2nd Forum on Fermented Foods

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School of Medicine, University of Málaga

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SUMMARY

Session 1. Diversity of FF. Production and consumption

Session 2. Safety of Fermented Foods

Session 3. M4SF Cluster

Session 4. Health promoting effects of Fermented Foods

Session 5. Booster and hurdles for innovation in FF

Welcome to the second Forum on Fermented Foods

It is a great pleasure to welcome you to the 2FFF, an international conference taking place in beautiful Málaga, Spain. This event, organized by the COST Action PIMENTO, focuses on the promotion of fermented foods in Europe and represents the culmination of the Action. It brings together the European academic and scientific community, the project's initiatives, and the business world, showcasing the strong synergies that have been established.

Málaga, in Southern Spain, with its rich history, vibrant culture, and stunning Mediterranean setting, offers a wonderful backdrop for this important event. We hope you have a productive and enjoyable conference, and that you also take some time to experience the many delights Málaga has to offer.

Local organizer committee

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PIMENTO
Promoting Innovation of ferMENTed fOods

5th of February, Morning

08.00-09.00 **Registration**

09.00-09.10 **Ignacio SANTOS,** Dean of School of Medicine, UMA
Carmen GONZALEZ DOMENECH, Lecturer of School of Medicine, UMA
Christophe CHASSARD, INRAE, France
Welcoming

Session 1. Diversity of FF. Production and consumption

*Session Chairs. **Effie TSAKALIDOU** (AUA, Greece) and **Photis PAPADEMAS** (CUT, Cyprus)*

09.10-09.35 **Paul COTTER,** Teagasc, Ireland
Microbial diversity of fermented foods

09.35-10.00 **Photis PAPADEMAS**, CUT, Cyprus
WG2 results

10.00-10.25 **Emmanuella MAGRIPLIS**, AUA, Greece
Frequency Fermented Foods Questionnaire

10.25-10.40 **Chloé GAPP**, University of Lorraine, France and **Anna GRIZON**, INRAE, France
Top-down and bottom-up ecological engineering for the selection of autochthonous ferments for cheese production

10.40-10.55 **Vittorio CAPOZZI**, ISPA-CNR, Italy
Italian Fermented Foods with a Geographical Indication status. cheese, bread, table olive, semi-dry sausage, and wine

10.55-11.10 **Bastien RENARD**, PhD INRAE, France
One process applied to different cereals leads to specific microbial profiles. the case of traditional fermented couscous from Senegal.

11.10-11.25 **Baltasar MAYO**, IPLA-CSIC, Spain
Microbial diversity in the blue-veined Cabrales cheese by culturing and metataxonomics

11.25-11.55 Coffee break

11.55-12.10 **Òscar BORONAT NIELSEN**, CETT, Spain
Enhancing Food Aromas Through Novel Fermentations of Upcycled By-Products

12.10-12.25 **Marina CRETENET**, University of Caen, France
Is French cider a bad wine?

12.25-12.40 **Muge ISLETEN HOSOGLU**, Gebze Technical University, Turkey
Fermentation of microalgae as a way to increase the implementation of algae-based products in food industry

12.40-12.55 **Eugenia PERTZIGER**, Agroscope, Switzerland
Dietary Intake of Live Microorganisms and Fermented Foods in Swiss Adults

12.55-13.10 **Elif SEZER**, Sakarya University, Turkey
Incorporating Spore-Forming Probiotics and Dietary Fibers in Ice Cream Production

13.10-13.25 **Milan CERTIK**, STU, Slovakia
New types of fermented-based foods. challenge for fungal solid-state fermentation?

13.40-14.40 **Networking**
Networking Platform - One-to-one meetings
https://www.networking.fermentedfoods.eu

13.30-15.00 **Lunch and poster session**

5th of February, Afternoon

Session 2. Safety of Fermented Food

Session Chairs. ***Zuzana CIESAROVA*** *(NPPC, Slovakia),* ***and Carmen GONZALEZ*** *(UMA, Spain)*

15.00-15.25	**Kalliopi RANTSIOU**, University of Turin, Italy *Microbial safety aspects of fermented foods*
15.25-15.40	**Ricardo ASSUNCAO**, National Institute of Health Dr. Ricardo Jorge, Portugal *From Health Impact to Green Choices. The Dual Impact of Fermented Foods*
15.40-15.55	**Daniela NIKOLOVSKA NEDELKOSKA**, University St. Kliment Ohridski-Bitola, North Macedonia *The impact of fermentation on acrylamide levels and bioactive profile of pumpkin and soft wheat composite bread*
15.55-16.10	**Zuzana CIESAROVA**, National Agricultural and Food Centre, Slovakia *Is fungal solid-state fermentation a safe method for food valorization in terms of acrylamide formation?*
16.10-16.25	**Bojana BOGOVIC MATIJASIC**, University of Ljubljana, Slovenia *New insights into the safety of fermented dairy products by analysing resistomes*
16.25-16.55	*Coffee break*
16.55-17.10	**Marta LARANJO**, UEVORA-MED|CHANGE, Portugal *Farm-to-fork transmission of antimicrobial resistance genes in fermented pork sausages*
17.10-17.25	**María QUINTANILLA-CARVAJAL**, Universidad de La Sabana, Colombia *Antimicrobial Activity of Fermentation Metabolites Produced by Limosilactobacillus fermentumK73 Isolated from Costeño Cheese Against Shigella spp., Escherichia coli, and Salmonella spp.*
17.25-17.40	**Abdelkader BOUTINE**, DOMCA, Spain *Revolutionizing Food Safety. Breakthroughs in Bacteriocinogenic Ferments from Spontaneously Fermented Mediterranean Foods*
17.40-17.55	**Marion DALMASSO**, University of Caen, France *Exploring the unknown. are phages present in cider?*
17.55-18.10	**Monica GANDIA**, University of Valencia, Spain *BEFERMED project. Development of fermented beverages from local Mediterranean plant-based raw materials*
18.10-18.50	**Networking** Networking Platform - One-to-one meetings https://www.networking.fermentedfoods.eu

6th of February, Morning

Session 3. M4SF Cluster

*Session Chairs. **Marta LARANJO** (UEVORA-MED|CHANGE, Portugal) and **Vittorio CAPOZZI** (ISPA-CNR, Italy)*

09.00-09.25 **Orla O'SULLIVAN**, Teagasc, Ireland
Fermented foods, their microbiome and its potential in boosting human health

09.25-09.40 **Kristin VERBEKE**, Faculty of Medicine, Belgium
HEALTHFERM project

09.40-09.55 **Stéphane CHAILLOU**, INRAE, France
DOMINO project

09.55-10.10 **Ricardo DIAS**, University of porto, Portugal
WHEATBIOME project

10.10-10.25 **Angela SESSITSCH**, Austrian Institute of Technology, Austria
MICROBIOMES4SOY project

10.25-10.40 **Tanja KOSTIC**, Austrian Institute of Technology, Austria
MICROBIOME project

10.40-11.10 Coffee break

11.10-13.00 Round table

Health - Future European Projects on FF

13.40-14.40 **Networking**
Networking Platform - One-to-one meetings
https.//www.networking.fermentedfoods.eu

13.00-14.30 Lunch and poster session

6th of February, Afternoon

Session 4. Health promoting effects of Fermented Foods

Session Chairs: ***Guy VERGERES*** *(Agroscope, Switzerland) and* ***Smilja TODOROVIC PRACER*** *(Institute of Biological Research, Serbia)*

14.30-14.55 **Robert HUTKINS**, University Of Nebraska, U.S.A
Dietary microbes. Persistence in the gut and impact on health

14.55-15.20 **Guy VERGERES**, Agroscope, Switzerland
PIMENTO WG3 - Summary of the activities, results to date, and short-term outlook

15.20-15.35 **Arghya MUKHERJEE**, Teagasc, Ireland
The impact of consumption of live dietary microbes on health. a scoping review

15.35-15.50 **Isabel MORENO-INDIAS**, IBIMA-Bionand Platform, Spain
Effect of Moderate Consumption of Different Phenolic-Content Beers on the Human Gut Microbiota Composition. A Randomized Crossover Trial

15.50-16.05 **Jekaterina KAZANTSEVA**, AS TFTAK, Estonia
The Effect of Fermented Vegetables on Human Health and Gut Microbiota

16.05-16.35 Coffee break

16.35-16.50 **Isabelle SAVARY-AUZELOUX**, INRAE, France
Development of a fermented food with validated beneficial impact on health. a case study with a cheese including a Lacticaseibacillus casei capable to enhance muscle insulin sensitivity

16.50-17.05 **Cornelia BÄR**, Agroscope, Switzerland
A new strategy for the development of functional fermented foods to support human health

17.05-17.20 **Konstantinos PAPADIMITRIOU**, Agricultural University of Athens
A multi-omics approach to study different Greek PDO fermented foods and wines

17.20-17.35 **Jonna KOPER**, Lesaffre Institute of Science and Technology, France
In vitro human gastrointestinal digestibility and colonic fermentation of whey, casein and edible yeast-based proteins

17.35-17.50 **Vincenzo VALENTINO**, University of Naples Federico II, Italy
Grana Padano and Parmigiano Reggiano cheeses microbiomes exhibit neuroactive potential and bioprotective activities

18.10-18.50 **Networking**
Networking Platform - One-to-one meetings
https.//www.networking.fermentedfoods.eu

7th of February, Morning

Session 5. Booster and hurdles for innovation in FF

*Session Chairs: **Marie-Christine CHAMPOMIER-VERGES** (INRAE, France) and **Magali CORDAILLAT-SIMMONS** (UCA, France)*

09.00-09.15	**Marie-Christine CHAMPOMIER-VERGES**, INRAE, France *PIMENTO WG4 - Federating scientists and Fermented Food producers to boost innovation for society*
09.15-09.40	**Magali CORDAILLAT-SIMMONS**, Université Clermont Auvergne, France *First lessons from a survey on bottlenecks for innovation in fermented food sector in COST countries*
09.40-10.10	**Antonio DEL CASALE**, Microbion, Italy Round table - *Towards an European Fermented Foods cluster initiative*
10.10-10.25	**Luca COCOLIN**, University of Turin, Italy *The challenge of microbiome preservation and storage*
10.25-10.50	**Bruno POT**, Yakult Europe, Netherlands *The (future) fate of "dietary microbes"! Is "dietary" the same as "challenging"?*
10.50-11.10	Coffee break
11.10-11.40	**Round table** - Multi-stakeholders *Discussion on 'Dietary Microbes'*
11.40-12.05	**Biljana TRAJKOVSKA**, UKLO, North Macedonia, **Elena GERBAUD**, INRAE, France and **Kathryn PIMENTEL-BURTON**, Agroscope, Switzerland *WG1 strategy*

Innovation awards

Chair : **Antonio DEL CASALE**, MICROBION, Italy

12.05-12.35	**Pitch communications**
12.35-12.45	**Votes & Awards ceremony**

Closing session

12.45-13.00 **Christophe CHASSARD**, INRAE, France and **Juana FRIAS**, ICTAN-CSIC, Spain
Closing remarks

13.10 **Lunch and poster session**

13.40-17.00 **Networking**
Networking Platform - One-to-one meetings
https://www.networking.fermentedfoods.eu

Session 1. Diversity of FF. Production and consumption

Plenary Lecture

Paul COTTER, Teagasc, Ireland

Microbial diversity of fermented foods

Revealing the microbial diversity of fermented foods can facilitate new innovations through the identification and harnessing of novel strains and activities, while also facilitating the establishment of new regulations relating to the microbial composition of specific foods. This presentation will summarise some recent developments with respect to understanding the microbial composition of a broad variety of different fermented foods as well as parallel investigation to more thoroughly characterise specific fermented products, such as milk kefir and water kefir. This information represents a significant resource that can be harnessed by academia and industry for many years to come.

WG2 Cartography of the current place of FF in the diet of COST Actio Countries

Effie Tsakalidou[1] and Photis Papademas[2]

[1]Department of Food Science and Human Nutrition, Agricultural University Athens, Department of Agricultural Sciences, Biotechnology and Food Science

[2]Cyprus University of Technology

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Abstract

The Working Group 2 of the PIMENTO Cost Action aims to produce a cartography of the current place of Fermented Foods in the diet of COST Action Countries. The data collected by 15 countries (i.e. Cyprus, Estonia, Finland, France,Greece, Italy, Lithuania, Morocco (Maghreb), North Macedonia, Portugal, Serbia, Slovenia, Spain,Switzerland and Turkiye) is presented and critically discussed while the difficulties encountered in data collection and the opportunities for future work are also mentioned. Dairy products (i.e. cheese,fermented milks, yoghurt) seem to be the driver of the fermented food sectors, while the cereal-based products (i.e. bread) are equally important. In the near future, it seems that the plant-based products(including legumes), non-alcoholic, non-dairy fermented products as well the fermented tea sectors will become even more important, globally, mainly because of their reported health benefits and sustainable production processes.

