethical concerns, healthy lifestyles choices and sustainability. There is also an increasing consumer interest in products with innovative and unique sensory and nutritional characteristics, compared to the traditional ones.

Fermentation is an ancient tool which can be used to improve sensorial profile of existing products, as well as to develop new products from different origins not limited to animal derived ones but also, legume-, cereal-, vegetable-, fruit-based and the combination of them.

In this study, High-Throughput Screening (HTS) methods and model systems have been applied to rapidly investigate the high and heterogeneous number of isolates present in an industrial culture collection composed of lactic acid bacteria, acetic acid bacteria and yeasts. HTS has been used to select strains for their acidification capacity, aromatic compound production, sugar consumption, texturizing properties and antinutrients reduction according to the specific attributes desired in the different plant-based and fruit matrices.

We observed that the behaviour of each strain is matrix dependent: due to the variability of each substrate in terms of type and amount of sugars, protein content, pH, acidity and presence of inhibiting compounds (also due to the different technological processes applied to these matrices), it is not easy to predict the capabilities and potentialities of the strains. Thus, the miniaturized laboratory-scale analysis represents a powerful tool to reduce the volume and the time of analysis, allowing rapid screening and selection of the most promising strain or combination, on specific complex matrices. Candidates selected in miniaturized systems are then validated on a more realistic and larger scale, including specific analyses of dissolved metabolites or volatile compounds, combined with expert tasting. The selection of suitable microorganisms must therefore be tailored to each specific substrate and objective: miniaturized systems help to explore the potential of a culture collection and select the best candidates for future fermentations.

Real-time, non-invasive volatilome profiling by direct injection mass spectrometry: an effective green tool to speed up fermentation research

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● Fermented foods are diverse and hold significant economic, social, and environmental value. To support R&D in this field, low-cost, time-efficient, and green analytical tools are needed. Volatile organic compounds (VOCs), produced during fermentation, reflect microbial activity and influence sensory properties. Real-time VOC monitoring during fermentation provides insights into (i) the ongoing bioprocess, (ii) product quality and safety, and (iii) flavour development. Direct Injection Mass Spectrometry (DIMS) techniques are green analytical approaches that enable direct, high-sensitivity, real-time measurement of VOCs without requiring extensive sample preparation, extraction, or chromatographic separation. In recent years, DIMS has also been successfully applied to study bioprocesses – especially fermentation – monitoring the activity of diverse microorganisms, including complex microbiomes. Here, we report integrated case studies on DIMS applications for the evaluation of VOCs in fermented products, foods, and beverages, offering a comprehensive overview of the analytical strategy's potential to support R&D in the food and beverage industry. Examples include dairy (e.g., kefir, yoghurt), cereal-based (e.g., sourdough), and complex beverage fermentations (e.g., wine, beer, kombucha), demonstrating the versatility of DIMS in enhancing process understanding and product innovation.

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Lactiplantibacillus plantarum as a model species for lactic acid bacteria volatilomics in food.

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● Lactobacilli are a versatile heterogeneous group of lactic acid bacteria with applications ranging from fermentations to agricultural applications, from health concerns to biocontrol. The volatilomics of these lactobacilli is interesting because it allows complementing other omics in the study of these biological systems and provides information of interest to evaluate the protechnological role of lactobacilli in fermentations and agri-food systems. Proton transfer reaction time mass spectrometry (PTR-ToF-MS) has proven effective in large-scale screening and real-time monitoring of VOCs (in model conditions and different food matrices). It allows multiple measurements with high sensitivity due to basic sample preparation, without extraction and destruction, with standards oriented to the principles of green analytical chemistry. With its large and flexible genome, *Lactiplantibacillus plantarum* is a microorganism with a nomadic lifestyle in agro-food ecosystems, with significant protechnological, probiotic and biocontrol interest; for these reasons, it is considered a model species in this type of studies. Using PTR-ToF-MS as an analytical approach, we investigated the volatilomics of *L. plantarum* WCFS1 under model conditions (culture medium) and in different food matrices, including bovine milk, plant milk (soy, almond and oat), fruits and vegetables.

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Fifty years of *Lactobacillus helveticus* adaptation: a comparative study in the context of dairy fermentations

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● This work is part of the Helv4DairHy project, aimed at studying the evolutionary dynamics of *Lactobacillus helveticus* (LH) in traditional dairy environments by comparing strains isolated 50 years ago from natural whey starters and never revitalized and those obtained recently from the same habitat with the aim to understand how environmental and technological pressures have influenced LH strain biodiversity over the past five decades. The project relies on two extensive LH strain collections, one isolated and preserved in 1970 and the other isolated in 2023 from the same area to compare historical and contemporary LH biodiversity under a common ecological and technological context.

Phenotypic and genotypic characterization of about 40 LH strains revealed remarkable biodiversity. Old strains showed higher performances when cultivated in cultural medium and milk, highlighting higher growth rate and shorter lag phase. The most promising LH were further studied for their techno-functional properties in dairy food models.

Using a miniaturized cheese model, old and new strains were compared under conditions simulating traditional manufacturing. Old strains showed a higher tendency to decrease in concentration, probably due to lysis, and accumulated lower amounts of acetic acid and 3-hydroxy-2-butanone (acetoin), but higher levels of alcohols after two weeks of ripening.

These models offered a robust framework to translate microbiological and genomic data into insights supporting the selection of LH strains for tailored dairy applications, emphasizing the value of preserving and exploiting microbial biodiversity as a biotechnological resource.

This research is supported by EU – NextGenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR)), program Prin 2022 with the project “A 50 years evolutionary history of Lactobacillus helveticus from traditional dairy environments: biodiversity of strains as an opportunity for technological exploitation and new products – Helv4DairHy” (CUP J53D23010690006).

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Lactiplantibacillus plantarum diversity and Apulian plant-based sources: characterisation of antimicrobial activity

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● Lactic acid bacteria (LAB) play a crucial role in food processing, as they can positively affect safety and global quality (i.e. sensory, nutritional, functional). Among LAB, in particular considering lactobacilli, the species *Lactiplantibacillus (Lpb.) plantarum* emerges as a key model for the nomadic lifestyle, metabolic versatility, potential in the stress response and for the relevance in the design of starter cultures, probiotic cultures, and biocontrol solutions. The recent interest in LAB isolation for fruits and vegetables led to renewed attention to plant-based matrices as unconventional reservoirs of new strains of *Lpb. plantarum* with distinct genetic and phenotypic traits, which might offer new opportunities for the development of bio-based innovation in the food industry. Here, we present an overview of a new collection of *Lpb. plantarum* strains from Apulian (southern Italy) plant-based sources that we are characterising, including also strains from other ecological niches, to improve our understanding of *Lpb. plantarum* as a bio-tool to support the green transition of food systems. In particular, a specific emphasis is on data of interest for biocontrol application, including antagonistic activities against bacterial pathogens and fungal spoilage.

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This project is supported by the ongoing projects i) Agritech, National Research Centre for Agricultural Technologies (European Union Next-Generation EU, Mission 4, Component 2, Investment 1.4, Project code CN00000022) and ii) ONFOODS (Next-Generation EU, Mission 4, Component 2, Investment 1.3, Project code PE00000003). Vittorio Capozzi was partially funded by CNR project “NUTRAGE FOE-2021 DBA.AD005.225”

Dietary microbes and Mediterranean diet.

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● Emerging trends in scientific research highlight the contribution of dietary microbes to human health and nutrition. The Mediterranean diet (MD) has been receiving outstanding interest for its balanced nutrition intake and health effects. We provide an overview of the content in dietary microbes for different MD components, with a special focus on fermented products. The objective was to support the design of microbe-depleted and microbe-rich diets for future experimental trials. In particular, integrating experimental data and information from the scientific literature, we explored the microbiological classification of food items within the Mediterranean diet and examined the abundance and diversity of the related microorganisms. We classified Mediterranean foods based on their microbial profiles using previous information opportunely integrated with original findings using culture-dependent methods. In addition, we also evaluated some case studies that consider the combination of different ingredients in complex preparations.

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This work was completed as part of the bEat project (We all eat microbes: diet as reservoir of microorganisms that preserve the ecosystem services of the human gastrointestinal microbiota) supported by the call “Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN) 2022” granted by the Italian Ministry for Universities and Research (MUR) (Prot. 2022TF9AHZ, CUP G53D2300574 0006). Vittorio Capozzi was partially funded by CNR project “NUTRAGE FOE2021 DBA.AD005.225”;. Mariagiovanna Fragasso is supported by the European Union NextGeneration EU [PNRR, CN00000022] within the Agritech National Research Centre for Agricultural Technologies.

Microbiota of fermented table olives in Türkiye: current knowledge and perspectives

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● Türkiye is one of the most important olive producers in the world, accounting for around 13.9% of global olive production. In addition, Türkiye alone stands out with a production of 605,000 tonnes of table olives in the 2022–2023 harvest period. Lactic acid bacteria (*Lactiplantibacillus plantarum, Lactiplantibacillus pentosus* and *Levilactobacillus brevis*) and yeasts (*Saccharomyces* spp., *Pichia* spp. and *Debaryomyces* spp.) are the dominant microorganisms in table olive production. However, table olives are produced in concrete ponds and by spontaneous fermentation. This can make it difficult to standardise the microbial and sensory quality of the product. In addition, it is clear that the development of starter cultures can enable the production of standardised products. For this purpose, it is essential to identify the microorganisms involved in the fermentation process in detail. Recently, especially with the use of new molecular technologies, the microbiota in products can be determined. In this study, an overview of studies on the determination of microbiota in table olives produced in Türkiye is presented. Thus, it aims to compile the microorganisms involved in the fermentation of table olives produced in Türkiye.

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[Keywords] Table olives, microbial diversity

Factors governing the dominance of lactic acid bacteria in vegetable fermentations.

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● Different types of vegetables can be fermented, impacting their organoleptic and microbiological properties. Bacterial community dynamics play an important role in the flavor and safety of these microbial foods, however the impact of various factors, such as vegetable substrate, addition of salt and carbon dioxide, on these dynamics are not yet systematically tested. In this study, 16S-rRNA amplicon sequencing was used to explore the impact of these factors on the active community dynamics during vegetable fermentations. Eleven vegetables and fruits from different plant sites (e.g. rhizosphere, carposphere, and phyllosphere) were fermented. The microbial community was consistently characterized by a dominance of lactic acid bacteria (LAB), with *Leuconostoc* being the most abundant genus. Substrate had the largest impact on the microbial dynamics, with rhizosphere substrates being more robust and LAB dominated. Additionally, an uncharacterized *Enterobacteriaceae* genus BIGb0383 was detected in tomato and fennel fermentations, worth researching for safety purposes.

Furthermore, the impact of salt concentrations on the microbial dynamics was examined. Reduced salt levels resulted in a slower LAB dominance and microbial succession of *Leuconostoc* and *Lactiplantibacillus*. Additionally, lower salt concentration led to a higher relative abundance of *Weissella* and various *Enterobacterales* taxa. CO_2 was explored as an alternative approach to lower the salt concentration while maintaining high LAB dominance. CO_2-injection reduced *Enterobacterales*' relative abundances and increased the overall abundance of *Lactobacillales*. Taken together, this study revealed the importance of vegetable substrates for specific LAB dominance and community dynamics and proposes a novel strategy to improve the safety of fermented vegetables.

Profiling the chemical diversity of European sourdough starters through untargeted LC-MS/MS metabolomics.

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● While conceptually simple as a fermented mixture of cereal flour and water, sourdoughs are produced with a variety of recipes in many countries. Driven by a variety of biotic and abiotic factors, the resulting chemical profile reflects an interplay between the ingredients, the microbial community, and the external environment. To be able to observe global trends between sourdough fermentation practices and the resulting metabolome, we performed untargeted RPLC-ESI-MS/MS metabolomics with both ionization modes on 660 sourdough starters from 10 countries in Europe collected as part of the HealthFerm citizen science project. We show the presence of around 400 level 2 and level 3-annotated metabolites from 69 chemical subclasses. Significant global differences were found when modelling for sourdough origin (household versus bakery) and cereal background (wheat, rye, spelt, multi-cereal mixes) despite geography-specific co-variates – among which sugar alcohols, peptides, and lipid classes served as key differential features. Intensities increased in peptides and decreased with lipids as sourdoughs spent more time in transport, revealing changes that occur during extended fermentation times. By applying multi-omics integration through supervised classification, we show that the metabolome can be used as a satisfactory predictor of the microbial community fingerprint, with better classification accuracy when using Random Forests versus PLS-DA. Overall, this study provides insights into how various factors shape the sourdough metabolome across diverse cultural and geographic contexts.

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Powerful selection of lactic acid bacteria strains with improved rheological properties.

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● Today, yogurt producers strive to make yogurt with the desired texture without the addition of thickening agents. A starter culture with improved capability to make texture of fermented milks is therefore an attractive solution.

Most antibiotics and antimicrobial agents target the bacterial cell envelope and interfere with the synthesis of peptidoglycan, membrane stability and permeability, and attachment of surface components. Here, we describe a powerful selection method developed to select for derivatives of lactic acid bacteria (LAB) with improved properties for dairy applications. Using inhibitory concentrations of several different cell envelope targeting antibiotics and antimicrobial agents, we observed that a fraction of the selected resistant LAB isolates had improved rheological properties. To validate the efficacy of the method and to understand the mechanisms behind the improved rheology, we performed genetic and physiological characterization of several improved derivatives. The results revealed an unexpected diversity of genetic changes affecting other cellular functions than the targeted cell envelope. The outcome of this work, documenting the versatility of this powerful toolbox for strain development, is presented here.

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Microbial diversity of natural whey starter used in Parmigiano Reggiano PDO cheese production explored through an integrated metagenomics and culturomics approach.

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● Natural whey starter (NWS) is a complex microbial consortium dominated by thermophilic, homofermentative lactic acid bacteria, including *Lactobacillus helveticus, Lactobacillus delbrueckii* subsp. *lactis*, and *Streptococcus thermophilus*. Used in the production of Parmigiano Reggiano PDO cheese, NWS is prepared daily by back-slopping sweet whey under controlled conditions following each cheesemaking cycle. Despite its industrial relevance, the microbial diversity of NWS remains underexplored, especially using integrated metagenomic and culturomic approaches.In this study, 48 NWS samples were collected during Winter and Spring and subjected to physicochemical analysis, total DNA extraction, whole-genome sequencing (WGS), and culturomics. Metagenomic profiling revealed two dominant microbial patterns: type-H (enriched in *L. helveticus*) and type-D (enriched in *L. delbrueckii*), with temporal shifts observed between seasons. Type-D samples were more prevalent in Winter and correlated with higher fermentative activity, a key parameter for efficient milk acidification. Culturomic analysis yielded 238 isolates, all identified as either *L. helveticus* (92.44%) or *L. fermentum* (7.56%), indicating a bias against *L. delbrueckii* in standard lactic acid bacteria media. A subset of 120 *L. helveticus* isolates was genotyped using $(GTG)_5$ REP-PCR to evaluate intraspecies diversity and dereplicate the clone library. High strain-level diversity was found even in *L. delbrueckii*-dominant samples. Selected *L. helveticus* strains were further characterized for their growth under low pH, bile salts, and lysozyme stress, demonstrating a wide range of adaptability.

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Microbial ecology and nutritional features in liquid sourdough containing hemp flour fermented by lactic acid bacterial strains.