Mapping Fermented Food Intake: Development of the Fermented Foods Frequency Questionnaire (3FQ)

E. Magriplis[1], T. Smiliotopoulos[1], S. Kotopoulou[2], N. Myrintzou[1] and M. Syrpas[3]

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[3]Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19A, 50254 Kaunas, Lithuania

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Abstract

Fermented foods, produced through the action of microorganisms, o6er a diverse rangeof products with potential health benefits. Assessing these benefits is challenging due tovarious fermented foods and regional consumption patterns.

Europe lacks specific consumption recommendations through a PIMENTO sub-projectwe aimed to explore.

This presentation introduces the study on mapping the Fermented Foods Frequency Questionnaire (3FQ), a tool designed to quantitatively estimate the frequency of fermented food intake across four European regions. The 3FQ comprehensively assesses the frequency and quantity of fermented food intake across all main food groups,addressing a crucial gap in research. The tool targets fermented foods, minimizingsystematic errors common in general food questionnaires. The tool is now beingvalidated for accuracyand repeatability, using robust statistical analysis techniques, andthe main results are presented.

Main findings suggest that 3FQ provides accurate and comparable data across EuropeanRegions, essential for future research on the potential associations between fermentedfood intake and health outcomes also paving the way to specific recommendations.

Top-down and bottom-up ecological engineering for the selection of autochthonous ferments for cheese production

C. Gapp[1*], A. Grizon[2*]

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Abstract

Microorganisms play essential technological roles in cheese production, particularly in milk acidification. To achieve optimum control over production, cheesemakers use selected ferments. However, these ferments are mostly generic, as they are used by many cheesemakers in many countries and continents, and are therefore disconnected from the terroir. In the past decade, significant research were dedicated to developing autochthonous ferments as alternatives to generic ones. However, empirical approaches were mostly used, often overlooking key biotic factors. The principles of ecological engineering can be used to develop a new generation of autochthonous ferments. Different engineering approaches can be employed: the top-down approach based on the application of environmental pressures to select adapted communities, and the bottom-up approach, which consists of assembling isolated microorganisms based on the knowledge of their ecology to obtain a microbiome with desired functions. Each of these approaches has both advantages and disadvantages, and both approaches can be used in a combination for an integrated design of autochthonous ferments. Combining these approaches in the -omics era enables the rational assembly of microbial communities, considering both biotic and abiotic factors of ecosystems.

Italian Fermented Foods with a Geographical Indication status: cheese, bread, table olive, semi-dry sausage, and wine

V. Capozzi[1*], M.L. de Chiara[1], G. Spano[2], M. Fragasso[2]

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Abstract

Italy has a significant gastronomic heritage with a large number of Geographical Indications. Many of these products are fermented, including cheese, bread, table olive, semi-dry sausage, and wine, representing the diversity of traditional Italian foods transformed through a desired development of yeasts, bacteria and filamentous fungi in the raw matrix. Here, we propose an overview of the variability of these foods and beverages in terms of categories and geographical distribution, providing information on the valorization of biodiversity (plants, animals and microorganisms), the technological regime used and the management of microbial resources. This diversity is observed through the scientific literature, proposing a picture of research activities that promote innovation in this sector, in line with trends oriented towards improving the quality and safety of production. In a sector that is intrinsically oriented towards environmental, economic, and social sustainability, a particular interest has been given to the reduction of 'footprints', also throughout the valorisation of microorganisms [1]. Selecting tailored case studies, a special discussion is dedicated to the selections and characterisations of bacteria, yeasts and filamentous fungi associated with these productions and to the emerging trends that combine the valorisation of microbes/microbiomes with the protection and promotion of traditional, typical and artisanal fermented foods [2,3]. Finally, an analysis of numerical evaluations, an overview of stakeholders involved and future perspectives are proposed to support evidence related to the emerging trends in the field. Particular emphasis is given to *i)* fermented foods as elements for the design of diets with a high content of microorganisms and *ii)* the importance of culture collection to support knowledge and innovation in the field [4].

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One process applied to different cereals leads to specific microbial profiles: the case of traditional fermented couscous from Senegal.

B. Renard[1], J. Tap[2], V. Chochois[3, 4], J. Balland[1], P. Tisseyre[1], C-T. Willane[1, 5], A. Diouf[5], M-C. Champomier-Vergès[2], C. Humblot[1*]

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Abstract

Spontaneous fermentation is a process widely used around the world for millennia. Although studies have characterized the microbiota and the impact of fermentation on the nutritional composition of several traditional cereal-based fermented foods, they have not yet studied fermented couscous, a staple food in Senegal, made from a variety of cereals.

Thus, this study aims to characterise the microbial composition of such fermented couscous from Senegal and the effect of the fermentation on their nutritional qualities.

Forty samples from flours of different cereals (sorghum, maize, millet, and a mix of maize and millet) were collected in Senegal before and after fermentation (flours moistened into small balls, fermented for 8 hours). We analysed microbial diversity and metabolic functionalities using shotgun metagenomic sequencing. We also measured vitamin B9 content, as fermentation can significantly alter its levels.

Each flour had a specific microbial diversity, with maize flours exhibiting the lowest diversity. *Lactobacillaceae*, especially from the genus *Weissella*, were in high proportions in all flours. Millet flour contained potentially pathogenic bacteria from *Enterobacteriaceae* family. Surprisingly, besides the expected presence of *Lactobacillaceae* after fermentation, a low proportion of species from *Acinetobacter* genera, previously undetected in flours, appeared. We detected only species of yeasts from the *Saccharomycetales* order in 28% of flour samples.

Despite the presence of potential folate-producing microbes, vitamin B9 content decreased during the fermentation. Analysis of functions from metagenomic data will help better understand the metabolic interactions within the traditional microbial food ecosystem and help to know more about their potential nutritional properties.

Acknowledgments

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Microbial diversity in the blue-veined Cabrales cheese by culturing and metataxonomics

Baltasar Mayo[1,2], Javier Rodríguez[1,2], Paula R. Suárez[1], Souvik Das[3], Lucía Vázquez[1,2], Sonam Lama[3], Jyoti Prakash Tamang[3], and Ana Belén Flórez[1,2]

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Abstract

By culturing and metataxonomic analyses, two and 16 long-ripened, high-quality Cabrales cheeses from independent producers were subjected to a complete microbiological characterization, which included both prokaryotic and eukaryotic populations. The microbial diversity detected by molecular and culturing methods was then compared and discussed. In general, the two methods returned similar results in terms of diversity, albeit with notable numerical differences. A wide microbial diversity, including 44 and 52 phylotypes of bacteria and 51 and 43 phylotypes of yeasts and moulds, was detected by culturing and molecular techniques, respectively. Further, molecular and culturing analyses showed the cheeses to be microbiologically safe for consumption. *Tetragenococcus* and *Staphylococcus* species constituted the majority of bacteria, followed by *Brevibacterium* and *Corynebacterium* species; these organisms were shown to be abundant by both molecular and culturing techniques. In contrast, lactic acid bacteria species, of which *Lactococcus lactis* was used as a starter, were only detected by the metataxonomic analysis, suggesting they were in a non-cultivable state. The starter *Penicillium roqueforti* was the most abundant eukaryotic organism in all the studied samples, followed by the yeast species *Pichia norvegensis*, *Debaryomyces hansenii*, *Geotrichum candidum* and *Yarrowia lipolytica*. The knowledge gathered in this work may serve to develop synthetic mixtures, which may act as robust microbiota-based starters that could contribute towards increasing the overall quality of Cabrales cheese.

Acknowledgements

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Enhancing Food Aromas Through Novel Fermentations of Upcycled By-Products

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Abstract

Every year, around 190 million tons of by-products from the agri-food industry are discarded due to their low commercial value.[1] To address this issue and reduce food waste, researchers and businesses are increasingly exploring innovative upcycling technologies. Among these, novel fermentation techniques have shown great potential.[2]

Our research focuses on the fermentation of various local by-products, including okara, tiger nut co-product, and brewer's spent grain. Given their distinct compositions, specific filamentous fungi – *Aspergillus oryzae*, *Rhizopus oryzae*, *Rhizopus oligosporus* and *Neurospora sitophila*- and tailored environmental conditions were employed for each substrate to develop new textures and flavors. Two-stage fermentations, such as miso-making, were carried out to evaluate interactions between solid substrates and the spontaneous microbial communities.

Preliminary results revealed the formation of diverse volatile compounds during secondary metabolism and palatable profiles in both texture and taste. Aromatic compounds, often associated with desirable food aromas such as fruity, floral, and olive-like notes, were absent in the raw substrates. This process not only enhances the sensory appeal of the by-products but also opens the door to create plant-based products that are nutritious and without the need to use artificial flavorings nor texturizing agents.

This research highlights the potential of solid-state fermentation as a powerful tool for reducing food waste while creating nutritious, appealing, and sustainable food products.

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Is French cider a bad wine?

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Abstract

Cider is a fermented beverage whose production practices vary enormously from country to country. However, it can be simply defined as a fermented alcoholic beverage made from apple juice. The wide disparities in production conditions make it a less homogenous fermented product than wine and an opportunity for researchers to dive into its microbial diversity. Unlike wine, production methods remain largely traditional without the addition of ferments, thus preserving spontaneous dynamics of microbial communities, particularly in cider for distillation. Physicochemical analyses, diversity analyses by high-throughput sequencing (bacteria and yeasts) and aromatic analyses were carried out on 10 cider fermentations at three different times. Metabarcoding analysis revealed the presence of 40 bacterial and 48 fungal genera in cider for distillation (Misery et al., 2021). Microbial community composition was shown to be specific to each producer. The study of the microbiota of those ciders together with chemical and aromatic profiles revealed a correlation between bacterial communities, especially lactic acid bacteria, and the aromatic profiles of cider distillates. Isolations of lactic acid bacteria from cider also revealed two new species of *Oenococcus sp* (Cousin et al., 2019). These studies show that the microbial ecology of ciders is complex, comprising several genera, species and strains of yeasts and bacteria. The sensory profile of cider is significantly associated with microbial activity and indigenous microorganisms can actively contribute to the expression of cider typicity. The role of lactic acid bacteria (LAB) in these products is still to be further investigated.

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Fermentation of microalgae as a way to increase the implementation of algae-based products in food industry

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Abstract

Microalgae biomass can be considered a very attractive source for creating sustainable, healthy food formulations. Becoming the most produced microalgae biomass, *Spirulina* stands for its highest protein content, balanced amino acid composition and bioactive properties. Fermentation of *Spirulina* biomass could be a process that can be used to enhance the flavor, nutritional as well as bioactive properties of the biomass through the metabolic activities of several microorganisms. This study presents researches describing to produce fermented *Spirulina* (FS) products, including the screening of several lactic acid bacteria, *Bacillus* and yeast species capable of making desired changes (Sahin B et al., 2022; Kurt et al., 2023), manufacturing process steps including flask and bioreactor level optimizations, revealing the changes in chemical compound, flavor profile and bioactive properties of FS and unfermented *Spirulina* (unFS) products (Yay et al., 2024). The effects of FS and unFS protein hydrolysate fractions, and also the *in vitro* digestion products on the cell viabilities of the HT29 and HUVEC cells were also determined. Additionally, their anti-inflammatory activities were revealed by on RAW264.7 cell line. LC-Q-TOF analysis identified 33 bioactive peptides in RP-HPLC Fraction 1 and 71 in Fraction 2, exceeding 50% PepNovo Score. BIOPEP-UWM database screening revealed notable activities, including ubiquitin-mediated proteolysis activation and prolyl endopeptidase inhibition, relevant for cancer therapy. While the industrial use of FS is still in its early stages, the enhancements in flavor, chemical composition, and bioactive properties observed in this study highlight the promising potential of FS for future applications in the food industry.

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Dietary Intake of Live Microorganisms and Fermented Foods in Swiss Adults

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Abstract

Foods with live microorganisms and fermented foods may be beneficial for human health, but their overall consumption in population-based studies is not well-defined. This study aimed to categorize and estimate the intake of foods with live microorganisms and fermented foods among Swiss adults. Data from the Swiss National Nutrition Survey menuCH (2014-2015) on 2,086 adults (ages 18-75) were used to classify foods by microorganism levels (low, medium, or high) and fermentation characteristics, including fermentation status, fermented ingredients, and core microbiota. We analyzed intake by population groups, food groups, and nutrient contributions. Swiss adults had a mean daily consumption of 269 g (8% of total intake) of medium- or high-microorganism foods, mostly from fruit, vegetables, and fermented dairy, accounting for 12% of daily energy intake and over 20% of nutrients such as vitamins A, C, B12, folate, calcium, and saturated fat. Fermented foods contributed a mean daily intake of 717 g (21% of total intake), mostly from coffee, bread, alcoholic beverages, and fermented dairy, contributing 27% of daily energy and over 30% of nutrients such as calcium, zinc, sodium, vitamins A and B12, and saturated fat. The intake of medium- or high-microorganism foods differed by sex and age, with no differences by linguistic region, while fermented food intake varied across all demographic groups. We identified 186 distinct microorganisms in fermented foods. This study comprehensively classified and quantified the consumption of live microorganisms and fermented foods in Switzerland, providing a foundation for future assessment of their health effects.

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Incorporating Spore-Forming Probiotics and Dietary Fibers in Ice Cream Production

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Abstract

This study examines the effects of incorporating milk fermented with dietary fibers (wheat fiber and inulin) and spore-forming probiotics (*Shouchella clausii* and *Heyndrickxia coagulans*) on ice cream quality over a 90-day storage period at -25°C. Two ice cream bases were developed: a fermented milk mix with added dietary fiber and probiotic culture and a traditional ice cream base. The final product combined these bases to create a functional ice cream formulation. The analyses included microbial viability, spore counts, sensory attributes, and physical and chemical properties. Results showed that ice cream containing inulin and *S. clausii* maintained the highest probiotic viability, with counts above 8 log CFU/g throughout storage. *S. clausii* exhibited more excellent stability than *H. coagulans* at all time points ($P < 0.05$). Sensory evaluations indicated no statistically significant differences among samples regarding color and appearance, structure and consistency, taste and smell, and general acceptability ($P > 0.05$), with consistently high scores across these attributes. In the 9-point evaluation, taste and smell ratings ranged from 6.96 to 7.52, while general acceptability scores were between 7.41 and 7.71. These results suggest that incorporating dietary fibers and spore-forming probiotics effectively preserved sensory quality, ensuring consistency in taste and overall acceptance. Dietary fibers contributed to increased viscosity and reduced overrun, with wheat fiber also enhanced hardness ($P < 0.05$). Overall, these results suggest that spore-forming probiotics and dietary fibers can effectively enhance the functional properties of ice cream without compromising sensory quality, providing a promising approach for probiotic-enriched frozen desserts.

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New types of fermented-based foods: challenge for fungal solid-state fermentation?

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Abstract

Cereals and legumes, as major components of the human diet, are important sources of several macronutrients (proteins, carbohydrates) and micronutrients (minerals, vitamins), but they are limited in several biologically active compounds. The increasing commercial demand for naturally enriched cereals and legumes with bioactive compounds has led to the development of accessible biotechnological methods. Attention has been focused on solid-state fermentation (SSF) processes in which Zygomycetous filamentous fungi (*Thamnidium* sp., *Cunninghamella* sp., *Mucor* sp., *Umbelopsis* sp., *Mortierella* sp.) are able to effectively utilize and transform cereals and legumes into fermented bioproducts containing polyunsaturated fatty acids (PUFAs), fungal sterols, carotenoid pigments, coenzyme Q, glycolipids, dietary fibers, various enzymes and amino-polysaccharides. Fungi also improved content of dry matter, proteins and ash in fermented products while reducing levels of anti-nutrient compounds (e.g. phytic acid, precursors of acrylamide). A number of fermented cereal-based bioproducts have been prepared and successfully used for the production of cereal-based products (e.g. rolls, bread and pasta). Application of these new biomaterials not only enriched breads with new bioactive compounds, but also changed the rheological and nutritional properties of the dough (increased water absorption, decreased dough rise, prolonged dough development time, higher dough softening degree) and sensory properties of the final cereal products (brownish color, crispiness, fungal flavor). The quality of the prepared breads was acceptable to consumers in sensory tests. These tailor-made bioproducts with improved nutritional and functional properties represent a challenging and potentially rewarding subject for preparation of novel fermented foods with required nutritional design.

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Effect of fermentation on cultured coffee cell cultures

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Abstract

Recently, plant cell cultures have been suggested as more sustainable alternatives for conventional agriculture products. For example, biomass from cell cultures may reduce the need for land, water and/or other resources compared to products from conventional agriculture. However, biomass from cell culture does not necessarily have all desired properties for the intended end use. Typically, biomass from cell cultures differs from conventional agricultural counterparts for example in their structure, appearance and/or flavor.

There is hence a need to find ways to process e.g. by fermentation biomass from cell culture to improve or modify its properties with the view in intended end use for example in food and/or beverage applications.

Therefore, we examined bioprocessing and fermentation of cultured Coffea arabica cells. Selected lactic acid bacteria and yeasts alone and in combination were used for fermentation of the coffee cell material. Fermentation was monitored by following growth of the inoculated starter cultures and by formation of volatiles with GC-MS. Sensory analysis was performed for selected hot brewed coffee drink samples.

Overall, this study shows that the fermentation increased flavor compound production in the cultured coffee cells.

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Fermentation and debittering of grapefruit juice

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Abstract

Grapefruit juice can serve as a substrate for the growth of probiotic bacteria. Lactic fermentation of fruit juice produces "functional food" rich in bioactive compounds such as fiber, oligosaccharides, and beneficial bacteria that support intestinal microflora. However, the bitterness of citrus juices, caused by naringin, reduces consumer acceptance. Debittering can be achieved by hydrolyzing naringin using naringinase, an enzyme complex with α-L-rhamnosidase and β-D-glucosidase activities. Naringin can be hydrolyzed to rhamnose and prunin by α-L-rhamnosidase; afterward, β-D-glucosidase catalyzes the hydrolysis of prunin to glucose and tasteless naringenin.

This study aimed to develop a simultaneous process for bitterness removal and probiotic grapefruit juice production through combined fermentation using naringinase and probiotic bacteria.

Grapefruit juice was fermented with a monoculture of *Lactobacillus* casei subsp.rhamnosus. Both fresh juice (pH 2.74) and juice adjusted to pH 5.0 with 1M Na_2CO_3 were tested. The juice was enriched with a naringinase preparation from *A. niger* and fermented at 30°C for 4 days, resulting in a 49% reduction in naringin concentration. After fermentation, cell counts increased to approximately 2×10^6 bacteria/mL. Antioxidant activity slightly decreased, but the changes were insignificant, while pH dropped from 2.74 to 2.71 in fresh juice and from 5.0 to 4.47 in neutralized juice. This result is promising for the development of technology for the production of probiotic grapefruit juice.

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Effect of dairy and fermented food diet on the urinary metabolome of youn and elderly men – a controlled, crossover intervention study

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Abstract

Molecular biomarkers can complement dietary questionnaires through an objective quantification of food intake. The validation of biomarkers of food intake requests that the impact of extrinsic (e.g., food transformation) and intrinsic (e.g., human metabolism) factors be controlled. To investigate how food fermentation and consumer age impact on markers of dairy intake, a randomized controlled crossover intervention study was conducted in 14 young and 14 old men. Acute changes in the urinary metabolome after intake of milk and yogurt were measured using three metabolomic platforms. The impact of age on the postprandial response was also determined. The specificity of the urinary markers was further evaluated with a 19-d dietary restriction phase preceding the postprandial test during which dairy and fermented food consumption was reduced. Profiling of the metabolome revealed a large number of features whose urinary concentration was modified by the milk fermentation status or age. Molecules known to be present in dairy products, such as lactose and its metabolites or the aldehydes heptanal and nonanal, were among the 31 postprandially active metabolites identified. The validity of these candidate markers of dairy intake should be further investigated, eventually using multi-markers panels, under real life situation in observational cohorts.

The nutritional composition of main fermented foods from Senegal

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Abstract

In Senegal, fermented foods are very diverse, derived from animal and vegetable products and consumed as condiments or staples foods. They can contribute significantly to daily nutritional intakes but their nutritional composition and the microbial actors of fermentation have never been described.

The aim of this study was thus to estimate the nutritional quality of all fermented foods consumed in Senegal as well as to describe their microbiota.

We identified 34 fermented foods from the data of national consumption surveys. Nutritional composition of these foods was obtained using food composition table for West Africa and Mali based of 100g edible portion on fresh matter.
They were mainly of animal origin (71%). Many of them were prepared from fish products (47%), milk and cereals (23% each) and only a few from pulses and meats (<1%). Mineral content was highly variable with cheese and netetu (from legumes) rich in calcium and zinc. Data on vitaminic content of most fermented foods was lacking with the exception of fermented milk products such as butter, which contained interesting amounts of vitamin A (864µg) but little amount of vitamin B9 (5µg) and vitamin B12 (0,16µg). Analysis of microbial actors of fermentation is ongoing.

Nutritional quality of Senegalese fermented foods needs to be revisited, especially for micronutrients affected by fermentation such as vitamin B. Microbial composition analysis of these fermented foods will help to better understand the role of microorganisms on the nutritional quality of these foods. Nutritional impact of fermentation should also consider the consumption levels.

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The MetaOlive project: meta-omic approaches for traditional table oliv fermentation

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Abstract

The microbiota of table olives and their brines (4 to 8% NaCl) is usually dominated by lactic acid bacteria (mostly *Lactiplantibacillus* spp.) and yeasts (e.g., *Saccharomyces cerevisiae, Candida boidinii, Wickerhamomyces anomalus*) which are able to stand the highly selective conditions (high NaCl and phenols, low nutrients) and cause desirable changes but their role is not always clear-cut. The MetaOlive project aims to:

- Create the widest and most comprehensive map of the microbiome and volatilome of commercial table olives in the Mediterranean area;
- Understand spoilage dynamics;
- Carry out an in-depth analysis of the sources of contamination and of the microbial dynamics during fermentation and storage of the Italian PDO "Oliva di Gaeta", identifying the main reasons of the ecological success of beneficial microorganisms during olive fermentation and highlighting genes and functions relevant for the fermentation and for successful interactions.

More than 300 samples from different countries (Italy, Spain and Greece) were collected and a meta-ohmic approach was used to have a full characterization. Preliminary results show that *L.pentosus* is the main current species among acid lactic bacteria. Yeasts, such as *C.boidinii* and *W.anomalus,* are strictly correlated with product's alteration. The outputs of MetaOlive project will contribute to the understanding of key ecological processes in microbial ecology of table olive (e.g., contamination, dispersal, selection) and to the knowledge of the genetic determinants of the success of microbial strains in olive fermentation. The results will also be valuable for stakeholders potentially contributing to the identification of markers for olive varieties.

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Drivers of dairy farm microbiomes under changing climate and farmin practices, and implications for the microbiome of raw milk cheese

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Abstract

A better understanding of the driving forces behind microbiota in the food chain is essential to address the microbiological quality of fermented foods and global health issues. Evolving agricultural practices aimed at mitigating the effects of climate change raise questions about their impact on the microbial and sensory characteristics of the food matrix, which is particularly relevant for raw milk cheese production. To address these issues, we explored microbial transfers across dairy farm environment to raw milk cheese, in four farming systems with varying grazing proportion in cow diet, in relation with the characteristics of the derived cheeses. The bacterial and fungal communities in 736 samples collected from soil to cheese were characterised by 16S rRNA and ITS genes high-throughput sequencing. Dissimilarities in microbiota composition according to farming system were assessed using Bray-Curtis distance.

Microbial transfers were analysed based on Amplicon Sequence Variants (ASVs) shared between different ecosystems in the food chain. Cheeses from cows fed the more grazed grass were the yellowest and had the most pronounced flavors, in line with their higher counts of heterofermentative lactobacilli and lactic acid bacteria. These differences could be related to the microbial community profiles along the food chain from soil to cheese, where 12-28% of the variance was explained by farming system. Diversity hotspots for downstream microbiomes were identified, as animal bedding material, with cascading effects into the microbiota of cheeses. Ongoing analysis of shotgun metagenomic data will enable more precise identification of functional responses and of microbial transfers at the intra-species level.