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● The aim of this study was to investigate the microbiota of two liquid sourdoughs (SLs) based on hemp (*Cannabis sativa*) or a wheat–hemp flour mixture, before and after spontaneous or piloted fermentation (*Lactiplantibacillus plantarum* ITM21B or *Weissella cibaria* C43-11 used as starters). Culture-dependent and -independent (high-throughput sequencing of V3-V4 regions of the 16S rRNA gene) methods, were used to evaluate the microbial community. The effect of fermentation on the bioactive molecules content (polyphenols, organic acids, proteins, amino acids) was also investigated. Results indicated that the microbial community of all SLs was mainly (99.7 ÷ 100%) composed of *Firmicutes* and *Proteobacteria*, and the latter was the unique phylum before fermentation in formulations produced exclusively with hemp flour. Two PCoA plots (Adonis test pseudo-F, $p > 0.05$) showed no significance difference between the microbial communities of the formulations. However, the relative abundance variation at the family level in the wheat–hemp-based mixture SLs showed a significant enrichment of the *Lactobacillaceae* family (KW test, $p = 0.04$). Results confirmed hemp seed flour as a suitable fermentation substrate to obtain microbial consortia allowing for an increase in organic acids, especially lactic acid (9.12 ± 1.22 and 7.45 ± 0.75 mmol/kg with *Lpb. plantarum* and *W. cibaria*, respectively), in both piloted fermentations, and in polyphenols by 21% and amino acids by 158% in SL fermented by the C43-11 strain. These preliminary results highlight the need for further investigations to better characterize the key players responsible for the fermentation steps driving these metabolic activities.

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Microbial-based biotechnological applications for more efficient re-use of agro-food waste in the food industry.

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● The valorization of wastes and by-products through microbial-based biotechnological solutions for more efficient re-use in the food industry represents one of the crucial issues in the green transition of food systems. Using apple, orange, tomato, broccoli, white cabbage, carrot, potato, buttermilk, scum, spinning water, and whey as model matrices and a panel of ten different lactic acid bacteria (LAB) strains, we evaluated the combination of fast screening and digital solution as emerging approaches to optimize the management of the strains in the different matrices/conditions. For each of the matrices, after fermentation with a selected LAB strain, we proposed a case study of re-use in the food industry. In the framework of evaluating the feasibility of these optimization strategies, different physical, chemical and biological contaminants were studied to investigate the safety of the treated wastes/by-products.

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Study of cultivability and inter-species interactions within the microbial communities of the whey starter used for Parmigiano Reggiano cheese production.

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The aim of this study was to investigate the role that different amino acid sources and interspecies interactions may play in the cultivability of lactic acid bacteria (LAB) from the natural whey starter (NWS), an undefined microbial consortium used for Parmigiano Reggiano PDO cheese production. Two NWS samples type-H (dominated by *Lactobacillus helveticus*) and type-D (dominated by *Lactobacillus delbrueckii*) were subjected to metagenomic analysis and viable cell counts on MRS agar supplemented with 12 different nitrogen sources. The media were evaluated for their ability to estimate the cultivable fraction (Log_{10} CFU/mL), to maintain the viability and cultivability of isolates (percentage of viable isolates), and to preserve the original species ratios. All conditions altered species ratios and failed to cultivate L. delbrueckii subsp. lactis effectively, even in type-D whey where it dominated, indicating its recalcitrant to growth despite nitrogen supplementation. Cross-feeding experiments in milk were performed using three sets of axenic cultures, each composed of one strain of *L. helveticus* (Lh), *L. delbrueckii* (Ld), and *S. thermophilus* (St), tested in mono-culture, co-culture, and tri-culture combinations Tri-cultures, followed by Lh-St and Ld-St co-coltures, showed faster, more efficient acidification compared to monocultures, while the Lh-Ld combination did not. Analysis of lactate isomers revealed that St positively influences Ld without reciprocal benefit. Moreover, Ld and Lh showed negative interactions, likely due to sugar competition, as both are proteolytic.

These findings demonstrate the complexity of microbial interactions in NWS and highlight the limitations of traditional culture-dependent methods in preserving microbial biodiversity ex situ.

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Unveiling bioactive traits of sourdough lactic acid bacteria: a polyphasic insight into exopolysaccharide, riboflavin, and antifungal activity

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● Sourdough is a traditional fermented dough made through the metabolic activity of lactic acid bacteria (LAB) and yeasts. LAB plays a central role in sourdough fermentation by producing organic acids, antimicrobial compounds, and bioactive metabolites that improve dough texture, flavor, shelf life, and nutritional quality. In our work we screened, through in vitro assays, twenty LAB strains belonging mainly to *Fructilactobacillus sanfranciscensis, Furfurilactobacuillus rossiae, Limosilactobacillus fermentum*, on the basis of their capability to synthesize exopolysaccharides (EPS), riboflavin, and to inhibit the growth of bread spoilage-associated filamentous fungi such as *Penicillium chrysogenum* and *Aspergillus japonicus*.

In particular, EPS production was determined in the presence of different carbohydrate sources (i.e., glucose, fructose, sucrose, maltose, lactose). Strains were considered prototrophic for riboflavin if able to grow in a riboflavin-free medium. Screening for the antifungal activity was performed by using the overlay assay. The antagonistic effect of the cell-free supernatants (CFSs) and volatile organic compounds (VOCs) were also evaluated.

Nine out of 20 (45%) strains showed the ability to produce capsular EPS. *F. rossiae* isolates were found to produce riboflavin, while *L. fermentum* showed vitamin B2 production as a strain-dependent feature. *F. sanfranciscensis* strains did not exhibit this capability. CFS and VOCs were both responsible for the inhibition of the tested molds, although at different levels depending on the LAB strain analysed.

In conclusion these results suggest that Italian traditional sourdoughs are a reservoir of LAB strains carrying out beneficial traits that should be further exploited for potential applications in the bakery sector.

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Unlocking the applicability of microbial players in kombucha.

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● The growing popularity of kombucha, a fermented tea beverage produced by the metabolic activity of a complex Symbiotic Consortium of Bacteria and Yeasts (SCOBY), underscores the need for a deeper understanding of its microbial dynamics to ensure product safety, quality, and innovation. Establishing a curated collection of microbial bioresources from kombucha thus represents a strategic asset, enabling the characterization of individual strains through taxonomic identification, functional property analysis, and safety profiling.

This study aimed to set up a collection of the main microbial players in kombucha by investigating the microbial diversity associated with the fermentation of an artisanal product manufactured at a facility in Verona, Italy. Fermentation was monitored in four different tanks (~1 m diameter, 600 L capacity). For each tank, six representative samples (including both SCOBY and liquid kombucha) were collected and pooled at three key time points: at the beginning, middle and end of fermentation. Selective growth media were employed under various cultivation conditions, mimicking the fermentation environment (i.e., pH 4.8), to isolate the major active fermentative groups, mainly acetic acid bacteria (AAB), lactic acid bacteria (LAB), and yeasts.

The resulting collection serves as a platform for the applicability of kombucha-associated microorganisms. Beyond their unequivocal taxonomic identification and the characterization of their pro-technological features, strains are comprehensively assessed for their safety, a prerequisite for their consideration under the EFSA Qualified Presumption of Safety (QPS) framework. This body of knowledge is crucial to support the deliberate inclusion of these microorganisms for the production of safer, higher-quality kombucha products.

Ontologies for fermented foods: tools for research and innovation

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● Research on fermented foods requires the integration of vast knowledge across diverse domains, ranging from microbial, plant and animal species to their metabolites, the biological and chemical pathways they're involved in, their effects on human health, and more. Doing so effectively and efficiently – for example, when interrogating documents or databases, analyzing results, or designing and testing models – is a challenge for research and industry alike. To this aim, ontologies are precious tools: their formalization of entity properties (such as definitions and synonyms) and relationships (including hierarchies) in a machine-readable framework facilitates the integration of diverse data sources to support semantic search, knowledge graph construction, and other IT applications. Hence, ontologies allow for much richer information content and potential for discovery compared to unstructured resources such as lists or taxonomies. Well-established public ontologies include GO (Gene Ontology), FoodOn (Food Ontology), CHEBI (Chemical Entities of Biological Interest), MONDO (Mondo Disease Ontology), HP (Human Phenotype Ontology), and NCBITAXON (an ontology representation of the NCBI species taxonomy), all of which are highly relevant for fermented foods research and ideal tools to interrogate and integrate diverse -omics data. Where public ontologies are lacking or there's a need to integrate specific types of entities, semantic technology can provide solutions. SciBite operates in this space, and has developed a technology stack and ontology content for working with resources pertinent to fermented foods. These have been used, for example, to demonstrate the potential of functional foods as therapeutic agents.

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Comparative genomics of *Lactiplantibacillus plantarum* strains isolated from cocoa fermentation processes worldwide.

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● Lactic acid bacteria (LAB) have been extensively used in food fermentation to enhance preservation, texture, and flavour. They are critical in the production of many fermented foods, such as cheese, salami, sourdoughs, and cocoa. During the cocoa fermentation process, specifically, *Lactiplantibacillus plantarum* is one of the most commonly found LAB species. It contributes to the fermentation process by converting sugars and citric acid into lactic acid, acetic acid, ethanol, and carbon dioxide. In this study, the genomes of 15 *Lactiplantibacillus plantarum* strains, isolated from cocoa fermentation processes performed in Brazil, Ecuador, Ghana, and Malaysia, were sequenced using Oxford Nanopore Technologies' long-read sequencing platform. Subsequently, a comparative genomic analysis was performed to assess potential differences in the genomic structure of these strains, including the presence or absence of genes important for their role in cocoa fermentation. Also, genomic structural variants were investigated that could affect gene expression, such as SNPs and genomic synteny, using a variety of state-of-the-art bioinformatics tools and pipelines (e.g., Anvi'o, MAKER2, and longshot). The results of this study indeed highlighted genomic differences among the strains analysed, leading to potential differences in their functions during cocoa fermentation. Interestingly, these differences did not coincide with the strains' origin of isolation, suggesting that the strains investigated underwent a selection for cocoa fermentation.

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Phenotypic and genomic assessment of sugar assimilation in the pomegranate yeast *Hanseniaspora valbyensis*.

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● Among non-conventional yeasts, *Hanseniaspora* is known for its high capacity to produce aroma compounds during fermentation and for its apparent reduced ability to completely utilise sugars in some ecosystems, due to sensitivity to ethanol or antimicrobials produced by other yeasts. Although the capacity of *Hansenisapora* species (e.g. *Hanseniaspora valbyensis*) to grow on fructose was previously reported, no fructose-specific transporters were identified. This study aimed to characterize fermentation behavior and scout potential fructose transporters of *H. valbyensis* biotypes, isolated from pomegranate. Well characterised strains of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were used as references.

The capacity to assimilate diverse sugars was first assessed using agar minimal medium (MM) supplemented (at 2%) with glucose, fructose, lactose, xylose, maltose, or sucrose. *H. valbyensis* grew at highest level on MM plates containing glucose or fructose. Starting from this result, growth, sugar consumption and metabolite production were investigated during liquid cultivation on glucose, fructose, glucose and fructose. Among the yeast species under investigation, only *H. valbyensis* was able to consume fructose and glucose after cultivation on MM broth, thus suggesting potential fructophilic behavior. Whole genome analysis was performed to identify potential sugar transporters of *H. valbyensis*, and a phylogenetic tree was built. The analysis showed that some sugar transporters of *H. valbyensis* were close to Ffz1 fructose facilitator of *Zygosaccharomyces bailii*. Ffz transporters are phylogenetically distinct from all the other previously characterized hexose transporters and more similar to drug transporters. Further research will focus on the role of this candidate Ffz1-like transporter in *H. valbyensis*.

Mid- and long-term preservation strategies of natural whey-starter microbiomes.

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● Microbial consortia (MC) play a fundamental role in many fermented foods. To date, unlike axenic cultures, the preservation of MC is still challenging, due to several constraints that may compromise viability and functionality.

In the Project Micro4ever (PRIN-PNRR 2022, n. P2022RJYCN), we evaluated the effect of cryopreservation (CrP) and freeze-drying (FD) on the survival (differential plate counting, fluorescence microscopy), composition (AT-HTS using 16S rRNA and ITS as target) and functionalities (EcoPlates® and OmniLog® Phenotype MicroArrays; acidification rate) of MC of 5 natural whey-cultures (NWC), to identify the best operating conditions to ensure maximum protection of all microbial fractions, after 4 and 8 month of storage (mid- and long-term preservation).

The survival of microbial groups (mesophilic and thermophilic LAB, yeasts) depended on the type of preservation and protective agent (DMSO or glycerol for CrP; skimmed milk or sucrose for FD). High survival (especially for thermophilic LAB) was observed after 4 and 8 months of storage, in both CrP and FD samples. Fluorescence staining (cFDA/PI) were effective and rapid in assessing MC viability.

Metataxonomic and meta-phenomic analyses confirmed that composition and functionalities of preserved NWCs were correlated to the storage process and cryo-/lyo-protectants. The acidifying capability was impaired after long-term storage (mainly for FD samples) and was related to the MC composition. The results confirm the effectiveness of CrP and FD in mid- and long-term storage of NWC; however, further studies are needed to verify the reuse and performances of preserved MC in food matrices.

Genotypic and phenotypic characterization of the IRC7 gene in commercial brewing yeast strains.

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● The IRC7 gene encodes a β-lyase enzyme that converts odorless cysteine-conjugated compounds into aromatic thiols, valuable in brewing for enhancing aroma while reducing hop use and environmental impact. Two allelic forms exist: the functional IRC7^L (400 aa) and the truncated, non-functional IRC7^S (340 aa, due to a premature stop codon).

Fifteen commercial brewing yeast strains were genotyped via nested PCR to detect IRC7 alleles. Eleven strains carried only IRC7^L , one only IRC7^S , and three were heterozygous. For heterozygous strains, allele segregation was attempted through sporulation and monospore isolation or, when unsuccessful, via cloning in E. coli. Sanger sequencing uncovered unexpected genetic diversity, notably the T185A mutation, which impairs cofactor binding and β-lyase function. In silico predictions using I-Mutant2.0 indicated that T185A and other detected mutations reduce protein stability and activity. Phenotypic β-lyase activity was evaluated spectrophotometrically by monitoring NADH oxidation with L-cysteine, S-ethylcysteine, and S-methylcysteine. Significant variation in enzymatic activity was observed, with the IRC7^S variant and T185A mutants showing marked reductions. Monosporic strains carrying IRC7^L displayed higher activity than heterozygous or IRC7^S homozygous strains. This study reveals the dominance of functional IRC7^L in brewing yeasts and identifies promising candidates for hybridization strategies aimed at increasing thiol production. These findings support the development of sustainable brewing practices by enhancing aroma profiles while lowering hop resource input.

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Innovative approaches in fresh-cut produce: an overview on microbiomes, edible packaging, and biocontrol

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● Packaging solutions play a vital role in limiting microbial growth, a key factor in maintaining the quality and extending the shelf life of fresh produce. Recent innovations suggest that microorganisms can be integrated into packaging systems, offering novel, sustainable strategies for food preservation. Lactic acid bacteria (LAB) and microbiomes (e.g. milk kefir grains) isolated from fermented foods have been incorporated into packaging materials (including edible packaging) demonstrating potential for biocontrol activity, spoilage reduction, and support of environmentally friendly food systems. Combining experimental results and literature information, we also evaluated the strengths and limitations of microbiome-based packaging compared to conventional approaches, within the context of two ongoing research projects: "INTelligent, ACTive MicroBIOme-based, biodegradable PACKaging for Mediterranean food (INTACTBioPack)", funded by the PRIMA Programme Section 2 (2023), and "ON Foods – Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security (OnFoods)" (Project code PE00000003), funded by NextGeneration EU under the Mission 4, Component 2, Investment 1.3 initiative. Additionally, this review examines the role of hurdle technology and integrated solutions involving physical postharvest strategies, assessing their impact on the preservation of fresh-cut produce (de Chiara et al., 2024). Unlike conventional static methods, microbiome-based approaches offer dynamic, biodiversity-driven solutions for food preservation. In conclusion, this study highlights the transformative potential of microbiome-integrated packaging and biocontrol technologies in postharvest management. However, challenges remain, particularly regarding regulatory approval, microbial stability, and scalability. Future research should aim to optimize the compatibility of microbiomes with packaging materials, ensure controlled microbial activity, and assess consumer perception and acceptance.

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Italian fermented foods with a geographicalindication status: cheese, bread, table olive, semi-dry sausage, and wine

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● Packaging solutions play a vital role in limiting microbial growth, a key factor in maintaining the quality and extending the shelf life of fresh produce. Recent innovations suggest that microorganisms can be integrated into packaging systems, offering novel, sustainable strategies for food preservation. Lactic acid bacteria (LAB) and microbiomes (e.g. milk kefir grains) isolated from fermented foods have been incorporated into packaging materials (including edible packaging) demonstrating potential for biocontrol activity, spoilage reduction, and support of environmentally friendly food systems. Combining experimental results and literature information, we also evaluated the strengths and limitations of microbiome-based packaging compared to conventional approaches, within the context of two ongoing research projects: “INTelligent, ACTive MicroBIOme-based, biodegradable PACKaging for Mediterranean food (INTACTBioPack)”, funded by the PRIMA Programme Section 2 (2023), and “ON Foods – Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security (OnFoods)” (Project code PE00000003), funded by NextGeneration EU under the Mission 4, Component 2, Investment 1.3 initiative. Additionally, this review examines the role of hurdle technology and integrated solutions involving physical postharvest strategies, assessing their impact on the preservation of fresh-cut produce (de Chiara et al., 2024). Unlike conventional static methods, microbiome-based approaches offer dynamic, biodiversity-driven solutions for food preservation. In conclusion, this study highlights the transformative potential of microbiome-integrated packaging and biocontrol technologies in postharvest management. However, challenges remain, particularly regarding regulatory approval, microbial stability, and scalability. Future research should aim to optimize the compatibility of microbiomes with packaging materials, ensure controlled microbial activity, and assess consumer perception and acceptance.

Preservation of microbial consortia associated with table olives: towards the conservation in culture collections

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● Microbiomes associated with spontaneous fermentation and/or selected through backslopping processes are crucial resources for artisanal, typical, and traditional foods. Moreover, these complex microbial communities can be exploited to design innovative and nature-inspired bio-based solutions in the food sector. Scientific research and biotechnological valorisation of specific microbial consortia require the preservation of their viability and functionality, which are the traits guaranteed by Microbial Culture Collections. Currently, new insights into the long-term maintenance of microbiomes are gained from research activities, developed in the "SUS-MIRRI.IT" project, aimed at assessing the best practices in promoting the conservation and revitalisation of complex microbial communities associated with food. In this context, fermented table olives are plant-based products of considerable interest in the Mediterranean area. Including prokaryotic and eukaryotic microorganisms, consortia associated with fermented table olives are a good model food for research activities in this field. Here, we report original research data on the cryopreservation of microbiomes associated with table olives and brines, evaluating the effectiveness of protocols that tested two different storage temperatures and the use of glycerol and DMSO as cryoprotectants. The conservation has been assessed using culture-dependent approaches, RNA-based metabarcoding analysis, and metabolic profiling evaluation by Biolog Ecoplate®. Project SUS-MIRRI.IT "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy" Area ESFRI "Health and Food", granted by the European Commission NextGenerationEU Code N° IR0000005.

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Promoting innovation of ferMENTed fOods (PIMENTO) – COST ACTION CA20128

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● Present in all European diets, fermented foods (FF) hold a strategic place due to the benefits they offer in terms of nutrition, sustainability, innovation, cultural heritage and consumer interest. The potential of FF for improving human health, but also driving food innovation and local production in the next decades, has become highly relevant. Here, we summarise the public repositories and publications where the results and outcomes of PIMENTO project, a COST Action CA20128 (Promoting Innovation of ferMENTed fOods), which started in November 2021 and will end in November 2025, are available. COST Action CA20128 is supported by COST (European Cooperation in Science and Technology). The project has been conceived to face the challenge of federating the scientific community and key stakeholders working on FF, with the aim of collectively advancing scientific evidence of their health benefits, building a benefits/risk approach in order to promote multi-modal innovation and respond to the expectations of European communities. The achieved results and outcomes supported the long-term goal of PIMENTO, which is to place Europe at the spearhead of innovation on microbial foods, promoting health, regional diversity, local production at different scales, contributing to economic and societal development as well as food sovereignty. The project is the result of a collective contribution, summarising the efforts of the PIMENTO Community (PIMENTO Core Group and the PIMENTO Members)

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Changes in bioactive compounds of cauliflower, broccoli, and green pea byproducts after lactic acid fermentation.

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● Vegetable byproducts are rich in valuable bioactive compounds, which have potential applications as natural antioxidants, preservatives, and supplements in food, cosmetics, and pharmaceuticals. This study aimed to enhance the value of cauliflower, broccoli, and green pea byproducts by investigating the effects of fermentation with two selected strains of *Lp. plantarum*. The strains were selected based on preliminary screenings in which phenolic compounds and glucosinolate standards were added to MRS broth and analyzed before and after fermentation with various lactic acid bacteria. Metabolic changes in byproduct purees before and after fermentation were determined using UHPLC-HRMS (Q-Orbitrap). Additionally, next-generation sequencing (NGS) assessed the impact of starter cultures on microbial ecology compared to natural fermentation. Fermentation led to a reduction in glycosylated phenolic compounds, such as kaempferol glucoside, and a decrease in caffeic acid, *p*-coumaric acid, protocatechuic acid, quinic acid, and methyl gallate. Accordingly, levels of deglycosylated metabolites, such as kaempferol, increased. Metabolites such as dihydrocaffeic acid and phloretic acid were also detected, likely resulting from the microbial conversion of caffeic and p-coumaric acids. On average, 79% of the glucosinolates in broccoli and cauliflower byproduct purees remained after fermentation, with variations observed between the two *Lp. plantarum* strains. This study confirms that *Lp. plantarum* fermentation modifies phenolic compounds, resulting in more bioactive or bioavailable metabolites while retaining glucosinolates, demonstrating the potential to add value to vegetable byproducts through lactic acid fermentation.

Evaluation of the impact of milk-derived exosomes on bacterial growth.

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● Milk is a nutrient-rich source containing bioactive compounds, including exosomal miRNAs and proteins, which play a critical role in gene regulation, metabolism, and cell-to-cell communication. Milk exosomes (MEs) are extracellular vesicles (30–150 nm), that have shown potential in modulating bacterial growth and gene expression, making them valuable for investigating their application in fermented dairy production. This study aimed to isolate and characterize bovine MEs using ultracentrifugation and advanced analytical techniques such as Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), and Western blotting while also assessing their impact on bacterial growth. The isolated MEs exhibited high purity, a size distribution of 20–50 nm, and the presence of key exosomal protein markers, such as CD9, CD63, TSG101, and Tubulin. After this, the effect of MEs on the growth kinetics of lactic acid bacteria (LAB), specifically *Streptococcus thermophilus, Lacticaseibacillus rhamnosus*, and *Lactobacillus paracasei*, was investigated. Growth studies revealed significant improvements in Gompertz parameters, including reduced lag phase, increased exponential growth rates, and enhanced stationary phase populations. Confocal microscopy also confirmed the uptake of MEs by LAB and demonstrated positive interactions after co-culturing. Additionally, bacterial stress responses, with and without MEs, were assessed using the OmniLog Phenotype MicroArray (PM) technology, revealing notable differences in their metabolic activity. These findings suggest that MEs could act as natural enhancers of LAB metabolism and growth performance. However, further research is needed to understand the specific mechanisms through which MEs influence bacterial metabolic pathways.

In vitro functional assessment of probiotic fermented hemp flour toward the human gut microbiota.

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● Dietary strategies to positively influence gut microbiota are gaining attention, with hemp flour emerging as a sustainable, nutrient-rich option due to its high fiber and bioactive compound content. This study explored whether fermenting hemp flour with probiotic strains could enhance its ability to promote in the human gut beneficial microbiota shifts and the production of health-related metabolites. The hypothesis was that probiotic fermentation would improve beneficial taxa and increase the generation of bioactive microbial compounds, as short- and medium-chain fatty acid (SCFA and MCFA). Organic hemp flour was fermented using *Bifidobacterium breve* BR03, *B. animalis* subsp. *lactis* BB-12, *Lactiplantibacillus plantarum* 325, or through spontaneous fermentation. Samples were subjected to simulated digestion (INFOGEST) followed by in vitro colonic fermentation (MICODE). Microbial changes were analyzed via qPCR, and volatile metabolites were profiled using SPME GC-MS. Results showed that hemp flour fermented with *B. breve* BR03 and *L. plantarum* 325 promoted the growth of beneficial bacteria such as bifidobacteria, ruminococci, and lactobacilli. These fermentations also increased SCFAs and MCFAs production and reduced levels of potentially harmful metabolites like p-cresol and 2,4-dimethylbenzaldehyde. In summary, fermented hemp flour demonstrates promising potential as a functional food ingredient for gut microbiota modulation and intestinal health support. These results from the in vitro model are fully translatable to in vivo condition and would serve as a solid foundation for clinical applications and nutritional intervention trial and also serve as a tool to reduce animal testing in food science, according to the Directive 2010/63/EU and the Regulation (EU) 2019/1010.

Proteomics insights into the role of lactic acid bacteria fermentation in whey and pea protein valorization.

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● Lactic fermentation offers a strategic way to valorize by-products and alternative protein sources, supporting sustainable food systems and the protein transition – key focuses in current research and industry. In this context, understanding how lactic acid bacteria respond to diverse substrates is essential for selecting optimal strains. In this study, label-free LC-MS/MS proteomics, along with metabolite analysis, was employed to unravel the metabolic response of *Lacticaseibacillus paracasei* AF43 – a proteolytic strain with proven bioactive peptide-releasing capacity – during growth in three distinct substrates: whey protein added with lactose (W), pea protein with glucose (P), and their 1:1 mixture (WP). By providing extensive insight into the strain proteome, we revealed a strong substrate-dependent metabolic activity. Enhanced glucose metabolism and ribosomal activity supported rapid growth in P and WP. In contrast, the strain in W exhibited slower growth, indicating adaptation to more complex carbon sources, such as lactose. The growth of the strain in the single-source media induced specific amino acid pathways, while the broader amino acid availability in WP, confirmed by analytical data, led to downregulation of several pathways associated with this metabolism. Changes in expression of the proteolytic system were also mapped. The WP media upregulated proteases with various specificities compared to the others, while peptide transport was greater in W. These findings elucidate how substrate composition drives bacterial functionality and protein metabolism, while also providing a scientific foundation for designing tailored, protein-based fermentates with improved nutritional and functional profiles. In this regard, proteomics is a powerful and innovative tool for deeply understanding bacterial metabolism in diverse environments.

Exploring the effects of novel probiotic strains on intestinal barrier function: In vitro tests on human Caco-2 cell-line

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● The intestinal epithelium plays a crucial role in the maintenance of gut homeostasis by acting as both a selective barrier and an immune-mediated interface. Specifically, the positive influence of probiotics on intestinal barrier function and immune responses has been demonstrated, offering potential benefits in the management of gastrointestinal disorders. In this regard, the current research focuses on the identification of novel potential probiotic strains belonging to different bacterial taxa (e.g. *Streptococcus spp., Lactobacillus spp.*, and closely related genera) and yeasts from several substrates (e.g. plants) with the long-term objective of improving the regulation of intestinal barrier function. A total of 20 bacterial and yeast strains will be examined and selected for their resistance to simulated gastric and intestinal fluids under *in vitro* conditions, as well as auto-aggregation capacity and hydrophobicity characteristics. Afterwards, in order to investigate the potential immunomodulatory and barrier-enhancing properties of the selected viable potential probiotic strains, and their corresponding supernatants, the human Caco-2 (ATCC® Cat. No. HTB-37™) cell line will be employed as an established *in vitro* model of the human intestinal barrier. Gene expression profiles will be analysed using quantitative real-time PCR (RT-qPCR) to assess the potential ability of selected probiotic strains to regulate the gene expression of tight junction proteins (e.g. Occludin, ZO-1) and inflammatory mediators (e.g. NF-kB1, IL-6, IL-8). Ultimately, the future objective will focus on the integration of probiotic functions into a novel multifunctional pharmaceutical formulation, thereby demonstrating their combined synergistic efficacy in strengthening intestinal barrier integrity.

Fermentation as an effective and sustainable approach to increase the extractability and bioactivity of apple phenolics.

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● Phenolic compounds are functional molecules that have attracted the most interest from researchers. Microbial-mediated apple bioprocessing is considered an effective biotechnology to improve the extractability of bioactive phenolics, and can be used alongside standard or unconventional extraction procedures. In this perspective, mixed fermentations with both lactic acid bacteria (LAB) and yeasts enable the full exploitation of the huge metabolic potential of microorganisms. Therefore, this study aims to establish an effective, sustainable bioprocess for processing fresh apples and upcycling apple by-products, through a precision fermentation process using selected autochthonous LAB and yeasts, contributing to the enhancement of their bioactive potential for possible applications in food, nutraceutical, and functional ingredient industries. Facing this background, 34 different samples (fresh apples and apple by-products of different varieties) were characterized for their physicochemical, microbiological, and biochemical properties, total phenol content, and *in vitro* antioxidant activity using the DPPH assay. Following microbial isolation process from all samples, a total of 27 LAB and 9 yeast strains were identified. Based on the phenolic compound content determined across all 34 samples, 5 different substrates were selected for fermentation experiments. Specifically, after assessing growth and acidification kinetics and ability of the 27 LAB and 9 yeast strains to change the phenolic compound profiles on the selected substrates, the most promising starter cultures will be selected. The outcomes will provide insights into the role of microbial metabolism in enhancing phenolic extractability and bioactivity.

Fermentation of a wine pomace and microalgae blend to synergistically enhance the functional value of protein- and polyphenol-rich matrices.

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● In the quest for sustainable, healthy and nutritious sources, functional food ingredients that are highly bioavailable and bio-accessible are becoming increasingly valued. To achieve this, the integration of microalgae and wine pomace through fermentation was explored to enhance the nutritional value and bioactive properties of the resulting novel food ingredient. Five lactic acid bacteria (LAB) and five yeast strains were variously chosen for their species diversity, origin, and metabolic potential. During fermentation, the combination of *Chlorella vulgaris* and wine pomace overcame the limited growth observed in pomace substrate, with all LAB and yeasts effectively utilizing sugars and synthesizing microbial metabolites. Additionally, the synergistic interplay between the substrates, alongside the enzyme specificity of the starter cultures, improved the bioavailability of phenolic compounds, particularly flavanols, flavonols and procyanidins, while simultaneously generating unique peptides in the formulated ingredients. In some cases, these metabolic changes were associated with enhanced antioxidant activity, improved protein digestibility, and overall protein quality. Our findings highlighted the potential of fermented mixed substrates as new functional ingredients, with promising health-promoting benefits and significant potential for applications in the food industry.

Influence of smear microbiota on the survival of *Escherichia coli* in semi-hard raw milk cheeses.

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● Shiga toxin-producing *Escherichia coli* (STEC) are a food safety issue for raw milk dairy products. Optimal development of starter cultures generally controls the growth of generic E. coli, thereby preventing spoilage by early blowing. However, the infectious dose of highly pathogenic STEC is low, making their presence in food problematic even at minimal levels. The growth and survival of *E. coli* in the core of raw milk cheeses has been described, but their behaviour in cheese smears remains to be investigated.