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From Tradition to Trend- Exploring Albanian Students' Perceptions of Fermented Foods

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Abstract

Fermented foods represent a promising intersection between health and sustainability, appealing to a growing demographic of environmentally conscious consumers (Florence et al., 2022; Saint-Eve et al., 2021; Vinderola et al., 2023). This study examines young adults' preferences for the health and sustainability aspects of fermented foods in Albania, focusing on 18–24-year-old students at the Agricultural University of Tirana. As a critical demographic shaping future agricultural policies and consumer trends, their knowledge, perceptions, and behaviours offer valuable insights into adopting healthier and more sustainable dietary habits. One hundred eighty students participated in an online survey. Most respondents (32.1%) study food science, biotechnology, and veterinary and agriculture sciences (16.1%, respectively). Most respondents believe fermented products most positively impact digestive health (46%) and immunity (45.1%), followed by energy and vitality (41.6%). Fewer associate them with weight management (29.2%) or mental health and well-being (21.2%**.** The most frequently consumed fermented product among respondents is yoghurt (22.1%)**,** followed by pickled vegetables (11.5%)**,** while other products like kefir and cheese are less frequently consumed. Finally, we analysed the framing effect on students' perceptions of fermented foods, comparing their importance as "natural substitutes for health supplements" and "less expensive alternatives to supplements." Results showed stronger agreement with the health-focused framing, indicating that emphasising natural health benefits is more effective than cost-based messaging in promoting fermented foods. Also, the mere exposure of the Albanian population to fermented foods, rooted in cultural heritage and shaped by limited historical food storage infrastructure, is reflected in students' consumption of these products and underscores the importance of connecting traditional practices with modern educational campaigns to raise awareness and promote the health and sustainability benefits of fermented food.

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The effect of cold plasma on the bioaccessibility of phenolics in red cabbage pickles

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Phenolics have a multitude of health effects, but they should be bioaccessible and bioavailable to exert their health effects. Red cabbage is a good source of phenolics, particularly anthocyanins. The red cabbage pickles were prepared containing a commercial probiotic powder, which contains prebiotic fiber (inulin) and starter cultures (Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Leuconostoc mesetorides ssp. cremocis, Streptococcus diacetilactis). Prior to cold plasma treatment, the pickles were freeze-dried. In this cold plasma treatment, low pressure (0.1-1 bar) cold plasma was used to treat the samples. Freeze-dried pickles were subjected to 6 different cold plasma treatments. The power of all treatments was 100 W and atmospheric gas was used. These cold plasma treatments were 1, 15, 30 minutes without rotation and 1, 15, 30 minutes with rotation. The INFOGEST static in vitro digestion procedure was applied to both untreated and cold plasma treated freeze-dried pickles. We determined TPC and TAC in cold plasma treated and untreated red cabbage pickles, and the bioaccessibility of phenolics and anthocyanins in cold plasma treated and untreated red cabbage pickles. All cold plasma treatments increased the TPC and TAC of red cabbage pickles. The bioaccessibility of TPC was 8.23% for untreated red cabbage, all cold plasma treatments increased the bioaccessibility of TPC to values varying between 36.3-99.8%. The bioaccessibility of TAC in untreated red cabbage pickles was 1.82%, while cold plasma treatments with rotation increased it to varying values between 4.17-9.36%.

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The power of fermentation to transform fruit and vegetable juices

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Abstract

Non-alcoholic fermented beverages are gaining significant attention due to their ability to enhance gut microbiota, provide bioactive metabolites with health-promoting properties, and address the increasing demand for sustainable and nutrient-dense functional foods.[1] The application of fruit as substrates for lactic fermentation has the advantage of the incorporation of flavors and nutrients specific to each type of fruit, resulting in products with differential sensory and physicochemical characteristics, appreciated by consumers.[2]

In this study seven natural juices of apple (A), beetroot (B), carrot (C), and their combinations(AB,AC, BC, and ABC) were fermented. Fresh juices were inoculated with *Leuconostoc mesenteroides* subsp. *dextranicumhe* at an initial density of 5 × 10^7 CFU/mL, and the samples were incubated for 12 hours at 30 °C. Samples were analyzed pre- and post-inoculation. The study aimed to evaluate the impact of fermentation on key physicochemical properties,including cell viability, pH, total acidity, total soluble solids, color, total polyphenol content and,antioxidant activity to better understand how different juice formulations influence the fermentation process and final product quality.

The initial composition of the juices played a crucial role in determining the characteristics of the fermented products, resulting in distinctly different outcomes despite the use of the same microorganism. This study underscores the versatility of *Leuconostoc mesenteroides* subsp. *dextranicum*, highlighting its potential to produce a diverse range of non-alcoholic fermented products from various juice bases.

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Characterisation of Murciano-Granadina goat milk kefir

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Abstract

The Murciano-Granadina, Spain's most important dairy goat breed, originated in southeastern Spain by hybridising the Murciana and Granadina breeds in 1975. With over 500.000 milking females producing up to 500 litres each one annually, these medium-sized goats are valued for milk production and adaptability to arid, nutrient-poor regions1. Their milk is valued for cheese production and typically contains around 5.6% fat and 3.6% protein2. However, there are no studies of the bacterial communities inhabiting the Murciano-Granadina milk. Here, we study the bacterial composition of milk collected in six farms in the Campo de Cartagena region in May 2024 using Illumina sequencing. Additionally, we describe the population dynamics when fermented with commercial kefir grains and we correlate them with metabolite changes measured using LC-MS. This is, to the best of our knowledge, the first report characterising the bacterial composition of the Murciano-Granadina milk.

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Delving into the development of *Lachancea thermotolerans* x *Candida boidinii* (*Lt* x *Cb*) hybrids to improve table olive fermentations

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Abstract

A first screening was carried out to select several strains belonging to Lachancea thermotolerans (Lt) and Candida boidinii (Cb) species with the best technological characteristics. Briefly, resistance to salt, pH and oleuropein was assayed. Then, a hybridization of spores and vegetative cells of those selected strains (Lt x Cb) was performed to carry out a genetic improvement. Possible interspecific hybrids were selected from crosses of vegetative cells of BMA46, 61, 66, 228, 339 (Lt) and BMA17 (Cb) based on the genetic markers of each parent, resistance to cycloheximide (CYHR) of BMA17 and killer phenotype of all Lt strains. With these potential interspecific hybrids, technological characterization was carried out again to assess if they had a better performance than their parents. Although different hybrids displayed good resistance to all stressful conditions, only BMA61×BMA17-9, BMA228×BMA17-8 and BMA339×BMA17-4 improved significantly the behavior of the parental yeasts. The identity of the hybrids was analyzed by sequencing different regions of the rDNA, the cell morphology of the yeasts in vegetative state and their spores, and different genetic markers (Killer, CYHR, SO2R and CuR) present in the parental yeasts. All selected Lt × Cb hybrids showed typical cell morphology. However, all hybrids which did not show a killer phenotype were discarded. With all these hybrids, table olive micro-fermentations were carried out with high salt content (10%, p/v) and pH 8u. A strategy of co-inoculation was implemented in combination with a reference Lactiplantibacillus plantarum strain. BMA339×BMA17-4 showed the best fermentative process, even more than the control test with Wickerhamomyces anomalus, achieving microbial safety conditions in less time than the other yeasts.

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Multiobjetive Optimization of Lactic Fermentation of Murta Juice: An Innovative Approach with Lactobacillus Monocultures and Cocultures

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Abstract

Response surface methodology (RSM) and the desirability function (DF) approach have been widely used for multiresponse optimization in lactic acid fermentation processes. However, multiobjective optimization (MOO) can provide a broader range of compromising optimal cultivation conditions. This study applied multiobjective optimization of murta (Ugni molinae Turcz) juice lactic fermentation under monoculture and co-culture conditions. Total Phenolic Content (TPC), Lactic Acid Bacteria (LAB) count, and Lactic Acid (LA) concentration were optimized simultaneously. Decision variables were temperature, initial pH, and inoculation percentage. Several multicriteria decision-making methods (MCDM) were applied to select the optimal compromising responses, considering 80% priority for TPC, 15% for LAB, and 5% for LA. The highest TPC (723 ± 15 mg/L) was achieved at 35.3 °C, a pH of 5.9, and 2% inoculum in monoculture. The highest viable cell count (6.70 log CFU/mL) was obtained at 34.6 °C, pH 6, and 2.7% inoculum in monoculture. The highest lactic acid (0.48 ± 0.03 g/L) was achieved at 35.6 °C, pH 5.9, and 2.1% inoculum in co-culture. Applying the simple additive weighting (SAW) method made it possible to prioritize the solutions more effectively according to the priorities of each answer. These optimal conditions were validated, showing that monoculture fermentations were closer to the identified optimal points. Additionally,monocultures achieved higher TPC and LAB values than co-cultures. Although MOO and DF yielded the same Pareto front, the former approach was faster and produced a more populated and diverse one.

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Shared Fermented Food Heritage of Portugal and Spain

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Abstract

The common historical and cultural heritage for Portugal (PT) and Spain (SP) has flourished in a myriad of fermented foods shared between both countries. Through analysis of databases like eAMBROSIA, GIview, and direct contact with producers, this study maps a landscape dominated by dairy products (mainly cheeses) and alcoholic fermented beverages (mainly wine) from the Iberian Peninsula, many of which possess Protected Designation of Origin (PDO) certifications. Thus, 27 and 18 PDO cheeses were from SP and PT, and over 130 PDO wines from SP and PT, respectively. Legumes, cocoa, tea, and coffee were less prevalent. Both countries also share similarities in food products with Protected Geographical Indications (PGI), particularly wines(44 from SP and 14 from PT), and meat-based products, especially from Portugal (29 *vs.* 11 from SP).

Although traditional methods are still widely used, innovative approaches are beginning to emerge, such as sourdough and multigrain bread, digital winemaking, and the revival of ancient cheese-making techniques. Interestingly, newer fermented drinks, such as kefir and kombucha are also gaining popularity in Spain, with over 3,000 hectolitres annually produced.

This study contributes to a general overview of the fermented foods produced in both countries as well as to promoting the study and consumption of this type of food with great health benefits.

The production of tannase by probiotic bacterial strains and its application in the food industry

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Abstract

Tannase (EC 3.1.1.20) catalyzes the hydrolysis of ester bonds in hydrolyzable gallotannins, releasing glucose and gallic acid, and is widely used in the food industry to improve the quality of food products. Tannase has been recognized by the United States Food and Drug Administration (FDA) as a safe enzyme, which allows its use in the pharmaceutical and food industries.

The study aimed to review tannase production using probiotic bacteria and its application in food production.

Tannase production has been identified in lactobacilli species of *Lactobacillus plantarum, Lactobacillus paraplantarum*, and *Lactobacillus pentosus*, which have been reported to play an important role in the production of many fermented foods, thus being proposed as a potential probiotic.

Tannase is used in fermentation to improve the flavor and quality of food products by breaking down tannins, which can otherwise impart a bitter taste. This process also helps produce gallic acid, which has antioxidant, anticancer, and cardioprotective properties. Tannase improves the quality of beverages such as tea, coffee, beer, and wine. It enhances the extractability and cold water solubility of compounds in instant tea. It helps remove haze and unflavored phenolic compounds from beer and wine, improving clarity and taste. The enzyme is applied to minimize haze formation and bitterness in fruit juices, enhancing their quality and consumer appeal. Tannase is used to synthesize propyl gallate, an important antioxidant food preservative that helps extend food products' shelf life.

In conclusion, microbial tannase, particularly from bacterial sources, holds significant potential for enhancing food quality and processing. Continued research into optimizing production and understanding the enzyme's properties will be crucial for expanding its applications in the food industry.