In the present study, raw milk spiked with generic *E. coli* was processed into semi-hard smear cheese. Survival in the core and in the smear was monitored during ripening. The smear allowed better survival of *E. coli* than the cheese core, with ≤ 2.5 log and ≥ 4 log decrease after 105 days, respectively. Complex smear microbial consortia isolated from four ripening facilities were used to inoculate smear brines, mimicking the old-young smearing process. *E. coli* survival was dependent on the consortia used, with reductions ranging from 1 to 2.5 log. Shotgun metagenomics of the original consortia revealed the presence at dominant level of the genus *Advenella* (order Burkholderiales) in the most antagonistic smear.

The study demonstrates that *Escherichia coli* survival in raw milk semi-hard cheeses is influenced by the smear microbial consortia. As plant-associated Burkholderiales strains have been shown to inhibit gram-negative bacteria in a contact-dependent manner, future work will explore the potential of *Advenella* strains as natural antagonists to enhance food safety in cheese production.

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Enhancing overall quality and safety of fermented African cereal beverages: innovations in *Umqombothi* (opaque beer) production

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● Global challenges of food security and malnutrition pose a significant burden on many economies, making it increasingly important to consider sustainable solutions that address both the nutritional quality and functional aspects of food. In this context, *umqombothi*, a fermented traditional sorghum beverage from South Africa, with its cultural significance, offers untapped potential to contribute to these solutions. This study highlights *umqombothi's* nutritional profile, fermentation dynamics, processing challenges, and recent innovations, while also addressing key safety concerns, particularly its relation to mycotoxin contamination. Research suggests that sustainable and innovative brewing techniques could offer a promising approach to enhancing the nutritional and functional benefits of *umqombothi* while mitigating safety issues. Exploring bioprocessing tools, including the use of starter cultures and optimized fermentation protocols, while incorporating smart computational tools, can improve the safety and quality of indigenous fermented foods. This will not only safeguard consumer health but also improve the overall food safety standards. In this context, the European Union (EU) - African Union (AU) Partnership for Resilient, Inclusive and Safe food systems for Everyone (UP-RISE) project is part of a broader food safety effort, which aims to reduce mycotoxin contamination, particularly from the five major mycotoxins, i.e., Aflatoxin B1, Fumonisin B1, Ochratoxin A, Deoxynivalenol, and Zearalenone, along the African agri-food chain. Through collaboration between the EU and AU partners, this initiative focuses on innovative and sustainable strategies to improve the quality of agricultural products and safeguard consumers' health by exploiting fermentation techniques using selected microbial starters.

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Bioactive potential of lactic acid-fermented microalgae: evaluation as a functional food supplement in a zebrafish model

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● Obesity and its comorbidities are a global health challenge, prompting the search for novel bioactive compounds. Microalgae and cyanobacteria are particularly promising for their rich secondary metabolite profiles, while lactic acid bacteria (LAB) can enhance bioactivity through fermentation. This study investigates the effects of methanolic extracts from LAB-fermented microalgae on lipid metabolism, using the zebrafish Nile Red fat metabolism assay (NRFMA) and the 2-NDBG assay to assess glucose uptake. Six microalgal species approved as food sources, including *Chlorella vulgaris, Tetraselmis chui*, and *Arthrospira platensis*, were successfully fermented with six LAB strains belonging to the University of Parma Culture Collection (UPCCO). The NRFMA measured lipid accumulation, while the 2-NDBG assay assessed glucose uptake. Extracts were dissolved in DMSO (10 mg/mL) and were administered at 25 µg/mL, with resveratrol (NRFMA) and emodine (2-NDBG) as a positive control and DMSO as the solvent control. Fluorescence intensity was quantified using a fluorescence microscope and the fluorescence intensity in individual zebrafish larvae was quantified. Fermentation markedly enhanced the metabolic activity of microalgal extracts, in contrast to their unfermented counterparts, which showed no significant effects. Six fermented extracts reduced lipid accumulation, and nine improved glucose uptake. Bioactivity varied with both the microalgal species and lactic acid bacteria (LAB) strains used, highlighting the critical role of fermentation and starter culture selection in developing functional fermented foods. Untargeted LC-MS/MS analysis identified metabolite families potentially linked to bioactivity, including fatty acids, triterpene saponins and chlorophyll derivatives. These results support lactic acid fermentation as a strategy to boost the metabolic benefits of microalgae, offering potential for anti-obesity functional supplements.

The in vitro intake of *Sambucus nigra* extract promisingly impacts the human gut ecosystem in an individual-dependent way.

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● Modern diets, often characterized by polyphenol intake, are linked to gut dysbiosis and chronic metabolic disorders. Plant-based polyphenols supplementation shows promise as “duplibiotics”, combining antimicrobial and prebiotic effects. However, their structure, metabolism, and dependence on individual microbiota limit generalizations, particularly in clinical and animal studies. Our study examined the impact of a novel polyphenol-rich (22.45%) extract (EBE) from European black elderberry (*Sambucus nigra* L.) on two distinct gut ecosystems, using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). Two SHIME® units (S1 and S2), simulating the stomach, proximal colon, and distal colon, were inoculated with faeces from two healthy donors (out of $n = 40$) Mediterranean diet adherents. After stabilization, a two-week control, two-week treatment with 600 mg/day EBE, and one-week washout were conducted. Lumen samples collected at these timepoints were analyzed for short-chain fatty acids, polyphenols, and microbiome composition (shotgun metagenomics). S1 was *Bifidobacterium*-dominated while S2 was *Prevotella*-dominated. Despite baseline microbiota influencing the response to EBE intake, both SHIME® units showed a reduced Firmicutes-Bacteroidetes ratio ($p < 0.05$), common modulations of *Enterococcus durans, Leifsonia* sp. C5G2, and *Phytobacter diazotrophicus*, increased acetic acid and total polyphenol content ($p < 0.05$) in at least one colon tract. S1 and S2 showed increased genes for glycine, serine, threonine metabolism, plant secondary metabolite biosynthesis, and glutathione metabolism. While S1 had increased genes for sphingolipid metabolism, S2 had increased genes for aminoacyl tRNA biosynthesis and vitamin B6 metabolism. The differential and some common shifts in S1 and S2 indicate the individualized, potentially beneficial impact of EBE intake on gut health, highlighting its promise for personalized nutrition interventions.

Development of a colorectal cancer-simulated in vitro gut model for evaluation of dietary effect

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● The global burden of colorectal cancer (CRC) continues to grow, with rising incidence and mortality rates closely linked to Westernized diets and excessive consumption of ultra-processed foods. As diet strongly influences gut microbial composition, there is increasing interest in developing food-based strategies to prevent CRC by modulating the intestinal environment. To support this effort, we established an in vitro fecal fermentation model that reflects key microbial and metabolic features observed in CRC patients, enabling rapid screening of medicinal food candidates. Shotgun metagenomic data from 697 CRC patients and 684 healthy individuals were analyzed to identify CRC-associated microbial taxa. A machine learning-based approach revealed consistently enriched species in CRC, including *Fusobacterium nucleatum* and *Peptostreptococcus stomatis*. Based on these findings, we constructed a simulated CRC microbiota and applied it to an in vitro fermentation system designed to mimic the human colon. The model reproduced microbial shifts and metabolite profiles similar to those reported in clinical CRC samples. To evaluate biological relevance, we treated colorectal cancer cell lines with sterile-filtered supernatants and heat-killed microbial fractions derived from the fermentation. This approach allows for a comprehensive assessment of dietary influences on gut microbial composition, metabolite profiles, and cancer cell responses within an integrated, sequential experimental framework. Collectively, these findings demonstrate that the CRC-targeted in vitro fermentation model effectively mimics the tumor-associated gut environment and offers a practical platform for screening food-derived interventions with potential applications in colorectal cancer prevention.

Development of a novel sour beer by incorporating the probiotic strain *Levilactobacillus brevis* ACA-DC 1705.

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● The development of functional alcoholic beverages to promote human health is an emerging field for both academia and industry. Beer, in particular, presents a challenging matrix for probiotics delivery because of the presence of ethanol and hops. In the present study, the application of the probiotic strain *Levilactobacillus brevis* ACA-DC 1705 in sour beer production was examined. The strain was selected among 11 LAB probiotic strains from the ACA-DC Collection (http://www.aca-dc.gr/; Agricultural University of Athens) based on viability in hopped wort. During a 10-day co-fermentation at 20°C with *Saccharomyces cerevisiae* SafAle S-04 in unhopped wort, *L. brevis* ACA-DC 1705 maintained high counts (>8.00 log CFU/mL), indicating that its growth was not affected by the presence of yeast. This symbiosis was further supported by the observed pH (3.40) and °Brix (7.0) values. Subsequently, isomerized hop extract was added to the wort at a concentration of either 1 or 10 IBUs and the brews were stored at either 4 or 20°C. At the end of the storage period (40 days), the best viability of *L. brevis* ACA-DC 1705 was observed in the brew with 1 IBU stored at 4°C (7.82 log CFU/mL). GC-MS analysis showed differences in volatile compounds profiles between bacterium-yeast co-culture and yeast and bacterium monocultures (controls) brew samples, while sensory analysis revealed no significant differences compared to a commercial sour beer. The results suggest that *L. brevis* ACA-DC 1705 is a promising candidate for probiotic sour beer production under appropriate brewing and storage conditions.

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Development of a predictive model to define the digestibility of sourdough bread: Identification of key-factors affecting the in vitro protein digestibility

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● In vitro protein digestibility (IVPD) is a critical indicator of the nutritional quality of breads. However, IVPD analysis is highly labour-intensive, requiring complex enzymatic and chemical procedures. Therefore, this study aimed to develop a predictive model to assess IVPD in sourdough and baker’s yeast breads based on their biochemical and nutritional variables. Experimental sourdough and baker’s yeast breads were prepared using various flour and ingredient combinations. All breads (n = 56) were characterized for biochemical, nutritional, and textural properties, including pH, total titratable acidity (TTA), organic acids, moisture, starch components, total free amino acids (TFAA), and antinutritional factors. A random forest modelling was employed to predict IVPD, with optimization steps to identify the most significant variables. As predicted, sourdough breads generally exhibited higher IVPD and TFAA levels compared to baker’s yeast breads. Key predictors for IVPD included total free amino acids (TFAA), TTA, lactic acid, starch hydrolysis index (HI), glucose, and total protein content. The developed model achieved a prediction efficiency (R^2) between 50 – 70%, with a mean squared error (MSE) indicative of moderate predictive performance. While the model demonstrates promising predictive capability, its precision could be enhanced by expanding the amount of bread samples, more diverse ingredient profiles and incorporating broader IVPD ranges. Further variables such as fermentation and baking conditions could also be considered in future iterations to refine model robustness.

Antimicrobial activity of lactic acid bacteria strains isolated from fermented vegetable products.

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● The integration of fermented vegetables into our diet has gained significant attention due to their high nutritional value and the presence of live lactic acid bacteria (LAB), which are widely recognized for their health-promoting properties. Naturally fermented, non-pasteurized vegetable products are particularly valued for their distinctive sensory qualities as well as their alignment with sustainable, low-input production practices. However, the absence of preservation steps poses microbiological challenges, including spoilage and undesired biofilm or slime formation, which can compromise product quality and safety.

This study aimed to address these issues by isolating and characterizing LAB strains from non-pasteurized, naturally fermented vegetable products to identify predominant microbiota and their dynamics. LAB strains were isolated from various stages of the fermentation process, at the end of which occasional biofilm or slime formation in final products was observed. An initial screening of the antimicrobial activity of selected LAB strains was conducted *in vitro* using a dual agar overlay assay. The inhibitory effects of these strains were tested against common bacterial biofilm formers (*Bacillus* spp. and *Leuconostoc* spp.) and yeasts (*Saccharomyces* spp., *Pichia* spp. and *Kazachstania* spp.). Notably, several LAB strains exhibited inhibitory activity against *Bacillus* spp.. Building on these findings, future research will focus on the detailed characterization of LAB strains exerting inhibitory effects on adverse microbiota and their potential to mitigate spoilage and concomitant undesired quality effects.

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Fermentation: a safe and engaging gateway to microbiology for the high school curriculum

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● Recent curriculum changes in Flanders (Belgium) have introduced research skills and microbiological literacy into high school curricula across age groups and education systems. As a result, STEAM teachers are increasingly in need of safe, engaging teaching material and research projects. However, traditional microbiology demonstrations often rely on tests designed for pathogen identification. When asked to design a research project, most students -and even teachers- tend to default to plating samples from different surfaces or environments and comparing colony counts. This approach carries the risk of exposing untrained students and teachers to large numbers of unknown, potentially pathogenic microorganisms.

Vegetable fermentations offer a safer and more controlled alternative for unexperienced microbiologists. Even without the use of starter cultures, the fermentation environment is self-limiting, providing a safe space to explore physical parameters of the fermentations, colony growth, and even single-cell metabolism within a basic high school context.

The “Ferme Scholen” project is a citizen science initiative designed to support this approach. Participating teachers are invited to set up fermentations with their students, supported by background material, essential hardware and online support to develop their own lesson plans. Participating classes send samples from their fermentations to the university lab for sequencing and receive individualized reports on the microbial composition of their fermentations. Additionally, these samples contribute to ongoing research on the microbial communities and dynamics involved in vegetable fermentations.

So far, the project has generated strong enthousiasm and engagement from both teachers and students, while also yielding valuable new insights into microbial communities present in home and classroom vegetable fermentations.

Impact of fermentation on food safety: a study on *Listeria monocytogenes* in sauerkraut

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● This study investigates the effects of different fermentation methods on the reduction of *Listeria monocytogenes* in sauerkraut, addressing critical food safety concerns and exploring effective fermentation techniques. The aim of the study was to evaluate the impact of spontaneous and induced fermentation on *L. monocytogenes* populations in sauerkraut and achieve a 5-log reduction as per U.S. legislation, ensuring the safety of the final product.

Sauerkraut was prepared using natural and commercial fermentations, inoculated with *L. monocytogenes* at 0,10^5 and 10^7 ufc/mL. The process involved using organic cabbage and non-iodized sea salt, with fermentation carried out in glass containers equipped with airlocks.

The pH of all samples dropped below 4.4 within 48 hours, except for the natural control, which reached a pH of 3.9 after 4 days. The presence of *L. monocytogenes* did not affect the population of lactic acid bacteria (LAB), as similar patterns were observed across fermentation types. *Listeria monocytogenes* populations decreased significantly, achieving a 5-log reduction by day 7, and were undetectable thereafter.

Lactic acid fermentation effectively eliminates *L. monocytogenes* within 7 days, regardless of initial concentration or fermentation type, without impacting LAB populations. This study confirms that proper fermentation can ensure the safety of sauerkraut and highlights the potential of using controlled fermentation processes to enhance food safety in fermented products.

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Effect of Sardinian type I sourdoughs on nutritional quality and digestibility of bread.

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● In recent years, bread obtained with sourdough starters has been revalued by consumers, especially for its health and nutritional aspects, which include a better balance of taste, nutritional quality and benefits for the digestive tract. The aim of this study was to examine the effect of three Sardinian type I sourdoughs on the nutritional quality and digestibility of the resulting breads (BSD81, BSD82 and BSD84) by in vitro digestion simulations, microbial characterisation, metabolite production and bread quality parameters. The pH values of bread samples ranged from 4.68 ± 0.03 to 4.89 ± 0,06, compared to pH 5.64 ± 0,14 of the control bread obtained by using a commercial yeast starter (BCont). Improvement in the nutritional profile of the sourdough breads were related to a decreased phytic acid content, from 0.05 ± 0 to 0.07 ± 0.01 g/100 g. Additionally, in vitro protein digestibility (IVPD%) showed notable enhancement in BSD81 (78.88%) compared to the control (70.18%). A significant reduction in the hydrolysis index (HI) was observed in BSD82 and BSD84 (35.27 and 37.67, respectively) compared to BCont (51.67), leading to lower predicted glycemic index (pGI) values (59.08 and 60.39 versus 68.08 in the control). These findings provide comprehensive insights into how different sourdoughs can modulate bread's biochemical and nutritional properties.