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Development of Artisanal Probiotic Yoghurt Using Novel Starter Cultures from Turkiye

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Abstract

This study aimed to isolate novel starter strains from 13 artisanal yoghurt samples collected in the Urla region of Izmir, Turkey, for probiotic yoghurt production. A total of 453 lactic acid bacteria (LAB) strains were isolated, with 5 *Streptococcus thermophilus* and 26 *Lactobacillus delbrueckii* ssp. *bulgaricus* strains identified as potential probiotic candidates. These strains were characterized using biochemical, physiological, and molecular methods, including PCR-RFLP and PFGE-RFLP for differentiation (1). The probiotic properties of the isolates were assessed through tests such as bile salt tolerance, cholesterol assimilation, antimicrobial activity, and adhesion to Caco-2 human intestinal cells. The results showed that all strains were acid- and bile-resistant, adhered well to Caco-2 cells, and grew well with prebiotics (2). They also exhibited antimicrobial activity against *Escherichia coli* and *Listeria innocua*. Sixteen probiotic yoghurt combinations were created using these isolates, and the yoghurt products were evaluated for physical, chemical, rheological, and organoleptic properties. Key findings included acetaldehyde concentrations ranging from 5.61 to 15.38 mg/L, viscosity between 261 and 608 mPa.s, and hardness values from 3.81 to 6.71 N. Aroma compounds like acetaldehyde, ethanol, acetone, and diacetyl were present in varying concentrations (3). Sensory evaluations confirmed the potential of these probiotic starter strains for producing high-quality, functional yoghurt. Overall, this study successfully identified and characterized a range of traditional yoghurt LAB strains with both starter and probiotic features. A combination of cocci and bacilli isolates was developed as probiotic/starter strain combinations, which could be effectively utilized in the dairy industry to produce high-quality, functional, and probiotic-rich yoghurt.

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Effect of lactic acid fermentation on plant-based protein hydrolysis in quino and chickpea blended beverages

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Abstract

The consumption of plant-based beverages has been increased in the last decades. However,these products face nutritional challenges related to their low protein hydrolysis, which negatively impacts their protein bioaccessibility. In this study, three beverages formulated with quinoa and chickpea flour blends were fermented using *Lactobacillus acidophilus* LA-5 to assess the effect of lactic acid fermentation on the degree of hydrolysis of plant-based proteins. Additionally, the impact of quinoa and chickpea blends on the protein content in the beverages was evaluated. Fermentation was completed within 10 h, resulting in a decrease in the pH (<4.3) and an increase in titratable acidity and lactic acid (>0.37 % and > 1.7 g/L),respectively. SDS-PAGE and the O-phthalaldehyde method revealed hydrolysis of quinoa and chickpea proteins. A quinoa-to-chickpea ratio of 50 % exhibited the highest protein content(>2 %) and hydrolysis (35.9 %) after fermentation , indicating that an increase in chickpea improved these parameters in the prepared PBBs. Overall, fermentation using *Lactobacillus acidophilus* increased plant protein hydrolysis, and legume addition improved the protein content and the nutritional value of plant-based beverages.

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Optimization of the fermentation process of caperberries

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Abstract

This study investigates the optimization of caperberry fermentation processes to address quality variability observed between production plants in Spain and Morocco. The objectives were to (i) compare the impact of different brine compositions and processing conditions on fermentation parameters, (ii) assess the role of microbial dynamics in product quality, and (iii) recommend improvements for process standardization and product consistency.

Pilot-scale fermentation experiments were conducted using caperberries processed under eight treatments varying in salinity (7–14% NaCl), buffering agents (sodium acetate), and acidification (acetic and lactic acids). Physicochemical (pH, salt, and acidity), microbiological (lactic acid bacteria counts), and sensory attributes (color and texture) were monitored.

Optimal fermentation occurred in treatments with lower salinity (7% NaCl) and sodium acetate as a buffering agent, achieving a stable pH of 3.7 and a lactic acid concentration of 1.06%. These conditions favored the growth of *Lactobacillus brevis*, resulting in improved product texture and color. Treatments with higher salinity (14% NaCl) inhibited microbial activity and failed to develop sufficient acidity.

The study identified critical parameters to enhance caperberry fermentation, including reduced salinity and the use of buffering agents to stabilize pH. These recommendations aim to standardize production processes and ensure consistent product quality across facilities.

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Solid-state fermentation: A promising approach to producing meat analogues

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Abstract

Solid-state fermentation (SSF) is a promising approach for developing meat analogs by improving protein conversion, e-nhancing texture, and generating umami flavors. SSF use various microorganisms that exhibit proteolytic and amylolytic capabilities, including mold fungi (*Aspergillus oryzae*, *Aspergillus niger*, *Rhizopus oligosporus*) and proteolytic bacteria (*Bacillus subtilis*, *Lactobacillus plantarum*, *Bacillus amyloliquefaciens*). The proteolytic enzymes, such as proteases, hydrolyze proteins to free amino acids, and the amylolytic enzymes, comprising amylases, are involved in the breakdown of starches for improved texturing in finished products. In meat alternative production, such processes are crucial as they provide textural and nutritional characteristics similar to meats of animal origin.

Examples of successful applications include *Aspergillus oryzae* in Koji fermentation, *Rhizopus* species in tempeh production, and *Bacillus subtilis* in miso fermentation. This fermentation processes may result in the generation of free amino acids, mainly glutamate, which imparts the umami taste so desirable in meat analogs.

Although SSF has a lot of advantages, it also has some disadvantages. Maintaining enzyme stability, ensuring consistent flavor production, and optimization of fermentation conditions for scalability are some of the challenges. Besides, the scale-up time and resources involved in SSF production must be carefully managed to make the process cost-effective. Despite these challenges, SSF is a promising technique that could result in sustainable, flavorful, and nutritious meat substitutes with a low environmental impact to help reduce the environmental impact of food production.

The review presents research on SSF applications in meat analog production, highlighting its potential for creating sustainable and flavorful alternatives.

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Development of gluten free sourdough bread enriched with olive pomace

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Abstract

With a growing population and increasing food production, the global food supply faces constant challenges, including rising levels of waste. Developing sustainable food systems is essential to ensure a secure, waste-minimized food supply. In 2022, global olive oil production is projected to reach 2,511 tons, with 55% produced in Europe. Spain leads with approximately 680 tons, followed by Greece, Italy, and Portugal, producing 330, 240, and 126 tons respectively. Olive oil yield can vary between 15-20% of the fruit's fresh weight, depending on factors such as variety maturity, harvest year, and agronomic conditions. Pomace oil, a lower-value byproduct of olive oil, requires considerable time and energy to process. In the realm of gluten-free foods, bread is a staple that can be made with flours from gluten-free grains and legumes, such as rice, soy, corn, and buckwheat. This study aims to create a gluten-free sourdough bread using olive pomace at varying levels (0%, 5%, 10%, and 15%). During production, dough pH and temperature were monitored before and after fermentation. Additionally, fermentation and baking losses were recorded, followed by sensory analysis after cooling. Results showed that as olive pomace content increased, fermentation losses rose (from 0.96% in the control to 2.08% with 20% pomace), along with baking losses (from 8.35% in the control to 9.25% with 15% pomace). Olive pomace addition also lowered dough pH, enhancing its sourness, with bread containing 10% pomace exhibiting the best sensory qualities.

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Microbial diversity and characterisation of microbial strains isolate from local sauerkraut

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Abstract

Fermented vegetables have huge potential for health, combining the nutritional and phytochemical advantages of plant material with beneficial microbes and their metabolic products. The fermentation process also adds to the safety and shelf life of foods, having a positive impact on food security. Whole genome sequencing and metagenomic analyses allow a better understanding of the diversity of fermented foods (Leech et al 2020) and provide an opportunity to discover novel bacterial pathways and metabolic traits. Sauerkraut is based on lactic acid fermentation of cabbage, a good source of antioxidants, and contains live microbes (Tlais et al 2022). In this project we isolated bacteria and yeast from 5 locally available sauerkrauts and performed whole genome and shotgun metagenomic sequencing to assess the diversity and functional potential of their microbiomes.

34 species of bacteria were isolated using selective plating, with several potentially novel bacterial species. Most samples were dominated by lactic acid bacteria, but diverse Bacillus spp and rare Pseudomonas spp were also identified. Yeasts were isolated from 3 of 5 samples and all 4 species belonged to the genus Kazachstania. Enrichment for Salmonella failed to isolate potential pathogens, but some Bacillus isolates contained genes for diarrhoeal toxins. Metagenomic sequencing confirmed the predominance of lactic acid bacteria and also revealed a high abundance of tailed bacteriophages. No significant antimicrobial resistance genes were present in the metagenomes, although some were identified in sequenced isolates. We have mined these isolate genomes for probiotic traits and novel pathways.

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Desirability function-based optimization of apple pomace extraction for the production of a fermentable must for the development of NoLo beverage using central composite design methodology (CCD).

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Abstract

The production of cider, especially in the Basque Country, generates a significant amount of apple pomace byproduct, which is challenging to manage. However, apple pomace contains valuable compounds, making it suitable for further use in obtaining a fermentable must for the development of a non- or low alcohol (NoLo) fermented beverages.

The objective of this work was to characterize and maximize the extraction of sugar, malic acid and nitrogen content, from apple pomace by comparing different extraction conditions of maceration, assisted by time, application of different enzyme concentration and agitation. To evaluate the responses, a rotatable central composite design (RCCD) with a two-factor five-level was applied.

After the response results were studied, the experiments conditions were optimised by multi-response analysis, considered to achieve targeted and optimum output. The desirability function was used to optimize and select the apple juice extraction that will undergo the fermentations. R-project software was used for data analysis.

Significant differences were observed in sugar and malic acid release with different maceration times and agitation. By macerating apple pomace for 4.6 hours at 20 ºC a maximize fermentable must is obtained, composed mainly by sugars (26.0 g/L), malic acid (1.3 g/L) and nitrogen (29.6 mg/L) and aromatic compounds that provide the must with apple flavour.

Selected must could be used to develop NoLo beverages by alcoholic and malolactic fermentations, revalorizing food byproduct. Overall, this study shows the potential of the apple pomace enhancing the sustainability and achieving a circular economy and innovative approaches in cider industry.

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Yeasts association networks in fermented alcoholic beverages.

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Abstract

Alcoholic beverages are produced using raw materials (musts, mashes), which may (beer) or may be not (wine) submitted to a heat treatment; furthermore, in industrial fermentations, Saccharomyces spp. starters are used , which rapidly dominate the community. In artisanal or indigenous fermentations, a diverse assembly of non-conventional yeasts (NCY) may emerge, which might influence the characteristics of final products and could be useful to design novel starters. Inference of microbial association networks (MAN) may assist in the identification of emerging properties of microbial communities and, although their ability to detect interactions and keystone taxa has been questioned (Rottjers and Faust, 2018), a recent study (Junkers et al., 2018) claimed to be able to detect stable associations in fermented vegetables. We therefore selected 9 studies on fungal communities of alcoholic beverages from FoodMicrobionet (Parente and Ricciardi, 2024), inferred MAN using three methods (SparCC, CCREPE, SPIEC-EASI) and analysed network, edge and node properties.

Correlation based methods (SparCC, CCREPE) returned networks in which a modular structure was evident, with two or more groups of nodes connected by mutual exclusion relationships, reflecting communities associated with different niches (raw materials and environment with high prevalence/low abundance taxa, fermenting material with taxa with higher relative abundance). SPIEC-EASI networks were usually smaller, with a lower clustering coefficient,possibly because of the exclusion of indirect relationships.

Combining all the copresence edges identified with any given method and clustering allowed the identification of highly connected hubs and clusters of NCYs which were clearly associated to different niche preferences.

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Effects of fermentation conditions on kefir’s sensory, chemical an microbial profiles

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Abstract

Kefir, a traditional fermented beverage made with a symbiotic mixture of yeasts, lactic and acetic acid bacteria, has gained popularity for its unique flavour and potential health benefits (Walsh et al.,2024). This study aimed to investigate the impact of fermentation conditions, particularly temperature, on the microbiological, chemical and sensory properties of kefir.

We used 15 kefir grain samples from the UK, Germany and Slovenia and analysed microbial composition of both, coagulum and kefir grains, using MALDI-TOF MS; and measured the content of D- and L-lactic acid, acetic acid, lactose and glucose in kefir coagulum. Sensory evaluation was carried out according to ISO 22935-1/IDF99:2023.

The sensory analysis revealed that kefir produced at 21 °C had a better flavour and texture than kefir fermented at 25 °C, which had an off-flavour, increased acidity and undesirable texture. Significant changes were observed in the ratio of D- to L-lactic acid (p=0.0194), with higher temperatures increasing acidity and D-lactic acid production. A mixed-effects linear regression model showed that a higher acid-to-sugar ratio had a negative effect on flavour and sensory scores, while increased D-lactic acid production correlated with poorer sensory characteristics. Microbial analysis revealed that *Lactobacillaceae* were dominant in the grains, particularly species *Lactobacillus kefiranofaciens* and *Lentibacillus kefiri*, while *Enterococcaceae* and *Streptococcaceae* were more abundant in the coagulum, with *Enterococcus durans* and *Kluyveromyces marxianus* as the dominant species.

These results emphasise the complex microbial interactions in kefir and the role of fermentation temperature in microbial composition and sensory quality.

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Establishing a mini cheese system for monitoring the fermentation and quality of white brine cheese

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Abstract

PDO cheeses, such as Feta and Sfela, play an important role in the economy and cultural heritage of the regions they are produced. This fact highlights the importance of preserving their quality and traditional way of production. For the purpose of this study, miniature model cheeses were devised based on a reference PDO Feta and Sfela cheeses simulating the production conditions of a particular producer. The model cheeses were subsequently compared to a separate third PDO cheese from an alternative producer to validate the applicability of miniature cheese model system. Fourier Transform Infrared (FTIR) spectroscopy, Gas Chromatography Ion Mobility Spectrometry (GC-IMS) and metagenomic analysis, were employed to analyze the chemical and microbiological properties of the miniaturized cheese versus the cheeses produced at full scale. The FTIR spectra of both Feta and Sfela cheese, analyzed by Principal Component Analysis (PCA), demonstrated that the miniaturized cheeses were identical to the original PDO cheese and both these cheeses could be distinguished from a third control PDO cheese. The GC-IMS also identified profiles of volatile compounds that were practically identical between miniaturized and PDO reference cheeses samples and distinct from the third control PDO cheese. Moisture, as well as salt, protein and fat content of the mini cheeses adhered to PDO requirements for the Feta, while a slight difference was observed in the moisture content of Sfela in comparizon to the PDO requirments. All findings support the efficacy of the miniature cheese model system for the study of white brined cheeses.

Isolation and identification of potential starter cultures for oa fermentation

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Abstract

Oat fermentation using lactic acid bacteria (LAB) is a process leading to numerous nutritional benefits: a lower glycemic index, reduced antinutrient content (e.g. phytic acid), improved digestibility due to hydrolysis of complex carbohydrates, dietary fiber and proteins, enhanced bioavailability of minerals, and better antioxidant properties1, contributing to the mitigation of oxidative stress. Although fermentation can be carried out by commercial starter cultures, innovative starters can be isolated from the proper food matrix, which is of greater interest considering their adaptation to the substrate. This work consisted in isolating, characterizing, and identifying starter cultures from wholegrain oat flour for further fermentation. Isolation was performed by inoculating serial dilutions of oat flour and sourdoughs (200%, dough yield DY) incubated during 16, 21, 24, and 48 h at 32 °C, in different culture media supplemented with cycloheximide and maltose, when required. A total of 123 isolates were characterized as starter cultures based on their ability to acidify oat sourdoughs (200% DY) during 24 h at 32 °C, and typified through amplification of 16S-23S rDNA internal transcribed spacer (ITS). The isolates that exhibited the best acidification properties of fermented oat and different ITS patterns were molecularly identified. Enterococcus faecium species was the most abundant in oat flour while Weissella confusa predominated in sourdoughs. Although the strains of Lactiplantibacillus plantarum and Lactiplantibacillus argentoratensis species were minorly found in both flour and sourdoughs, they showed the best fermentative capabilities in the oat matrix.

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The fungal microbiota of table olives: a survey.

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Abstract

Table olives production is among the most ancient fermentation processes. It is concentrated in the Mediterranean area and use of raw materials and poor control of fermentation parameters results in high variability of microbial communities, whose composition is strongly affected by trade preparation. Addition of starter cultures is rare in artisanal and semi-industrial fermentations. The microbiology of table olives has been reviewed (Anagnostopoulos and Tsaltas, 2022; Tsoungos et al., 2023). Both lactic acid bacteria and yeasts are important in determining the quality of the product.

Amplicon targeted metagenomics has become the tool of choice for the study of the microbiology of table olives but most studies focus on one or few varieties. Therefore, we started a survey of table olives produced in Italy, Spain, Greece and Cyprus. We collected 193 samples from 21 varieties. DNA was extracted from olives and brine; the ITS2 region was sequenced using Illumina NovaSeq and processed using the DADA2 pipeline (https://github.com/ep142/FoodMicrobionet/tree/master).

For most samples, less than 50 genera were identified. Four (*Pichia, Candida, Wickerhamomyces and Saccharomyces*) had a prevalence >45% and a median relative abundance >10%. The composition of fungal communities for Spanish-style olives was usually more uniform than that of Greek-style olives. While for a few varieties the between-sample diversity was relatively low, for others the composition of the microbiota of samples obtained from different producers was very different.

Although more data are needed, house microbiota and dispersal may be more important than selective pressure in shaping the microbiota of olives.

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Selection of potential starter bacteria for carrot juice fermentation

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Abstract

Soft drinks based on fermented vegetable juices are gaining popularity as an alternative to the traditional soft drinks. They are rich in antioxidants, organic acids and vitamins, have a low sugar content and are enriched with lactic acid bacteria. Although fermentation of vegetable juices can occur spontaneously it is recommended to use a specific starter culture to ensure the production of high-quality fermented juice. The aim of this study was to isolate potentially probiotic bacterial strains with suitable organoleptic and technological properties for the fermentation of carrot juice. The study was conducted in collaboration with the fermented vegetable juice producer Raw Edge OÜ. Carrots from various sources were used in the experiments, and juice was prepared both at production and in laboratory conditions. The fermentation process was carried out spontaneously at 32°C and 5°C. A control fermentation using the starter culture *Lactiplantibacillus plantarum* TENSIA®, was used as a reference for organoleptic properties.

The fermentation of juices made from carrots purchased from different locations showed that the environment and raw materials significantly influence fermentation outcomes. Microbiological analyses of spontaneously fermented samples and identification of bacteria isolates revealed the presence of various lactic acid bacteria including *Leuconostoc mesenteroides*, *Leuconostoc citreum* and *L. plantarum*. Fermentation with isolated lactic acid bacteria strains was conducted at 32°C, followed by sensory analyses, which showed significant variation in taste and aroma. The best technological and organoleptic properties were provided by several strains of *L. plantarum*. Further studies will focus on their probiotic properties.

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Effect of various strains usage on profile of volatile organic compounds (VOCs) in fermented bee products

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Abstract

Many researchers identified bee products as natural goods with a high nutritional value and health benefits [1,2]. Except for the high content of bioactive components, several chemical classes of VOCs can be distinguished [3]. It was defined that the bee products' origin could be differentiated by comparing their VOCs profile [3]. Moreover, the content of VOCs in bee products can be modulated by the fermentation process [4]. Therefore, the aim of our study was 1/ fermentation of bee bread and bee pollen with: *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactococcus lactis*, *Saccharomyces cerevisiae*, 2/ determination of VOCs profile and content: using as a method of extraction solid-phase microextraction (SPME) and as a method of compounds separation and quantification the gas chromatography coupled with mass spectrometry (GC/MS). The fermentation was processed for 24 and 48 hours at 30°C, then samples were lyofilized and ready for analytical part of experiment. According to obtained results, the dominant VOCs in bee pollen were (*E*,*E*)-3,5-octadien-2-one, (*E*,*E*)-2,4-heptadienal, acetic acid and benzaldehyde, whereas in bee bread 1-hexadecene, (*E*,*E*)-3,5-octadien-2-one, furfural and acetic acid were determinant as the major volatiles. The significantly higher content of VOCs was noted in bee pollen as well as in bee bread after longer fermentation (48h) for all type of strains. In case of strains, *Lacticaseibacillus rhamnosus* and *Lactococcus lactis* were the most efficient in formation of VOCs and could be selected in the future study. It can be concluded that the propriete conditions of fermentation can influence on VOCs profile and furthermore on aroma of final product.

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Fermented Foods as a Staple Food in the Sustainable Healthy Diet

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Abstract

Sustainable Healthy Diets are dietary patterns that promote all dimensions of individuals' health and wellbeing; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable. The aims of Sustainable Healthy Diets are to achieve optimal growth and development of all individuals and support functioning and physical, mental, and social wellbeing at all life stages for present and future generations; contribute to preventing all forms of malnutrition; reduce the risk of diet-related NCDs; and support the preservation of biodiversity and planetary health (FAO and WHO, 2019).

Fermented foods (FF) have been consumed by humans for thousands of years. They have been intentionally produced as a stable source of vitamins, minerals, probiotics, calories. The nutritional value of FF is well-appreciated, especially in resource-poor regions where yoghurt and other FF can improve public health and provide opportunities for economic development. Preferences for FF and beverages depend on dietary habits of consumers, as well as regional agricultural conditions and availability of resources (Tamang et al., 2020). FF also contain live microorganisms that may improve gastrointestinal health and lowering the risk of type two diabetes and cardiovascular diseases and have emerged as an important dietary strategy for improving human health (Marco et al., 2017). Today, FF endure in kitchens, restaurants and markets, where an estimated 5000 types of FF are eaten worldwide, making up 5-40% of human diets as certain foods and beverages such as sourdough bread and kombucha rise in popularity and receive trend-worthy recognition (Plessas, 2022).

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Impact of Bioprotective Cultures on Yogurt Properties During Storage and Post-Opening Refrigeration

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Abstract

Bioprotective cultures in dairy products are increasingly applied to improve safety, extend shelf life, and enhance product quality. This research aims to determine the effects of *Lactobacillus rhamnosus* (FreshQ cultures FQ9, FQ10, and FQ11) on the properties of yogurt, with samples taken on days 1, 21, and 35 post-production, as well as on days 1, 3, and 5 after the yogurt was opened and stored in a refrigerator. The analysis included pH values, titratable acidity (°SH), water holding capacity (WHC), diacetyl concentration, viability of the bioprotective cultures, and sensory analysis using a hedonic scale.

The viability of the bioprotective cultures was maintained throughout the entire period. The pH of the yogurt gradually decreased over time, with the lowest value (4.12) recorded in the FQ9 sample on day 35, five days after opening, while titratable acidity increased, peaking at 48.01 °SH in FQ9, slightly higher than the control (43.6 °SH). Water holding capacity (WHC) also improved over time, with FQ9 exhibiting the highest WHC by the end of the storage period. The concentration of diacetyl, which contributes to the buttery flavor of yogurt, was highest on the 21st day of storage, with 0.2192 $mg \cdot l^{-1}$ measured in the FQ9 sample, significantly higher than the control sample (0.1309 $mg \cdot l^{-1}$). Sensory analysis showed no significant variations in product acceptability ($p > 0.05$) until the fifth day after opening. These findings highlight the potential use of bioprotective cultures to enhance yogurt production without compromising sensory characteristics.

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Prevalence of fermented foods consumption in a Serbian young adult population

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Abstract

Although it is known that fermented foods have a long tradition in human nutrition, there is not much available data on the prevalence of their consumption in general and specific population groups. This study aimed to assess the prevalence of selected fermented food consumption in a young Serbian population. A survey was conducted among 18–30-year-old university students in Belgrade during the 2023-2024 academic year. A total of 217 participants completed the validated food frequency questionnaire and a beverage intake questionnaire. More than 50% of dairy intake was from fermented dairy products. The main contributor was yogurt, followed by cheese and sour cream. Among cheeses, the main contributor was fresh cheese. The most consumed fermented alcoholic beverages were beer and wine, while about 10% of the contribution was from distilled spirits. There were observed variations in the mean intake of fermented beverages between dietary assessment tools. The lowest differences were for beer and distilled spirits, and the largest was for wine. Overall, this study provides valuable insights into the prevalence of fermented food consumption among young adults. However, the existing discrepancy in assessing fermented beverage intake among assessment tools highlights the significance of developing novel and specific methodologies for a more precise estimates of fermented food intake.