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Promoting sustainable diets with plant-based proteins and complex dietary fibers.

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● Plant-based diets are increasingly advocated for their environmental sustainability and associated health benefits. However, the structural complexity of plant proteins and the presence of antinutritional factors within plant matrices can impair digestibility, allowing undigested protein fractions to reach the colon. There, proteolytic fermentation can generate potentially harmful metabolites (e.g., branched short-chain fatty acids (bSCFAs) and amines), which are associated with gastrointestinal discomfort. The inclusion of complex and non-digestible carbohydrates may mitigate these effects by favouring saccharolytic over proteolytic fermentation. Additionally, expose plant proteins to fermentation for the development of fermented products may enhance digestibility and reduce colonic proteolysis. Nevertheless, research on these strategies remains limited.

This study aims to elucidate the colonic metabolic interactions between non-digestible carbohydrates as dietary fiber and plant-based proteins – fermented or not – and to assess the potential health implications of their combination.

Plant-based protein sources (faba, yellow pea, chickpea) were selected for their complementary aminoacid profiles and sustainable production. Autochthonous and allochthonous lactic acid bacteria and yeasts were screened for their proteolytic potential, and the most effective strains were used to ferment tailored protein blends. Protein hydrolysis, release of bioactive peptides and amino acids were assessed. The resulting fiber-protein blends were digested *in vitro* and their impact was investigated.

Overall, this study highlighted that combining complex dietary fibers with plant proteins – and their tailored fermentation – has the potential to modulate colonic fermentation, limiting proteolysis, and fostering beneficial metataxonomic dynamics.

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The role of fungal microbiota in regulation of gut inflammation: the example of food-born fungal strains with probiotic properties

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● The food industry has long relied on a wide variety of microorganisms, including fungi, in its production processes, as seen in traditional fermentations such as those used for kefir and kombucha. While these strains have been extensively characterized for their biotechnological properties, their interactions with the host remain poorly understood – particularly at a time when the intestinal microbiota is increasingly recognized as a key factor in various pathologies. The prevalence of inflammatory bowel diseases (IBD) has been rising steadily since the 1960s and now represents a global health concern, with significant incidence in Europe, North America, China, and Japan. Maintaining a balanced interaction between epithelial cells, the immune system, and the gut microbiota appears crucial in preventing these disorders. Although the role of bacteria in gut health has been well documented, fungi – despite their lower abundance – remain largely understudied. Among the microbial strains selected by the food industry for fermentation, flavor production, or heterologous protein expression, the diversity and potential health effects of foodborne yeasts are still poorly characterized. Over the past five years, our laboratory has undertaken a systematic selection and characterization of yeasts and filamentous fungi used in food production, aiming to evaluate their variable interactions with the host. Through this work, we have identified several fungal strains with promising probiotic properties, capable of reducing susceptibility to intestinal inflammation in a mouse model of ulcerative colitis. These findings open new perspectives on the role of foodborne fungi in gut health, suggesting that a deeper understanding of their properties could contribute to the development of dietary strategies and alternative approaches to disease prevention and treatment.

[Keywords] Microbiota, mycobiota, probiotics, intestinal inflammation

Integrating phenotypic and metabolic characterization to assess the effect of potential probiotic candidates on gut microbiota composition, functionality, and immune modulation.

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[Keywords] probiotics, lactic acid bacteria, yeasts, phenotypic microarray analysis, gut microbiota, SHIME, immunomodulation

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● Probiotics have long been recognized for their positive impact on gut health, with their ability to restore balance in the microbiota, improve digestion, and support immune function. Their effectiveness is enhanced in multi-strain formulations, where diverse bacteria and yeasts cooperate synergistically. Probiotics are typically isolated from human gut samples, while growing research highlights fermented foods (e.g. sourdoughs, cheeses, yoghurt, and pickled vegetables) as promising alternative sources for novel probiotic candidates. This study aims to evaluate the probiotic potential of various lactic acid bacteria and yeast strains isolated from different food sources through the characterization of their metabolic and functional traits. An initial screening of approximately 100 strains was performed to assess their tolerance to simulated gastric and bile salt conditions. Strains exhibiting high resistance were selected and further characterized for their ability to produce exopolysaccharides, as well as for their hydrophobicity and auto-aggregation capacity, crucial indicators of probiotic adhesion and colonization potential in the epithelial cells of the intestinal mucosa. Based on our-multi-step and cumulative scoring-based approach, five most promising strains will be characterized for their phenotypic fingerprints through Omnilog Phenotype MicroArray Technology using Prebiome plates, to better understand their metabolic responses to various prebiotic substrates. In the final phase, the best-performing strain and/or combination of strains will be in vitro evaluated using the Simulator of Human Intestinal Microbial Ecosystem (SHIME) and human intestinal epithelial cell lines to further investigate their impact on gut microbiota composition and functionality and their immunomodulatory potential. This multi-tiered approach integrates physiological, phenotypic, and metabolic analyses to identify robust potential probiotic candidates with enhanced gastrointestinal survival and functional attributes. The findings of this study will contribute to the development of next-generation probiotics with improved efficacy for gut health applications.

Fermentation-driven protein hydrolysis in tarhana.

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● Tarhana is a traditional Turkish fermented food produced by the fermentation of both plant-based (wheat flour and various vegetables) and animal-based (yogurt) materials by lactic acid bacteria and yeasts. It is commonly consumed as soup, especially during illness. Although various studies have investigated the technological and rheological properties of tarhana, its effects on human health have not yet been fully elucidated. In particular, the hydrolysis of proteins derived from flour and yogurt during fermentation and the resulting bioactive properties remain unclear. Accordingly, the main objective of this study was to determine the changes in the protein fraction during a 21-day tarhana fermentation process. For this purpose, four different tarhana samples were produced: one with sourdough, and three with proteolytic strains previously isolated from sourdough, namely *Lactobacillus delbrueckii* F5, *Lactiplantibacillus plantarum* 113, and *Levilactobacillus brevis* 2103. Protein hydrolysis was monitored throughout fermentation on days 0, 3, 5, 10, and 21. In all samples, the total essential amino acid content changed throughout the fermentation process, but remained stable after day 10. At the end of fermentation, all tarhana samples produced with starter cultures were more enriched in essential amino acids compared to the sourdough-based sample. Among them, the tarhana produced with *L. delbrueckii* F5 had the highest essential amino acid content. Similar to the amino acid composition, the peptide profiles of the samples also showed strain-dependent changes during fermentation. These results indicate that the use of proteolytic starter cultures in tarhana fermentation may improve the bioavailability of tarhana.

[Keywords] Tarhana, fermentation, proteolytic starter cultures, protein hydrolysis, bioavailability
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Impact of different starter cultures on the functional properties of tarhana, a traditional fermented food.

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● Tarhana, a traditional food of Turkish cuisine, has the potential to positively impact human health due to its multifunctional properties, as it is produced through the fermentation of raw materials with diverse characteristics (such as flour, vegetables, and yogurt) by a highly complex microflora predominantly composed of lactic acid bacteria and yeasts. In this study, the effects of using different starter cultures in tarhana fermentation on its health-related properties were investigated. In this context, five different tarhana productions were carried out: one with spontaneous fermentation (T1), one with Type 1 sourdough (T2), and three with starter cultures isolated from spontaneously fermented tarhana. The combinations used as starter cultures were as follows: *Lactiplantibacillus plantarum* LTF21 and *Saccharomyces cerevisiae* YTF1 (T3); *L. pingfangensis* LTF1 and *S. cerevisiae* YTF1 (T4); and *L. plantarum* LTF21, *L. pingfangensis* LTF1, and *S. cerevisiae* YTF1 (T5). Following fermentation at room temperature for 21 days, the tarhana samples were analyzed in terms of free amino acids, short-chain fatty acids, polyphenols, and DPPH radical scavenging activity. While the T5 sample showed superior performance in terms of DPPH radical scavenging activity, the T3 sample was the most successful regarding free amino acids, short-chain fatty acids, and polyphenols. These results highlight the importance of selecting and applying appropriate starter cultures in tarhana production and indicate that especially the use of *L. plantarum* LTF21 as a starter culture in tarhana fermentation has high potential for the release of health-promoting metabolites.

[Keywords] Tarhana, fermentation, starter culture, functional food, bioactive compounds
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Exploring the health benefits of kimchi, sauerkraut, and other fermented Brassica foods.

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● Cruciferous vegetables from the genus *Brassica*, such as cabbage, broccoli, and cauliflower, are commonly used to create fermented products like sauerkraut, kimchi and pao cai. These foods are rich in lactic acid bacteria and bioactive compounds, contributing to their potential health-promoting effects. A review of twelve clinical trials investigating the influence of kimchi and sauerkraut on health revealed that regular consumption alleviated irritable bowel syndrome symptoms, reduced inflammatory cytokines, supported weight loss based on anthropometric measurements, and improved metabolic health by reducing the total serum cholesterol, HDL-cholesterol and fasting glucose levels. However, most clinical trials were conducted in one geographical region – South Korea, with small sample sizes, limiting their statistical power and generalizability. Three studies involved approximately 90 participants each, the remaining trials enrolled between 10 and 30 participants. Seven observational studies further linked fermented Brassica foods to health benefits. Six of these observational studies associated kimchi intake with reduced obesity risk and other positive outcomes, while one study suggested sauerkraut and cabbage consumption might lower breast cancer risk. Despite these promising findings, the specific roles of microorganisms and bioactive compounds in these effects remain unclear. This review highlights the potential of fermented cruciferous vegetables as functional foods and underscores the need for additional clinical trials and mechanistic studies. Future mechanistic studies should focus on determining the impact and contribution of specific microbial strains from the ferments, identifying key bioactive compounds, and elucidating their synergistic effects within the whole ferment to fully understand and optimize their health benefits.

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Biotransformation of beeswax constituents via lactic acid fermentation resulting in enhanced release of antifungal bioactive metabolites.

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● Beeswax is a natural, hydrophobic substance rich in bioactive compounds such as esters, hydrocarbons, and fatty acids. Despite its potential in health-related applications, its complex composition limits bioavailability. Fermentation offers a natural way to releasing these compounds by enzymatic transformation. Lactic Acid Bacteria (LAB) have shown the capability to hydrolyze a broad range of phenolic esters to produce metabolites with different bioactivity. The aim of this study is to generate antifungal metabolites from beeswax via fermentation with selected LAB species. In silico analysis for the prediction of the esterase activities based on genome mining of related genes across various strains of LAB was performed using several databases, which in turn allowed the optimization of the esterase metabolic pathways (KEGG/MetaCyc/BRENDA) through substrate feeding. Then the *in vitro* antifungal activity of novel fermentation-derived compounds was evaluated. Genome mining has identified the est_1092 gene in *Lactiplantibacillus plantarum* strains, encoding an enzyme capable of hydrolyzing a wide range of phenolic esters, including hydroxycinnamic and hydroxybenzoic esters (Esteban Torres et al., 2015). Functional analysis of linoleate hydratase genes revealed that L. plantarum produces antifungal metabolites, 10-hydroxy-12-octadecenoic acid and 13-hydroxy-9-octadecenoic acid from linoleic acid, displaying activity against *Aspergillus niger* and *Penicillium roqueforti* (Chen et al., 2016). Enzyme deletion and *in silico* modeling helped clarify substrate specificity and fermentation conditions of the linoleate hydratases from *L. plantarum* (Aziz et al., 2022). Thus, we targeted selection and metabolic modulation of LAB, based on esterase gene profiles and fermentation behavior, enhancing the release of functional metabolites from beeswax. This integrated approach supports the development of antifungal food ingredients through sustainable microbial bioprocessing.

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The impact of arabinoxylan fermentations in different breads on the gut microbiota.

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● The human gut microbiome comprises the collective genomes of all the microbiota within the gastrointestinal tract, with the largest quantity predominantly in the large intestine. The interaction of fibre with the gut microbiota can produce beneficial responses to the hosts physiology, mainly known through production of short chain fatty acids (SCFAs).

Arabinoxylan (AX) is the main fibre present in wheat starchy endosperm, and it is common in our diets. Wheat breeding programmes have developed wheat lines with different ranges of AX content and solubility. Isolated AX has been shown to be beneficial via production of SCFAs and microbial composition. However, little is known about the flour and its fermentation within different bread types and its corresponding impact on the microbiome.

We have developed and used a high throughput digestion and colon models to stimulate AX flour digestion, with a focus on the gut microbiota response in healthy people. So far, our findings show that increasing the water extractable AX in wheat lines impacts the gut microbiota response resulting in increased butyrate production, a key SCFA. Current work involves investigating AX fermentation in sourdough bread created with different starters. This will be compared to the gut microbiota response to industrially style Chorleywood breads.

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Water kefir starters create functional dairy-like plant-based beverage.

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● The specificity of ingredients and fermenting microorganisms is critical in designing fermented plant-based beverage and maximizing their protective properties. Our study aimed to replicate kefir microbial ecosystem within a novel plant-based formulation, focusing on the synergistic interactions between starters (isolated from water kefir including *Leuconostoc mesenteroides, Lacticaseibacillus paracasei, Lactiplantibacillus plantarum*, and *Pichia bruneiensis*) and distinct substrates to enhance nutritional, functional, and sensory properties. 16-h incubation period was chosen as optimal for fermentation at 30°C. Various fermentation methods were employed. Compared to Raw-PBB (without microbial inoculum and incubation), fermentation with *L. paracasei* alone or the ternary LAB culture showed superior microbial growth, strong acidification (evidenced by maximal lactic acid production), and enhanced proteolysis as indicated by elevated peptide and free amino acid concentration. Although phenolic compounds metabolism largely depended on the starter composition, the same fermented beverages caused a significant release of phenolics (including catechin and rutin). Only ternary LAB culture achieved phytic acid degradation, while the beverage fermented with *L. paracasei* not only resulted in lowest fructan and raffinose content among all beverages but imparts organoleptic properties similar to dairy-like flavor profiles. Overall, our findings highlighted the potential of kefir microbial starters combined with plant-based formulation to provide nutritious and sustainable functional alternatives to dairy yoghurt.

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Production of baker's yeast from prickly pear waste.

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● Many studies were carried out to screen inexpensive substrates to produce several metabolites by fermentation in a viable and sustainable way. In fact, the use of agricultural residues as substrates could not only reduce fermentation costs but also minimize environmental pollution. Prickly pear waste of *Opuntia ficus-indica (OFI)* is one of the commercially feasible lignocellulosic biomasses and has a great potential of being sustainable feedstock for a range of products. With high concentration of sugar, *OFI* offers a potential substrate for fermentation processes.

The aim of the present study was to investigate the potential of *OFI* waste as carbohydrate feedstock for yeast biomass production by using *Saccharomyces cerevisiae*. From the experimental results, the *OFI* fruit peel is an appropriate carbon source for the production of baker's yeast. The maximum biomass concentration obtained was 17.3 g/L with a productivity of 0.7 g/L.h. The feasibility of producing yeast biomass from *OFI* cladode hydrolysate (OFICH) as a source of carbon was also investigated.

These results clearly indicate the high potential of *OFI* for baker's yeast production by *Saccharomyces cerevisiae* for subsequent industrial applications.

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[Keywords] Opuntia ficus indica, yeast biomass, Saccharomyces cerevisiae, fermentation

Understanding fungal and yeast fermentations of plant-based substrates.