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The role of *Lactococcus lactis* small non-coding RNAs in response to antimicrobial stress

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Abstract

Non-pathogenic Gram-positive *Lactococcus lactis* bacteria is used for food fermentation and preservation, contributing to the texture and flavour of products[1]. It also exerts inherent beneficial health effects and is increasingly being tested as vector-expressing peptides and proteins for oral or intranasal administration to combat viral and bacterial infection, metabolic disorders etc. *L. lactis* may encounter antimicrobial stress in all these applications, making it highly important to understand bacteria's response to such stressors. One common stressor in the human body is lysozyme, a 14.7 kDa protein that hydrolyses the β-1,4-glycosidic, disrupting the bacterial cell wall's peptidoglycan (PG) layer. Another group of stressors, β-lactam antibiotics, including penicillin G, are among the most broadly used antimicrobials. Their β-lactam ring irreversibly binds to penicillin-binding proteins, resulting in weakly crosslinked PG and rendering the bacteria highly susceptible to cell lysis. In the past decade, it has been shown that bacteria are able to cope with stressors in part due to 50–500 nt long small non-coding RNAs, sRNAs. Thus, to decipher the response of *Lactococcus lactis* subsp. *cremoris* MG1363 to the major cell wall-targeting antimicrobials - lysozyme and penicillin G - we analysed genome-wide changes in small non-coding RNA profiles following antimicrobial treatment. In parallel, functional screening identified *L. lactis* genome fragments conferring increased resistance to antimicrobials comprising the cell wall integrity. A few small RNA was then selected for a more detailed analysis of their mechanism of action.

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Selection of a starter culture for the production of a fermented beverage (mead)

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Abstract

For the selection process of yeasts as starter cultures for mead production, 98 strains belonging to eight different species isolated from beekeeping environments and products were used: *Saccharomyces cerevisiae, Candida albicans, Dekkera anomala, Zygosaccharomyces rouxii, Zygosaccharomyces mellis, Kazachstania unispora, Meyerozyma guillermondii* and *Saccharomyces unisporus.*

The selection of suitable strains for mead production focused on a fermentation with pure cultures and another with mixed cultures, thus observing if there were significant differences between them. To this end, the fermentative capacity of the yeasts and the organoleptic characteristics of the final product were studied. The results led to the selection of two strains: for 'pure culture fermentation' of honey (*Saccharomyces* spp. M11A2) and for mixed sequential inoculation (*K. unispora* M17A2). Both yeasts were isolated from honey and selected for their high fermentative capacity and ethanol resistance. To ensure the correct and controlled development of the mead fermentation, periodic measurements of weight loss, Brix, alcohol content, pH and yeast counts were taken.

A sensory analysis was performed between meads produced with these strains and a commercial product, using sensory profile analysis (ISO 13299:2016) together with a sorting test (UNE-ISO 8587:2010), carried out by expert tasters and olfactory and taste attributes, as well as the overall impression of the final product. The results indicated that the mead made with the pure *Saccharomyces* spp. culture (M11A2) obtained the best overall impression in the sensory evaluation.

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Effect of Quinoa Variety and Malt Addition on the Fermentation Characteristics, Quality, and Health-Promoting Properties of Wholemeal Quinoa Sourdoughs

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Abstract

Quinoa is a nutrient-dense pseudocereal renowned for its high protein content, essential amino acids, dietary fiber, and a range of bioactive compounds, including antioxidants and minerals. Fermentation can further improve the health benefits of quinoa-based products. This study investigated the effects of quinoa variety and malt addition on the fermentation, quality, and health-promoting properties of wholemeal quinoa sourdoughs. Quinoa flours (white, red, black) were analyzed for compositional properties, and sourdoughs were prepared with 0%, 5%, and 10% malt additions. Fermentation (46 hours at 30°C) was assessed through pH, total titratable acidity (TTA), dynamic viscosity, and lactic and acetic acid levels. Nutritional and functional properties, including dietary fiber, antioxidant activity, volatile compounds, and mineral content, were also evaluated.

Fermentation outcomes varied by quinoa type. White quinoa sourdoughs had the lightest color, the highest TTA, acetic acid, and sodium, magnesium, and copper content but lower protein, dietary fiber, potassium and manganese, and lactic acid content. Red quinoa sourdoughs had the highest viscosity, soluble dietary fiber, and zinc, but the lowest ash and magnesium levels. Black quinoa sourdoughs exhibited the darkest color, highest protein, ash, dietary fiber, lactic acid, manganese and potassium, but the lowest viscosity, sodium, calcium, zinc and acetic acid. Malt addition enhanced fermentation, increasing TTA, dynamic viscosity, lactic acid, and total dietary fiber while reducing protein content. Colored quinoa sourdoughs exhibited higher antioxidant activity compared to white quinoa.

Volatile compound analysis identified 25 compounds, with alcohols predominating. This study highlights the significant impact of fermentation and malt addition on the nutritional and sensory qualities of quinoa sourdoughs.

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Lactic acid bacteria diversity of traditionally produced hen mea fermented sausage

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Abstract

The production of fermented sausages from poultry meat can be challenging using traditional technologies and natural fermentation and drying conditions. In this study, fermented sausages were produced using meat and fat from COBB 500 hens and a nitrite-free salt and spice mixture. The sausages were stuffed into collagen casings, cold-smoked and matured under natural conditions for 90 days during the winter season. During maturation, isolates (n=88) of lactic acid bacteria were collected and subjected to MALDI TOF mass spectrometry identification. In addition, their ability to combat *Listeria* was tested against five strains using the agar well diffusion method. *Latilactobacillus sakei*, followed by *Lactococcus lactis* and *Enterococcus faecalis* were dominant at day 30 of processing, while *Leuconostoc mesenteroides* was dominant at day 60. In the final products, *Latilactobacillus sakei* and *Latilactobacillus curvatus* were the only species identified in the selected population. Inhibitory activity against *Listeria monocytogenes* strains and *Listeria innocua* was confirmed in the isolates of *L. sakei*, *Leuconostoc mesenteroides* and *Enterococcus faecalis* by both native and neutralized cell-free supernatants. In addition to the previously reported favorable chemical characteristics of the novel hen meat fermented sausage (low fat; 11%, high protein; 46%; composition and ratio of fatty acids), these results confirm the dominance of technologically and hygienically advantageous lactobacilli species in meat fermentation.

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Session 2. Safety of Fermented Foods

Microbial safety aspects of fermented foods

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Although fermented foods can be considered safe due to a combination of physicochemical and microbiological characteristics, they have been sporadically involved in events of foodborne illness. Accidental contamination combined with failures in the production process may lead to presence of foodborne pathogens in the final, ready to eat, fermented product, posing a risk for the consumers. Beyond this well documented, rare and confined circumstance leading to foodborne illness, new challenges to microbial food safety of fermented foods are emerging. This is due to alternative raw materials, novel formulations and technological interventions, often applied at non-conventional production settings (for example restaurants). At the same time, our understanding of microbial dynamics and the level of detail with which we can monitor fermentation processes has evolved dramatically thanks to high throughput sequencing approaches, bioinformatics and modelling. Additionally, we are experiencing important advancements in the areas of microbiome science and the design and use of microbial consortia.
The progress being made is translated into greater precision in the management of food fermentations and possibility to intervene proactively to further improve microbial food safety while at the same time diversifying the types of fermented foods available to consumers.

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From Health Impact to Green Choices: The Dual Impact of Fermente Foods

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Abstract

Fermented foods (FF) have been an essential part of human diets across cultures, cherished for their distinctive flavors and preservation capabilities. Beyond their culinary value, FF have shown significant potential health benefits (1). Studies link fermented foods to antioxidation, lower blood lipid levels, enhanced immunity, tumor inhibition, delayed aging, and prevention of gastrointestinal disorders (2). However, their consumption needs caution regarding potential risks, such as pathogenic contamination or the formation of harmful by-products during fermentation. The safety of fermented foods is currently being systematically revised within the PIMENTO CA20128 under WG3-Health benefits and risks of fermented foods. To navigate these complexities, risk-benefit assessment methodologies provide a robust scientific framework to evaluate the trade-offs, enabling evidence-based dietary recommendations (3).

Sustainability is another pivotal aspect of fermented foods. In the face of global challenges like food waste and climate change, fermentation emerges as a key strategy (4). For instance, kimchi and sauerkraut help preserve surplus vegetables, while yogurt and kefir optimize dairy utilization. Nonetheless, the production of certain products, such as fermented meats, underscores the need for strategies to mitigate their environmental impacts while maintaining their benefits.

This communication highlights the dual role of fermented foods in promoting health and enhancing sustainability. By bridging tradition and innovation, fermented foods hold the potential to support the transition towards sustainable food systems, paving the way for future diets that address pressing public health and environmental priorities.

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The impact of fermentation on acrylamide levels and bioactive profile o pumpkin and soft wheat composite bread

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Abstract

Ever since the International Agency for Research on Cancer (IARC) classified acrylamide as a probable human carcinogen in 1994 and even more after the discovery of acrylamide in food in 2002, much effort has been and continues to be put into reducing the levels of acrylamide in food products.

Bread, because of its high consumption rate, it is one of the main contributors to daily acrylamide intake in many countries. Hence, developing new concepts and mitigation processes for acrylamide reduction in breads is necessary for food sectors and safeguarding human health.

This study investigated the effect of prolonged proofing on nutritional and functional characteristics of pumpkin and soft wheat composite bread, especially on acrylamide content. For that purpose, the wheat-pumpkin bread samples (0 - 20% of the pumpkin flour) were prepared using two proofing times (60 and 120 minutes) of the dough before baking. Rheology, colour of crust and crumb, antioxidant activity, total polyphenolic content as well as reducing sugars and acrylamide content were assessed.

The results showed that prolonged proofing could modulate the content of acrylamide in the bread while maintaining or even improving the nutritional and bioactive profile of baked foods. Adding pumpkin powder to bread increases its total phenolic content and antioxidant activity, while prolonged proofing helps in maintaining the low levels of acrylamide generated during baking.

The study indicates that fermentation intervention strategies together with composite flours can contribute to lower acrylamide levels in the functional breads. This approach can be implemented on a large scale in the baking industries to reduce acrylamide production in their products.

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Is fungal solid-state fermentation a safe method for food valorization in terms of acrylamide formation?

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Abstract

Solid-state fermentation with filamentous fungi provides an effective approach for enhancing plant matrices with bioactive compounds such as essential fatty acids, phenolics, flavonoids, and for improving their antioxidative properties and digestibility. However, it is important to assess potential hazardous compounds, like acrylamide, a substance considered probably carcinogenic to humans, which may form during the heat treatment of plant matrices. Acrylamide primarily arises from asparagine, a free non-essential amino acid naturally abundant in plants. Legumes, valued for their high protein content and nutritional benefits, are increasingly used in the production of ready-to-eat snacks and biscuits, where heat treatment may lead to acrylamide accumulation. This study examined the solid-state fermentation of green pea, yellow pea, and red lentil with *Actinomucor elegans* and *Mortierella alpina.* The fermentation enhanced the matrices with polyunsaturated fatty acids and led to a notable increase in free amino acids, particularly essential ones. While fermentation with *Actinomucor elegans* resulted in minimal changes in asparagine content, significant reductions in asparagine levels were observed in all three legumes fermented with *Mortierella alpina*. In conclusion, solid-state fermentation with *Mortierella alpina* presents a promising method for valorizing protein-rich legumes and reducing the formation of undesirable acrylamide in heat-treated legume-based foods.

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New insights into the safety of fermented dairy products by analysin resistomes

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Abstract

Resistome analyses provide a good insight into the prevalence of antibiotic resistance that presents public health concern, in different environments. While human, animal, and environmental resistomes have been extensively studied, the resistomes of food samples, including fermented foods, have not yet been sufficiently investigated. In this study, the microbiomes of cheeses (raw or thermized, with or without starter cultures), probiotic food supplements and starter cultures were analysed using shotgun metagenome sequencing. We identified 689 ARGs in 62 samples, mainly conferring resistance to disinfectants, tetracyclines, aminoglycosides and macrolides. Of these, 32.2% were on mobile genetic elements (MGEs). Differences in the microbiome composition and the resistome profiles were found among the sample groups. Raw milk cheeses without starter cultures had the highest abundance and diversity of ARGs and MGEs. Thermal treatment of milk and starter cultures reduced microbiome diversity and ARG/MGE abundance. Starter cultures and probiotics had a significantly lower ARG load than raw milk cheeses. Bacterial composition correlated with the resistome, with most ARG-carrying contigs coming from Bacillota, particularly Enterococcus faecium and Lactococcus lactis, and some from Pseudomonadota, including potentially pathogenic species found in raw milk cheeses. This study improves understanding of resistomes in fermented dairy products and highlights the role of raw milk and raw milk products as reservoirs of antibiotic resistance. Production methods, in particular heat treatment of milk and addition of starter cultures, significantly influence cheese microbiome and resistome. The low abundance of resistance genes and MGEs in commercial starter cultures suggests their minimal contribution to food chain resistomes.