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● Global population growth and limited resources call for a protein transition towards more sustainable alternatives. Plant-based foods are a promising alternative to animal-based products, given their lower greenhouse gas emissions. However, the shift towards plant-based foods is hampered due to the presence of anti-nutritional factors and characteristic off-flavours, often described as beany or grassy.

Some fungal species, including yeasts, have been used extensively for centuries in fermentation processes to improve the quality of fermented food products, contributing to texture, preservation, and taste. Despite these promising results, there are at least two key challenges with respect to plant-based fermentations. First, there is a limited fundamental understanding of the conversion of plant-food raw material by fungal species and secondly, optimization of plant-based fermentation mostly relies on trial and error approaches.

In this project, we aim to expand our understanding of the role of enzymatic processes and metabolic pathways of selected food-grade fungal species to deliver rational design principles for fungal fermentations of plant-based substrates. We intend to use high-throughput yeast fermentation with different plant-based substrates to explore their capabilities to degrade off-flavours and antinutritional factors in plant-derived raw food materials. In conclusion, we will combine high-tech analytical methods with artificial intelligence to predict and validate the currently unknown functions. This will allow us to rationally design optimal fermentations to make more tasty, healthy, and sustainable plant-based foods.

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Enhancing bioactive profiles of elderberry through yeast fermentation: a pathway to low-sugar functional beverages

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● We investigated the effectiveness of yeasts in bio-transforming elderberry into a functional beverage. Initial screening of ten yeast strains for sugar metabolism and ethanol production in a synthetic medium and elderberry juice identified distinct metabolic performances. Five yeast species were then shortlisted and binary cultures, combining high and low to intermediate performers, were used to ferment elderberry juice for 36 h at 30°C. The co-culture of *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* with *Hanseniaspora opuntiae*, resulted in a more balanced metabolic profile, achieving high sugar reduction (80–88%) and modulating ethanol levels (1.38–1.57%). Although phenolic metabolism largely depended on the starter composition, the same cultures caused a significant release of phenolics (including chlorogenic acid, quercetin, hyperoside, and isoquercetin) with biological activity. Most of the binary cultures showed synergistic proteolytic activity, increasing the levels of free amino acids and promoting the release of bioactive amino acid derivatives such as dopamine and GABA, with GABA in combinations of *H. opuntiae* with *H. uvarum* and *Saccharomyces cerevisiae* with *H. uvarum.* Furthermore, the shaping of volatile organic compounds by *S. cerevisiae* favoring alcohol and acids production or non-*Saccharomyces* strains enhancing ester synthesis, further diversified the sensory profile when combined, contributing to aromatic complexity of generated functional beverages.

[Keywords] Low-sugar, binary cultures, phenolics, neurotransmitters, volatile organic compounds, sensory profile

Fermentation of oil pumpkin and butternut squash pulps: impact on chemical composition and rheological properties

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● This study explores the impact of lactic fermentation on the chemical composition, rheological properties, and nutritional profile of oil pumpkin (Cucurbita pepo var. *Styriaca*) and butternut squash (Cucurbita *Moschata*) pulps. Fermented and non-fermented samples were analyzed for β-carotene content, particle size distribution, viscosity, and colorimetric properties. Raw pumpkin puree samples from *Moscata* had the highest L (lightness) value at 49.57 ± 0.67, while raw *Stryaca* puree had a lower L value (41.16 ± 1.42). The particle size analysis of both *Moscata* and *Stryaca* pumpkin purees revealed that the mean particle size for fermented *Moscata* puree was 120.9 µm (± 16.2), while for fermented *Stryaca* puree it was 124.6 µm (± 17.6), which were significantly smaller compared to raw and blanched samples, with mean sizes of 174.9 µm (± 34.6) and 168.3 µm (± 21.1) for raw *Moscata* and *Stryaca*, respectively. The rheological measurements revealed that raw *Moscata* puree had the highest apparent viscosity (0.46 Pa·s) and consistency index (15.54 Pa·sn), while fermented *Stryaca* puree had the lowest viscosity (0.06 Pa·s) and consistency (0.73 Pa·sn). The hysteresis loop area was largest in raw *Moscata* puree (36,040 Pa·s). Raman spectroscopy and principal component analysis (PCA) revealed significant biochemical changes, particularly in carotenoid and phenolic compound profiles, distinguishing fermented from non-fermented pulps. The results demonstrate that fermentation modifies both the structural and functional properties of pumpkin pulps, with implications for their use in food applications.

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[Keywords] Pumpkin, fermentation, processing, physicochemical characterization

Nut-based fermented products as cheese-alternatives: exploitation of lactic acid bacterial consortia

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● The increasing interest in plant-based products has stimulated the development of cheese surrogates obtained through Lactic Acid Bacteria (LAB) fermentation of nuts or legumes. These products can pose safety (pathogens, spoilage microbiota) and quality (texture, aroma) challenges. This study, part of the InnoSol4Med project, evaluated the potential of autochthonous LAB strains as starter cultures for the fermentation of different nuts (cashews, almonds, hazelnuts, Macadamia nuts) to obtain spreadable cheese-like food. Five LAB strains belonging to *Lactiplantibacillus plantarum, Lactiplantibacillus paraplantarum, Pediococcus acidilactici, Latilactobacillus sakei, Leuconostoc lactis* species, isolated from artisanal fermented products, were tested for growth performances, alone or in combinations. The process was also optimized to inhibit spoilers. The results showed that one strain failed to outcompete the wild microbial population and that hazelnuts were unsuitable for obtaining a spreadable cream. Based on these findings, a hot water bath pre-treatment was introduced before nut soaking and the most promising LAB were used in combination to enrich the aroma profile and optimize the acidification rate. The results highlighted the potential of different LAB consortia to ferment raw materials reaching a safe pH value (<4.4). Moreover, aroma profiles were characterized by the presence of compounds able to confer cheese-notes (acetoin and diacetyl) that enrich sensorial appeal and favor sensory acceptability. This work provides valuable insights for developing innovative plant-based foods using microbial diversity from traditional fermented products.

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Maximizing the synergistic interactions between microbial consortia and plant-based matrices to design fermented cereal-pulse based beverages.

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● This study aimed to develop novel fermented cereal-pulse based beverages using four alternative yoghurt lactic acid bacteria starters. Following initial screening, we constructed three binary, three ternary, and one quaternary consortium keeping *Lacticaseibacillus paracasei* as the base strain due to its superior functional attributes, and proposed a fermentation conducted at 30°C for 20 h. The transition from single to mixed starter cultures marked a critical improvement in fermentation functionality. Most fermented beverages showed efficient release of microbial metabolites, peptides and amino acids achieving a yoghurt-like consistency compared to control dairy yoghurt. Although phenolic metabolism largely depended on the starter composition, fermentation generated a diverse array of phenolic compounds with enhanced antioxidant potential. Among the formulated consortia, binary combinations composed by *Lc. paracasei* and *Leuconostoc citreum* or by *Lc. paracasei* and *Levilactobacillus brevis* mainly revealed distinct nutritional advantages through antinutrients reduction and digestibility improvement. Volatile organic compounds analysis confirmed that substrate-starter interactions enhanced the aroma attribute complexity, while consumer preference skewed towards beverages in which *Lc. paracasei* was combined with *Levilactobacillus namurensis* or with *Leuc. citreum* and *Lv. namurensis*, which clustered sensory with the raw beverage control. Our findings highlighted the potential of tailored microbial consortia to provide nutritious and sustainable functional alternatives to dairy yoghurt.

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Lactic acid fermentation of non-conventional plant-based protein extract.

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● The growing demand for plant-based foods, calls for effective preservation strategies to maintain safety and quality. This study investigated biopreservation using eight plant-based protein extracts (PBPEs) pea, faba, soy, potato, pumpkin, hazelnuts, rice, and hemp, fermented with 12 lactic acid bacteria (LAB) strains from four species. Impedometric analysis revealed that biopreservation effectiveness varied by both matrix and LAB strain. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* showed the highest adaptability, particularly in soy, faba, and hemp extracts. Their performance highlights their suitability as biopreservative agents across different PBPEs. The impact of fermentation on sensory quality was also evaluated for the most effective strains. Notably, fermentation with *L. delbrueckii* subsp. bulgaricus 1932 and *L. plantarum* 4193 significantly improved aroma profiles in several PBPEs, including pea, faba, soy, pumpkin, rice, and hemp. Key off-flavor compounds such as hexanal and hexanoic acid were substantially reduced, enhancing the organoleptic quality of the final products. These results underscore the dual role of LAB fermentation as a natural preservative and flavor enhancer, offering promising applications in the development of high-quality plant-based foods.

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Fermentation-induced sensory modifications in high-protein, high-fiber plant-based (yo)gurts.

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● Fermentation is a well-known technology for inducing desired sensory and nutritional modifications in raw materials and has recently been widely used to improve plant-based formulations. This study characterized the sensory properties of four products: a fermented high-protein, high-fiber (yo)gurt made from oat and faba bean at lab scale; the same product produced industrially; a non-fermented porridge with the same composition as the (yo)gurt; and a low-protein, low-fiber oat (yo)gurt made at lab scale.

The starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus*) was selected to ferment the (yo)gurts until reaching a target pH of 4.5. Sensory evaluations were conducted individually in triplicate in a sensory laboratory (ISO 8589) using a 0–10 line scale (0 = not at all, 10 = very strong) by eight trained panelists, with reference samples provided. Assessors evaluated three appearance, six odor, two texture, four flavor, and six taste and chemesthetic attributes.

Sensory analysis revealed differences due to fermentation, with the increase of fruity and sour milk odors and flavors, but also composition, with the absence of faba bean resulting in lower bitterness and astringency. Differences between the (yo)gurts and porridge were evident in texture and appearance, with the porridge being thicker, lumpier, coarser, and less watery, and exhibiting stronger cooked starch, cereal, and cardboard odors and flavors. This study underscores the role of fermentation in generating dairy-like flavors typical of yogurts while maintaining the cereal-like flavors typical of plant-based protein sources.

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Screening of *Lactiplantibacillus plantarum* for the production of ɣ-aminobutyric acid in a legume-based fermented beverage.

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● The growing scientific interest in ɣ-aminobutyric acid (GABA) arises from its multiple beneficial effects. In addition to its role as the principal inhibitory neurotransmitter, GABA can be synthetized by several microorganisms, including lactic acid bacteria (LAB) which represent a promising strategy to enhance the functional properties of fermented foods, especially since the direct addition of GABA is not permitted.

This study investigated the GABA-producing potential of 20 *Lactiplantibacillus plantarum* strains, isolated from fermented foods, *in vitro* and *in situ* during a lab-scale fermentation to produce a legume-based beverage. After optimization of culture conditions (i.e. time, temperature) colorimetric assay, GABAse enzymatic assay and UPLC/MS-MS analysis have been performed.

Colorimetric results allowed the classification of strains into three groups, according to their GABA production levels (low, medium, high). Subsequently, for the GABAse assay six concentration (0–400 mM) of monosodium glutamate (MSG), used by LAB as GABA precursor, were tested, identifying 400 mM MSG as the optimal concentration to enhance GABA production. Four strains (C9S2, C9O4, LAB62, LT52) were selected as the best GABA producers and these findings were confirmed by UPLC/MS-MS analysis (21.94, 7.66, 4.86 and 3.96 mM, respectively). Then, they were combined (C1=C9O4-LT52; C2=C9S2-LAB62) and used as starters to produce a GABA-enriched legume beverage. UPLC/MS-MS analyses confirmed strain-specific GABA production *in vitro* (C1=2.53 mM; C2=4.54 mM) and in the final fermented product (C1=0.068 mM; C2=0.067 mM), supporting the importance of a careful strain selection to enrich, through fermentation, a high-quality nutrient matrix with potential health benefits.

Tailored fermentations to produce fava-based alternatives.

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● The increasing demand for sustainable protein sources is driving innovation in plant-based dairy alternatives, motivating the agrifood sector to explore new solutions. The valorization of underutilized crops like fava beans offers an opportunity to diversify plant-based raw materials beyond traditional sources like soy. This study aims to characterize the microbiological, technological, and functional properties of innovative fermented fava bean plant-based alternatives by fermentation. The protein fraction, extracted from fava beans, was thermally treated at 80°C for 15 minutes. After cooling to 30°C, the matrices were inoculated with *Lactiplantibacillus plantarum LB82, Lacticaseibacillus casei A13*, and *Lactococcus lactis LBG2* at 6 log CFU/g and stopped at reaching pH of 4.7. The fermented curds were molded, pressed, and then treated by high-pressure processing (600 MPa for 120 seconds). Plant-based products were stored at 8°C for 90 d and analyzed for microbiological, physicochemical, functional, rheological, and volatile molecule profiles. Freshly fermented curds showed a high viable count of lactic acid bacteria (8-9 log CFU/g), while, post-HPP, undesirable microorganisms were under the detection limits, and only lactic acid bacteria persisted at very low levels (2 log CFU/g). Microbiological stability was maintained over the storage. Fermentation enhanced proteolysis and modulated the volatile molecule profiles, increasing cheese-associated aromas in a strain-dependent manner. Significant changes, among samples, were observed in antioxidant activity and rheological parameters.

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Valorization of durum wheat milling by-products through the production of fermented bioingredients for bread-making.

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● The aim of this study was to valorize by-products from milling through fermentation in order to obtain fermented bioingredients for bread production. Wheat bran or wheat germ flour were fermented by *Lactiplantibacillus plantarum* ITM21B strain ($\log_{10}$ 5.0 CFU/g, 14 hours at 37°C) thus obtaining F21B-B (at dough yield DY500) or F21B-G (DY300), respectively. After fermentation the production of lactic acid and a concomitant significant reduction of total protein content (87% and 95% for F21B-B and F21B-G, respectively) due to proteolysis, was observed. Moreover, F21B-G formulation was significantly richer in total free amino acids, glutamate and exopolysaccharides respect to the start of fermentation. Freeze-dried bioingredients were used in bread formulation to replace wheat flour at 22% (bread 21B-B or bread 21B-G) and compared with breads containing unfermented bran or germ flour (bread-UB or bread-UG). Breads were evaluated for the textural properties and as expected, the addition of wheat bran or wheat germ flour influenced the structural characteristics of the bread. However, a significant positive effect attributed to fermentation was observed in fermented wheat germ bread, which showed improvements in hardness and chewiness compared to its relevant control (bread-UG). These results suggest that further studies are needed to optimize bread formulation and obtain high nutritional value products by upcycling wheat by-products.

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Fermented defatted durum wheat-by products enriched with potential prebiotic exopolysaccharides.

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● The aim of this study was to investigate the suitability of defatted wheat bran and germ, by-products of the wheat oil extraction process, as substrates for fermentation and exopolysaccharides (EPS) production. EPS are bioactive compounds produced by lactic acid bacteria during fermentation and are known for their prebiotic and technological properties making them valuable compounds in the food sector. In the current study, the EPS produced by strains *Weissella cibaria* C43-11 or *Leuconostoc mesenteroides* C43-2M in defatted wheat germ (GD) or defatted wheat bran (BD)-based bioingredients (flour:water ratio 1:4) containing sucrose (6% w/w), were extracted and subjected to *in vitro* gastric digestion to evaluate their prebiotic potential. After fermentation (14 h at 30°C) samples were analysed for physico-chemical parameters and results showed a significant acidification (as measured by pH and TTA), EPS production (ranging from 5.2 to 9.9 mg/g), and a concomitant increase of viscosity in all samples. As a consequence of the acidification, the proteolysis determined the reduction of total protein content. After simulated gastric digestion, some differences in the content of undigested EPS were recorded: the highest percentage (55%) of EPS potentially available for colonic microflora was obtained in Bio-GD C43-11. These findings suggest the high potential of the piloted fermentation in converting food wastes, no more exploitable in the food industry, into bioingredients with potential health benefits.

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Microbial and technological aspects of fermented plant based milks: a functional food perspective

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● The development and production of vegan milk alternatives are growing rapidly. Their low fat content, absence of animal derived allergens such as lactose, and health promoting components like antioxidants make plant based milks appealing not only to vegans but also to individuals pursuing a healthy diet. Numerous health benefits of plant-based milk include decreased risk of cardiovascular and gastrointestinal disorders, possible antibacterial properties, and improved immune system performance. Fermented products, including plant based kefir and kombucha, are derived from the milk of various plant sources, such as almonds, coconut, soy, and oats. Fermentation can reduce some of the undesirable off-flavors in these milks, increase the bioactive compounds, and act as a carrier for probiotic microorganisms. Lactic acid bacteria are commonly used in the fermentation process, but yeasts and acetic acid bacteria may also be used from time to time. While lactic bacilli are the primary fermentative agents, *Bifidobacterium* species are occasionally included to enhance probiotic functionality. Advances in food omics technologies now allow for a more comprehensive understanding of microbial diversity, metabolic interactions, and the bioactive metabolites produced during fermentation. On the other hand, improvements are still needed in terms of taste, texture and viability of probiotics in fermented vegetable milks. In the present study, the nutritional values, the microbiological composition and the technological developments of fermented plant based milks will be discussed. The development of such plant based fermented products offers nutritious alternatives not only for vegans and milk allergy sufferers, but also for people who want to eat healthy.

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[Keywords] Vegan Beverages, fermentation, health benefits, probiotic

Developing microbial consortia to produce new plant-based fermented foods as alternative of traditional animal-base products.

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● In recent years, the growing awareness regarding environmental and health impacts of animal-based products has led to an increasing demand for new plant-based foods. Fermentation can become a key process to produce this kind of foods: by using selected microbial consortia is possible to obtain new products with enhanced sensory, nutritional and safety characteristics.

In this project over 300 microbial isolates of food origin from University of Parma Culture Collection (UPCCO) and University of Bologna Collection were screened by genotypic techniques and characterized for their safety (antibiotic resistances and biogenic amine production). As a result, 30 lactic acid bacteria (LAB) strains and 15 staphylococci strains were selected for food application. Metabolic profiles of these 45 strains were tested using BiologGEN III microplates to assess their ability to utilize 71 different carbon sources, and to establish potential metabolic complementarity among bacteria. Integrating genotypic and phenotypic analysis, guided the development of microbial consortia, composed of two LAB strains and one *Staphylococcus*, for fermenting plant-based substrates, especially nuts and beans. Single LAB strains were screened for pH reduction capability on the substrate, and binary LAB combinations improved flavor formation and acidification. Addition of Staphylococcus enhanced volatile compound production. Best consortia, evaluated based on growth capability on substrates and flavor, were further optimized under various fermentation conditions (salt addition, time, temperature). The final products were analyzed using liquid and gas chromatography to assess the volatile compounds and metabolites produced during the fermentation.

This study highlights how selected consortia can improve the production of alternative plant-based foods through the fermentation process, addressing sustainability issues that nowadays increasingly concern the food sector.

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Computational approaches for enzyme prediction and targeted strain selection, to degrade off-flavours and anti-nutritional factors in plant-based foods.

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● The global food system is a major contributor to climate change, with animal-based products responsible for roughly twice the greenhouse gas emissions of plant-based alternatives. Transitioning toward plant-based diets is therefore essential to reducing our environmental impact. However, the acceptance of plant-based foods remains limited due to off-flavours and anti-nutritional factors (ANFs), which negatively affect both taste and nutritional value. Fermentation offers a promising natural strategy to address these challenges by degrading off-flavours and ANFs and producing beneficial compounds. Yet the enzymatic mechanisms underlying these conversions in microbial species remain poorly understood. Therefore, the metabolic screening of large numbers of microbial strains is required to select appropriate candidates to be used in starter cultures.

This project addresses this knowledge gap by developing computational approaches to predict enzymes involved in the biochemical conversion of off-flavours and ANFs. We apply two complementary strategies: (1) structure-based enzyme prediction using molecular docking and multiple structure alignments, and (2) enzyme-substrate prediction using transfer learning with pre-trained protein large language models.

The resulting enzyme predictions serve as markers to guide targeted strain selection for fermentation, enabling the rational design of microbial cultures and fermentation processes. A selected set of enzyme candidates will be experimentally validated in collaboration with partners within the FERMI consortium. Ultimately, this project contributes to the development of scalable, data-driven fermentation strategies that improve the sensory and nutritional properties of plant-based foods. In doing so, it supports broader efforts to promote climate-resilient, sustainable, and appealing food systems.

Mycoprotein production by *Aspergillus oryzae* for a meat-reduced diet through submerged fermentation using agro-food side streams.

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● Filamentous fungi are gaining attention as a sustainable and nutritionally promising alternative protein source in contrast to many ultra-processed plant-based meat alternatives with often limited nutritional value and environmental sustainability. This study investigated mycoprotein production by strains of *Aspergillus oryzae* through submerged fermentation using agro-food side streams, with an emphasis on minimal processing and circular economy.

Two strains of *A. oryzae* (BF-1 and A-Y) were shake flaks cultured in eight different agro-food side streams. Both strains exhibited similar growth in most substrates; however, strain A-Y demonstrated superior performance when grown in one specific medium (16.15 ± 1.1 vs. 4.04 ± 0.28 g dry matter/L for BF-1). Consequently, A-Y was selected for upscaling in 3 L stirred-tank bioreactors in three agro-food side-stream media. Among these, only one medium supported robust growth, achieving a biomass yield of 9.21 ± 1.18 g dry matter/L within the same growth time. A further upscaling to 10 L stirred-tank bioreactor yielded 8.05 g dry matter/L in this medium.

The nutrient composition of the resulting mycelial biomass was analysed and compared with a commercially available mycoprotein food product. The *A. oryzae*-derived biomass exhibited 20% less protein but 4.8-times the carbohydrate content, and a 12.5% increase in lipid content compared to the commercial food product.

These results demonstrate the feasibility of submerged fermentation using *A. oryzae* and agro-food side streams for sustainable mycoprotein production. Nonetheless, further research is required to address yield limitations, focussing on creating a monitoring system to determine optimal process parameters.

Harnessing the power of precision fermentation and single-cell protein for innovative food and feed solutions: the case of edible yeast-based proteins

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● Transitioning to more sustainable protein sources beyond carbon- and resource-intensive animal-based proteins has become a major focus in nutritional research. In addition to plant-based proteins, fermentation-derived yeast-based proteins (YBP) could represent a viable alternative with high nutritional quality and lower environmental impact. This study aims to investigate the nutritional profile, digestibility, and metabolic response of YBP compared to traditional animal-based proteins.

An *in vitro* 5-hour human digestion model was used to assess the digestibility and amino acid bio-accessibility of three YBP production batches, with whey and casein as reference animal proteins. Dialysate and ileal effluent samples were analysed for total nitrogen and free amino acids. The impact of the protein sources on human colonic microbiota was also evaluated through 48-hour Colon-on-a-Plate batch fermentation with faecal samples from 11 healthy donors. Microbial activity and composition were analysed by metabolomics and shotgun sequencing.

YBP, casein, and whey exhibited comparable digestibility. While YBP had a distinct metabolomic profile, microbial activity measured by short-chain fatty acid quantification was significantly increased for all protein sources compared to the blank, with no significant differences between them. Whey and casein resulted in higher or lower abundance of certain bacterial taxa compared to YBP.

The *in vitro* data suggests that YBP could be a relevant alternative to animal-based proteins. Further clinical studies are warranted to explore the potential of fermentation-derived single-cell protein as innovative, sustainable solutions for food and feed applications.

Culture growth stimulation in food bioprocesses by nanosecond pulsed electric fields (nsPEF) – oleaginous yeasts as a prominent case study.

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● The cultivation of microbial or mammalian cells for alternative and novel food products is a rapidly evolving sector in biotechnology, presenting unique challenges. While many promising food bioprocessing concepts are technically feasible, they still struggle with cost competitiveness. The efficiency of biomass expansion largely dictates process economy, as the cell or an abundant intracellular compound is typically the product of interest.

In this study, we explore nanosecond Pulsed Electric Field (nsPEF) treatment as an innovative and broadly applicable physical method to stimulate the growth of *Yarrowia lipolytica* cultures for alternative dairy cream products.

To achieve optimal outcomes, nsPEF parameters must be carefully chosen on a case-specific basis, and a flexible development platform is required to bridge the gap from high-throughput screening to industrial implementation. This study addresses both challenges successfully:

We systematically investigated the nsPEF-mediated increase in yeast growth rate (μ) and biomass yield (Yx/s), transferring the process from high-throughput well plates to shake flasks, and finally to an industrial-style bioreactor. At the end of the development campaign, nsPEF treatment consistently resulted in higher end-of-batch biomass, as indicated by various physiological metrics (e.g., up to 49.8% increase in CDW, 61.2% in OD, and 26% in cell count vs. the no-treatment control). This success was achieved through a new, integrated testing platform with tailored cell-biologic indicator assays and in close collaboration between a research lab, food startup and the leading tech supplier to ensure industrial readiness.

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Ferments du Futur: a public-private partnership to accelerate research and innovation on ferments, fermented foods and biopreservation

[1] **Madeleine Spatz** and Ferments du Futur team

● With the increase in environmental and climatic risks, society's expectations in terms of food have gradually changed towards a demand for safer, healthier and more sustainable food. Fermentation can be used to transform raw materials while adding new properties: taste, colour, texture, preservation, nutrients up to targeted health benefits. It is in this context that Ferments of the Futur (FdF) was launched in late 2022, with €48.3 m in funding from France 2030 over a 10-year period [1]. Its funding will provide unique, interconnected capabilities combining the very best in fermentation research and innovation. Focused on food, FdF will gradually expand into other sectors such as agriculture, health, feed and the environment.

To make ambitions a reality, FdF has brought together public-private ecosystem, currently comprising 41 members from higher education & research organisation and companies in the field of ferments, fermented foods and biopreservation. Twenty-five people are currently working in this vibrant ecosystem, spread over several sites and managed by FdF:

– A Ferments du Futur Innovation Centre or “CI2F”, located in the Paris-Saclay cluster and equipped with microorganism screening and characterization capabilities, multiple laboratory and pilot-scale liquid and solid fermenters, a state-of-the-art physico-chemical characterization platform and a food prototyping workshop.
– A “Distributed Platform” infrastructure, whose facilities reinforce existing scientific and technical platforms within several INRAE research units (MaIAGE, MGP, Micalis, SayFood, SPO, STLO, UMRF) specialising in microbiology, process engineering, nutrition-health and applied mathematics-computing.

This one-of-a-kind infrastructure is open to collaboration with international research labs and European companies.

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Reference: [1] https://www.fermentsdu-futur.eu/en/home/

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Solid state fermentation of medicinal mushrooms on buckwheat husk.

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● Medicinal mushrooms (MMs) are gaining attention for their content of bioactive compounds whose effects are relevant for human health. The possibility to exploit cultural residues for their cultivation represents a challenge to enhance the commercial use of MMs. These different substrates may also have positive impact on the fungi development with effects on their activities and final composition. The main byproduct of buckwheat processing (husk) is rich in fiber and has great potential as a source of bioactive peptides and polyphenols; however, buckwheat husk is primarily used for fuel since recovery of these compounds is hindered by the husk's complex supramolecular structure that also impairs it use in stapple food.

In this study we investigate the use of husk a substrate for *Ganoderma annularis* and *Pleurotus ostreatus* in solid state fermentation trials, with the final aim of obtaining a fungal/husk biomass matrix retaining bioactives and/or improving the content of bioactive compounds. The two strains colonized husks with different growth kinetic. Structural changes were also relevant, in terms of both the α-/β-glucan ratio and the soluble/insoluble fiber ratio. Bioactive compound profiling highlighted increased ergosterol levels and a marked peptide release. Polyphenol profiling showed a decrease in total phenolics, but an increase in free polyphenols, leading to an increase in their activity as α-amylase inhibitors and in full retention of their anti-inflammatory ability in a Caco 2 cell model. The overall data collected so far suggest that mushrooms/buckwheat husk biomass can be a sustainable ingredient in functional food formulations.

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Predicting the metabolome of a synthetic microbial community to reduce anti-nutritional factors and off-flavours in pulses.

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● The demand for plant-based, protein-rich, and sustainably produced food sources is growing. European pulses like faba beans and yellow peas are nutritious, cultivatable in temperate climates, and provide desirable functional properties for use in meat and fish analogues. This would make European pulses a great protein-rich food source. However, they contain anti-nutritional factors (ANFs) such as protease inhibitors and phytic acid, as well as off-flavours such as hexanal. ANFs and off-flavours can be modified by either chemical, physical or biological treatments such as fermentation. Since the latter shows the greatest potential, a systematic, knowledge-based design of synthetic microbial communities offers a powerful approach to optimise fermentation with the aim of reducing ANFs and off-flavours and to advance our understanding of microbial interactions in pulse fermentation. Here, we investigated all possible microbial communities formed by a range of microbes commonly used in fermented foods, including *Lactococcus lactis, Leuconostoc mesenteroides, Propionibacterium freudenreichii* subsp. *freudenreichii, Acetobacter aceti* subsp. *aceti,* and *Saccharomyces cerevisiae*, and addressed whether machine learning could predict the metabolic phenotypes of pulse fermentation. While prediction accuracy varied across individual metabolites, the model successfully identified trends in microbial metabolic activity. Analysis of model outputs revealed which species – or combinations of species – contributed to reductions in undesirable compounds. This work represents a first step toward understanding how phylogenetically distinct micro-organisms interact metabolically, with the ultimate aim of guiding the design of synthetic communities to reduce off-flavours and anti-nutritional factors in pulses.

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Producing new healthy, white lupine-based, dairy-like products with a selected starter culture.

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● Lupine seeds have been increasingly recognized as a valuable source of plant proteins, providing a more sustainable alternative to animal proteins. Their use in agri-food chains is therefore important to foster healthier diets and more sustainable food systems. This work belongs to a European Project "Divinfood" which has the aim, among others, to implement dairy-like products and formulations/recipes using white lupin flour as a raw material, alone or mixed with cow or plant-based milks. Specifically, the products will be fermented by selected lactic acid bacteria (LAB) to enhance nutritional and organoleptic quality and possibly reduce/minimize the levels of antinutritional compounds (such as phytic acid, alkaloids), generally present in lupin seeds. To this end, a deep, *in vitro* LAB strain selection and characterization was carried out, leading to the choice of strains of *Lactobacillus acidophilus, Lactococcus lactis* subsp. *lactis, Lactobacillus delbrueckii* subsp. *lactis,* and *Streptococcus thermophilus,* these latter three representing the acidifying starter. The matrices underwent mild or minimal processing methods, with the aim to eliminate unwanted microorganisms, preserving their nutritional value as well. Preliminary tests by mixing lupin flour and cow milk have been carried out. A ricotta-like cheese, produced at laboratory scale, showed interesting sensory features. The possibility to ferment raw, water-resuspended lupin flour will be explored as well. The final products will be chemically and microbiologically characterized. Further in-depth studies will also be carried out to improve the current understanding of the impact of food processing on composition, sensory properties, and consumer acceptance of lupine-based products.

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Solid-state fermentation of wheat bran for nutrient-rich food.

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● Wheat bran is abundant by-product of wheat milling. It has high levels of dietary fibre, minerals, bioactive compounds and significant amount of protein. However, antinutrients and insoluble bran layers limit its use as food ingredient. We compared potentially edible fungi from the genera *Rhizopus, Mucor,* and *Neurospora* for their ability to modify the properties of wheat bran. Moreover, *Rhizopus* was applied with lactic acid bacteria (LAB) or propionic acid bacteria (PAB). Sterilized wheat bran at 65% moisture was fermented at 30°C for up to 70 h. Nutritional analyses were performed on dried samples.

All strains exhibited abundant growth in 48 h. The pH levels remained near neutral, except in co-culture with LAB. SSF increased total and indispensable amino acid contents, particularly lysine. Protein was solubilized and protein solubility at pH 6.5 was enhanced. There was also a trend towards improved *in vitro* protein digestibility. The fatty composition and content improved as well. SSF with most strains increased dietary fibre content. Using *Neurospora*, the phytic acid content reduced by 50%. Co-cultivation with PAB produced vitamin B12.

In conclusion, SSF can enhance the nutritional properties of wheat bran in a strain-dependent manner. *Rhizopus* and *Neurospora* show the highest potential for upgrading nutritional properties. Co-cultivation with PAB provides the added benefit of vitamin B12 production.

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Bioconversion of off-flavor compounds in pea protein isolates using selected micro-organisms.

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● The global demand for plant-based foods is increasing due to their health benefits and reduced environmental impact compared to animal-based products. Plant-derived protein ingredients are particularly attractive for developing cheese- and meat-like products, owing to their high protein content. However, pulse protein isolates, such as those from peas, often carry off-flavors – commonly described as "beany," "green," or "earthy" – that hinder consumer acceptance. These undesirable notes are primarily linked to volatile compounds such as aldehydes, alcohols, and furans (e.g., hexanal, 1-octen-3-ol, 2-pentyl-furan).

This study investigates a short-duration, minimal-processing approach to mitigate these off-flavors via single-strain bioconversion, without added sugars or salts. Solutions containing 4% or 12% pea protein isolate (PPI) were prepared using two types of PPI: one obtained through industrial processing and another via an innovative method. Lyophilized cultures of three bacterial and three yeast strains were tested individually. Samples were incubated and analyzed for pH, microbiological profile, volatile compounds (via DHS-GC-MS), and sensory characteristics.

The flavor profiles obtained through GC-MS were similar across isolates, with only a few compounds detected exclusively in one type of PPI. Most strains significantly reduced hexanal, pentanal, and other off-flavor volatiles within 24 hours. One species of yeast and one of bacteria exhibited the highest efficiency, achieving notable off-flavor reduction and improved sensory perception after only six hours of bioconversion. However, key contributors to the beany flavor, including 2-pentyl-furan and 3,5-octadien-2-one, remained detectable after 24 hours.

Future work will focus on screening additional strains, process optimization, and scale-up using these promising candidates.

Brewer's spent grain as an alternative carbon feedstock for advancing sustainability of mycelium-based food and feed products.

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● Feeding a global population of 10 billion by 2050 challenges the sustainability of diets in high-income countries, which heavily rely on animal-derived products. Interest is rising in the production of fungal mycelium, with *Fusarium venenatum* currently dominating commercial mycoprotein production. However, its reliance on refined glucose syrup as a carbon source demands substantial arable land. Meanwhile, agriculture generates around 8 billion tonnes of underutilized lignocellulosic waste annually. This study explores using brewer's spent grain (BSG), an abundant by-product, as a carbon source for cultivating *Lentinula edodes* (Shiitake), a wood-degrading Basidiomycete. Building on a previously optimized growth medium for *L. edodes* (Feng et al.), three formulations were tested: (1) BSG replacing glucose entirely, (2) BSG + half the original glucose, and (3) maltose as a glucose substitute, with the control consisting of the optimized medium. Fibre fraction analyses were conducted on the samples to assess the actual degradation of lignocellulose. Our preliminary results showed that the medium containing only dried BSG as the carbon source supported faster mycelial growth and yielded up to 5 times more dry biomass than the control. As an additional approach to lignocellulose valorisation, next steps in the research will involve pre-treating and enzymatically hydrolyse the BSG, followed by testing the hydrolysate as a glucose substitute. These preliminary findings highlight the potential of fungal mycelium in valorising agro-industrial residues. Liquid-state fermentation of *Lentinula edodes* could represent a scalable and sustainable solution for converting by-products into high-value fungal biomass with potential applications in both human and pet nutrition.

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Impact of autochthonous non-conventional *Hanseniaspora valbyensis* on fermentation and quality of pomegranate cider-like beverage.

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● This study aimed to assess the differences, in terms of physico-chemical characteristics, antioxidant properties and flavour profile, among four pomegranate cider-like beverages (PCB), produced starting from two pomegranate juices, fermented by either commercial *Saccharomyces cerevisiae* or a non-conventional, autochthonous strain of *Hanseniaspora valbyensis*.

Although both starters were able to drive the fermentation of pomegranate juices, *H. valbyensis* reached higher cell densities four days earlier than *S. cerevisiae*. After fermentation, PCB were subjected or not to a clarification treatment (centrifugation or filtration) and matured for additional seven days. In the mature PCB, filtration caused a stronger decrease in yeast cell density compared to centrifugation. As for elemental composition, K and Ca increased during fermentation, whereas Fe and Cu decreased. A strong reduction of Cu, Zn, as well as Fe and Ni, was observed in clarified mature PCB. The use of *H. valbyensis* led to a more rapid consumption of monosaccharides, higher acetic acid and lower ethanol concentrations compared to *S. cerevisiae*.

The final profile of Volatile Organic Compounds (VOCs) was reached after three and seven days of fermentation with autochthonous and commercial yeast, respectively. After maturation, PCB obtained with *H. valbyensis* kept more complex VOCs profile, compared to *S. cerevisiae*. The panel test on centrifuged PCB did not result in any significant difference. The antioxidant activity of PCB was always high and not affected by fermentation, type of starter, and maturation. This study paves the way for better understanding fermentation capability and impact of *H. valbyensis* on food quality.

Bacillus spp. as promising microbial candidates for the bio-valorisation of pulses for meat alternatives.

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● The application of *Bacillus* spp. in traditional food fermentation is primarily recorded in Asia, Africa, and South America. However, the use of *Bacillus* spp. as functional microbes is relatively unknown and under-utilized in European countries, although certain species of *Bacillus* have been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA). Due to their ability to produce a wide variety of food-related enzymes, including proteases, lipases, and amylases, *Bacillus* spp. hold great promise for functional and flavour improvement in food fermentations. Therefore, the aim of this study was to evaluate 121 strains of QPS listed *Bacillus* species such as *B. subtilis, B. amyloliquefaciens,* or *B. licheniformis* for their effect on techno-functional, flavour, and health-related properties of plant-based substrates during fermentation. In a first phase of semi-quantitative high-throughput phenotypic screening assays, 16 strains with high proteolytic activity, 19 strains with high amylolytic activity, 53 strains with high exopolysaccharide (EPS) production, 14 strains with phytase activity and 16 strains with FODMAPs degradation capacity were found. In a second phase, strains with high potential are currently applied on plant-based substrates to improve their techno-functional properties, flavour, and nutritional values to finally identify strains that effectively increase an umami taste, improve texture, and decrease antinutritional factors in pulse-based meat alternatives. These findings will highlight the potential of *Bacillus* spp. to enhance the quality of fermented food products, particularly in the development of sustainable and healthy meat alternatives.

Lactic fermentation of banana pulp for the production of functional ingredients.

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● In recent years, growing attention to healthy food has encouraged the development of innovative strategies for enhancing the nutritional value of food ingredients. In this context, the present study investigates the potential of banana pulp as a substrate to produce a functional ingredient through lactic fermentation. Three commercial bacterial strains, *Lactiplantibacillus plantarum* (P), *Lactobacillus brevis* (B), and *Weissella cibaria* (W) were selected for their potential probiotic and technological properties. The microorganisms were first characterized in vitro for their ability to produce exopolysaccharides (EPS), lipolytic activity, antifungal activity, and sugar metabolism. Single strains and their different strain combinations (PB, PW, BW, PBW) were used for fermentation of banana pulp puree under controlled aerobic conditions (37°C, 150 rpm) for 24 and 48 hours. Microbiological counts were performed to assess natural and starter LAB growth and the main banana contaminants in each fermented product. In addition, untargeted UHPLC-HRMS metabolomics analysis was applied. The results showed good adaptability of the LAB strains to the vegetable substrate, with *W. cibaria* being the only EPS producer and all strains showing antimicrobial activity and a reduction in pH. Metabolomic analysis revealed that fermented pulp samples with blends BW and PBW showed a significant qualitative and semi-quantitative variation in the bioactive compounds production, including flavonoids, biogenic amines, and organic acids, and suggesting a potential improvement in the nutritional and functional value of the final product. The results demonstrated that banana pulp fermentation represents a promising strategy for the development of innovative and health-promoting ingredients for the food industry.

Formulation-driven modulation of cheese-like fermentation in gelled pea-based matrices.

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● With the growing global population and worsening climate crisis, there is increasing demand for healthier and more sustainable food alternatives, particularly plant-based products. Legumes are promising candidates to replace animal proteins due to their nutritional and techno-functional properties (e.g., high protein content, water holding capacity, gelling, etc). However, their acceptability remains limited by sensory defects, notably "beany" off-flavors. Fermentation holds great potential to improve the sensory quality of plant-based foods, yet the fermentation process in such matrices remains poorly described. This study aimed to assess the effect of pea-based food matrices formulation parameters on a soft bloomy cheese-like ripening process. We developed a modular model system supporting variable protein, fat, and salt content, and structured by acidification using glucono-delta-lactone (GDL). We optimized the GDL-to-protein ratio to achieve a fast acidification to pH 4.6 and ensure product safety. *Penicillium camemberti, Geotrichum candidum,* and *Debaryomyces hansenii* were assessed as adjunct cultures for the ripening of gelled pea-based matrices. Preliminary experiments with two model formulations (10% or 20% protein, 10% fat, 0.55% salt) showed successful fermentation. pH changes in the core and rind during ripening were consistent with those of Camembert cheese (i.e., pH 4.5 to 4.9 in the core and up to 7.8 in the rind). Distinct volatile compound profiles were observed, suggesting that matrix formulation influences the fermentation process and, most probably, microbial colonization. Ongoing work combining multi-omics approaches, structure and texture characterization will help us to elucidate the relationships between matrix structure and microbial activity in pea-based fermented foods.

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Adapting starter cultures for fermentation of plant-based milk using laboratory evolution.

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● The increasing demand for plant-based dairy alternatives has driven substantial growth in plant-based milk products, which now comprise approximately 15% of the liquid milk market. However, plant-based yogurts have not achieved similar market share, constituting only 3.5% of the yogurt sector. Why do consumers readily adopt plant-based milks but not plant-based yogurts? Several reasons have been proposed for this disparity, including differences in protein composition, fat content, and carbohydrate profile. Here, we specifically address the carbohydrate differences, focusing on the availability and utilization of fermentable sugars critical for yogurt fermentation.

We use oat milk as a representative case, Oat milk is rich in starch and starch-derived carbohydrates such as glucose, maltose, and maltodextrin. Starter cultures traditionally optimized for lactose fermentation, like *Streptococcus thermophilus*, exhibit limited ability to ferment these alternative sugars. To overcome this limitation, we employed adaptive laboratory evolution to enhance the maltose fermentation capability of *S. thermophilus*. Initially *S. thermophilus* was unable to utilize maltose, but evolved strains demonstrated remarkable improved utilization of maltose when cultured under carbon-limited, maltose-enriched conditions. The resulting evolved *S. thermophilus* strains, when used together with *Lactobacillus bulgaricus*, exhibited faster and more robust acidification during oat milk yogurt fermentation, as well as increased final viable cell counts.

Our findings demonstrate the potential of adaptive laboratory evolution to generate non-GMO microbial starter cultures tailored specifically for enhancing fermentation performance in plant-based dairy products.

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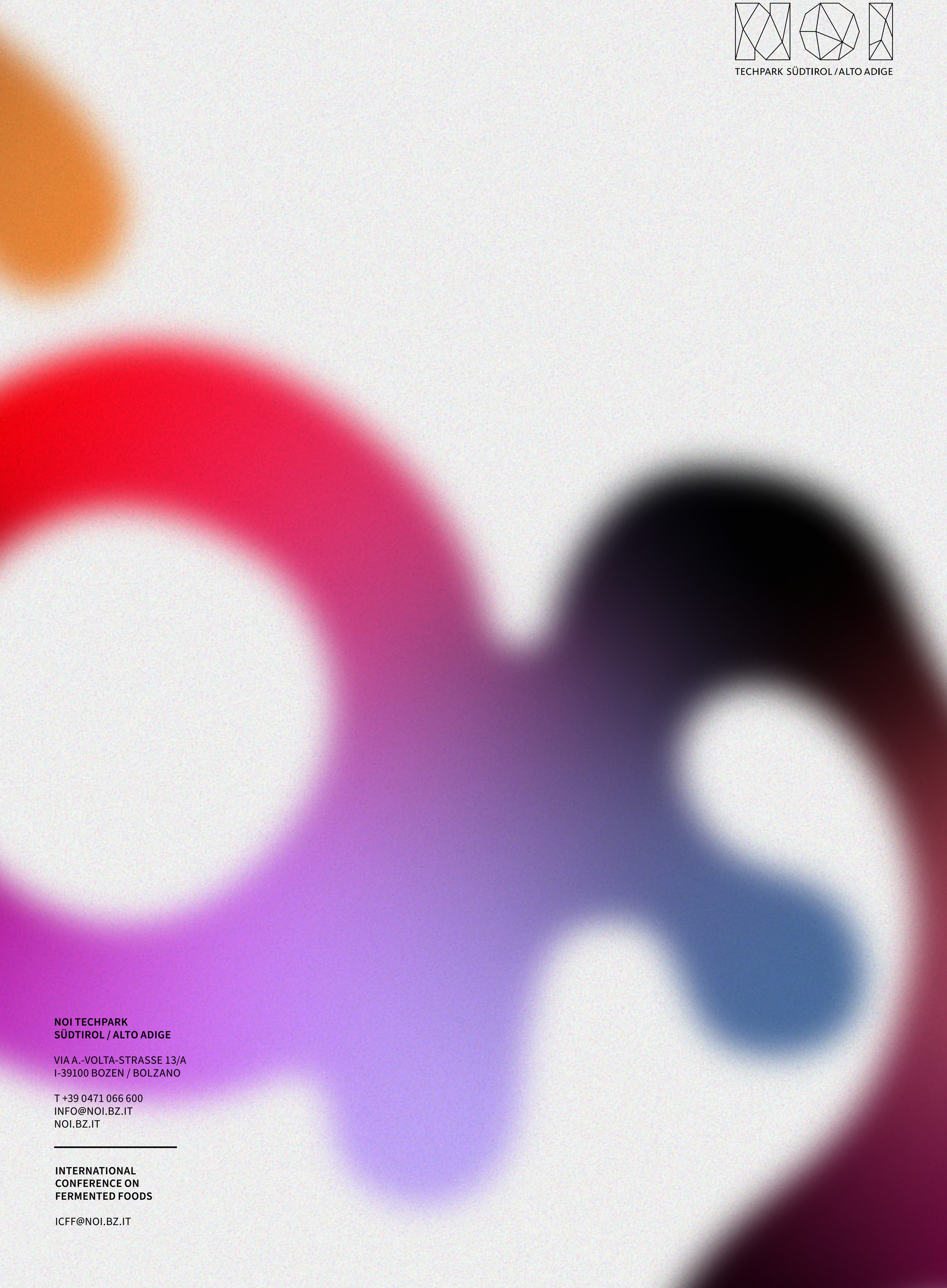

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