Acknowledgements

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Farm-to-fork transmission of antimicrobial resistance genes in fermented pork sausages

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Abstract

The farm-to-fork transmission of antimicrobial resistance genes (AMR) from food-producing animals to the consumer has been recently investigated [1]. The presence of these genes is linked to the misuse of antimicrobials in animal production, which contributes to an increase in antimicrobial-resistant bacterial populations, resulting in serious health issues in both in humans and animals. Because pigs are a huge reservoir for AMR, analysis of a fermented pork meat product was performed to understand how it can be a source of AMR genes. Analyses were performed throughout the manufacturing process, with two replicates per sample using two different approaches. In the culture-dependent approach, selective culture media with antimicrobials in their composition were used: CHROMID VRE (for the detection of vancomycin-resistant *Enterococcus*), CHROMID ESBL (to detect ESBL-producing Enterobacteriaceae) and CHROMID MRSA (for the detection of methicillin-resistant *Staphylococcus aureus*). For the culture-independent approach, next-generation sequencing (NGS) was used (minION MK1B, Nanopore Oxford Technologies), and the results were analyzed on the EPI2ME platform (Metrichor). No resistant bacteria were isolated with the culture-dependent approach. In the metagenomic analyses, several AMR genes were detected in different samples, such as *tetC*, *yojL*, *opmH*, *emrE*, which confer resistance to tetracycline, the peptide antibiotic microcin J25, triclosan and aminoglycoside, respectively. Apart from the *tetC* gene, with plasmid origin, all other AMR genes are chromosomal, and come from *Pseudomonas* or *Klebsiella*. The different results obtained are expected and the two approaches may be considered complementary, if we are to better understand the presence and potential farm-to-fork transmission of AMR genes.

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Antimicrobial Activity of Fermentation Metabolites Produced by Limosilactobacillus fermentumK73 Isolated from Costeño Cheese Against Shigella spp., Escherichia coli, and Salmonella spp.

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Abstract

Costeño cheese, a traditional Colombian dairy product, serves as a natural reservoir for diverse probiotic microorganisms capable of producing beneficial metabolites through fermentation. These bioactive metabolites, such as organic acids, bacteriocins, and other secondary compounds, play a crucial role in inhibiting the growth of pathogenic microorganisms[1]. Thus, this study aimed to evaluate the antimicrobial activity of metabolites produced by probiotic Limosilactobacillus fermentum K73 isolated from Costeño cheese against pathogenic bacteria, including Shigella spp., Escherichia coli, and Salmonella spp. Fermentation metabolites were obtained by centrifuge the food grade medium in which the probiotic growth for 10 h at 37 °C. Then supernatant which contained the fermentation metabolites was microfluidized at 5000 and 20000 psia for 1 to 4 cycles. Antimicrobial properties were tested using a BioScreen system to monitor bacterial growth kinetics during 24 h at 37 °C. Results revealed significant inhibitory effects similar to gentamicin which was used as control (10 µg/mL), with reduced growth rates and final biomass of all three pathogens, particularly Salmonella spp. However, there was not a significant effect of the pressure of the microfluidizer not the cycles in the inhibitory activity of the fermentation metabolites. These findings highlight the potential of probiotic-derived metabolites from Costeño cheese as natural antimicrobial agents, offering promising alternatives to conventional antibiotics. Further research is needed to characterize the active compounds and elucidate their mechanisms of action.

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Revolutionizing food safety: Breakthroughs in bacteriocinogenic ferments from spontaneously fermented Mediterranean foods

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DOMCA, a Spanish SME with extensive experience in the agro-food sector, has successfully led its participation in the PRIMA initiative through two flagship projects: BIOPROMEDFOOD and INNOSOL4MED. These success stories demonstrate how SMEs can play a pivotal role in international projects that drive innovation, sustainability, and food safety in the Mediterranean region.

In BIOPROMEDFOOD, DOMCA focused on the development of bioprotective cultures to extend the shelf life and improve the safety of perishable foods typical of the Mediterranean diet. This project leveraged the native microbial biodiversity of traditional fermented meat products to isolate bacterial strains with unique antimicrobial properties. These cultures not only inhibit pathogens such as *Listeria monocytogenes* and *Clostridium perfringens* but also reduce toxic compounds like biogenic amines, while restoring the traditional flavors and aromas of Mediterranean cured meats. Industrial-scale trials, conducted in collaboration with institutions such as the University of Bologna, have validated the effectiveness of these new fermentative cultures, contributing significantly to sustainability and food safety.

In INNOSOL4MED, DOMCA tackled the development of ready-to-eat (fourth-range) foods by formulating innovative matrices incorporating functional ingredients derived from sustainable sources and native microbial strains. The project explored fermented foods, such as pickles from Mediterranean countries (Spain, Morocco, Italy, Turkey, and Algeria), to develop biopreservation solutions that enhance quality and food safety. Additionally,disruptive technologies were integrated to meet modern consumers' demand for healthy,sustainable, and convenient products.

Both projects serve as benchmarks for agro-food innovation, highlighting the strategic role of SMEs in international initiatives. These success stories underscore how collaboration in programs like PRIMA enables small enterprises to make significant contributions to the research and development of sustainable food solutions, while preserving the rich culinary heritage of the Mediterranean region.

Exploring the unknown: are phages present in cider?

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Abstract

Bacteriophages (phages), the viruses of bacteria, are present in all microbiota. While some phages isolated from food products are well-described, phage communities (phageomes) as a whole are very poorly understood and explored in fermented foods, and especially in fermented beverages[1]. Their roles in the dynamics of microbiota that make up these unique ecosystems remain largely undiscovered.

This study is part of a project aiming to understand the impact of phageomes on the microbial communities of a traditional Norman fermented beverage, cider. The diversity of phageomes (lytic and lysogenic) was studied during the fermentation of several ciders combining a metagenomic approach and phage isolation. It was found that the temperate mode predominated in fermenting cider, while lytic phages were scarcely present. The diversity and abundance of prophages in metagenomic data fluctuated with their predicted host species[2]. One particular phage of interest, targeting the strain *Liquorilactobacillus mali* UCMA 16447, has been especially isolated and studied[3]. Other virulent phages are currently being isolated and characterized.

This work highlights the importance of acquiring knowledge about the abundance and diversity of phages in fermented beverages, to move toward a better understanding of phage-bacteria interactions in foods. This will contribute to improved control of fermentation processes and strengthen the production of sustainable foods in the future.

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BEFERMED project: Development of fermented beverages from local Mediterranean plant-based raw materials

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Abstract

The development of new functional fermented beverages from local plant-based raw materials contributes to progress towards more sustainable and healthy food production [1]. BEFERMED project proposes obtaining this type of fermented beverages with lactic acid bacteria from rice, tiger nut and carob evaluating their probiotic and antioxidant activities before and after the *in vitro* simulated digestion [2]. Their anti-proliferative and anti-inflammatory potential using cell cultures, as well as the sensory acceptance by the consumer is also assessed. Furthermore, the by-products generated in their production are intended to be used as substrates for the growth of filamentous fungi that produce important ingredients such as mycoprotein, which is considered an excellent protein alternative [3], thus contributing to the circular economy.

The project is related to different Sustainable Development Goals (SDG) such as SDG 3 (Health and well-being) as it will allow the development of plant-based beverages with potential health benefits, SDG 12 (Responsible production and consumption) by proposing the revaluation of by-products generated in the production of beverages, and finally, with SDG 13 (Climate action) since the use of plant-based raw materials from local producers favours the reduction of greenhouse gas emissions and the development of the local economy.

In general, this study will make possible to offer fermented foods that help to maintain health status or reduce the risk of disease, producing healthy, safe and high quality foods for both the general population and people with intestinal pathologies (inflammatory diseases) or specific nutritional needs (vegans, vegetarians, allergic or intolerant people).

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OLIVEPACK: Natural and biobased antimicrobial packaging materials t increase the shelf life of naturally fermented low-salt table olives

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Abstract

This work offers an innovative, circular economy-oriented bio-based antimicrobial food packaging material that is specifically tailored to increase the shelf life of table olive, a food product that is of high economic importance for the Mediterranean region. An innovative solution will be presented to the problem of the use of high salt concentrations or chemical additives, which are required to preserve naturally fermented olives, but seriously threaten human health.

Within the scope of OLIVEPACK, a biobased, biodegradable, antimicrobial bionanocomposite foam composed of natural components, that is designed to be placed into any table olive packaging as a packaging insert will be developed. Adapting a circular economy approach, the packaging inserts are composed of natural antimicrobial agents extracted from table olive wastewater and cellulose polymer matrix extracted from olive tree waste. Through a multidisciplinary approach integrating expertise in materials science, food microbiology, sensory analysis and food science, the food packaging inserts are being evaluated in terms of their antimicrobial activity, their effect on the nutritional, physicochemical and sensory properties of table olives, and also their market acceptance and socioeconomic impact. OLIVEPACK, through an interlinked, interdisciplinary, multi-actor approach, is making significant progress beyond the current state of the art by introducing a novel storage solution for low-sodium, preservative-free naturally fermented table olives. The novel bionanocomposite foam packaging inserts solely composed of natural and biodegradable components will not only help to reduce food waste but also ensure consumption of safer and healthier table olives with extended shelf-life, all without putting any burden on the environment.

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Innovative Approaches to Fermented Foods: Inhibitory Effects of LAB on *Candida parapsilosis*

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Abstract

Fermented foods have gained significant attention for their potential to improve human health, drive food innovation, and support local production. The application of lactic acid bacteria (LAB) in fermented foods not only enhances their nutritional and sensory properties but also offers a natural alternative to chemical preservatives (Figueroa et al., 2024; Poon and Hui, 2024). This study investigates the role of LAB in fermented foods and emphasizing their health benefits. This research focuses on the inhibitory effects of selected LAB cultures (commercial culture Fresco DVS 1010, *Lacticaseibacillus rhamnosus* GG, and *Lactobacillus acidophilus* 145) against the pathogenic yeast *Candida parapsilosis* ATCC 22019 in milk. The results demonstrate that the highest inhibitory activity on the growth of *C. parapsilosis* was observed with the Fresco DVS 1010 culture. Additionally, the inhibitory effect of LAB on the growth of *C. parapsilosis* was significant when LAB were added at an initial concentration of approximately 6 log CFU/ml or higher. The study also monitored the decrease in pH value of milk as a result of the metabolic activity of the selected LAB. These findings highlight the potential of LAB cultures in enhancing the safety and quality of fermented foods and promoting health.

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Amino Acid Profile in Legumes Fermented with Filamentous Fungi

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Abstract

Fermentation as a multi-beneficial process is considered a promising strategy to reduce acrylamide formation in baked cereal products, as it can decrease the content of asparagine, the main precursor of acrylamide. The present study provides a complex overview of changes in the free amino acid profile of legumes fermented with different filamentous fungi. The primary objective was to select a suitable strain of filamentous fungi to reduce asparagine levels in legumes, especially lentils, peas and beans. From a nutritional point of view, the potential impact of fermentation was found to increase the proportion of essential amino acids as well as crude and total dietary fibre in the raw materials, which contributed to the nutritional value of baked products with lower acrylamide content. This research underscores the potential of fermented legumes to enhance both the safety and quality of food products. The following sensory analysis and instrumental texture analysis represent a prospective tool to further evaluate the impact of fermentation on the quality of novel bakery products in the ongoing research of the project.

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The effect of milk protein variants and yoghurt starters on the immune status of the intestinal epithelium treated with fermented milk beverages

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Abstract

The aim of the study was to investigate how milk with different protein variants of kappa-casein (A or B), beta-casein (A1 or A2) and beta-lactoglobulin (A or B) variants fermented with different yoghurt starters impacts the immune status of the intestinal epithelium.

Fermented milk beverages (FMB) were developed using milk obtained from homozygotes HM1 (AA/A1A1/AA), homozygotes HM2 (BB/A2A2/BB), control heterozygotes C (AB/A1A2/AB). Milks were fermented with three starter cultures: a commercial YO-122, and K1 and K2 developed at IAR&FR. FMBs were digested (Brodkorb et al. 2019) and the low molecular weight fractions (<15 kDa) of digests were loaded onto fully differentiated Caco-2 cells. Proliferation, cell cycle, mRNA expression of inflammation-related genes and secretion of pro-inflammatory cytokines were determined after 24h incubation.

The lowest proliferation rate was observed for Caco-2 cells treated with C/YO122 (66%) and C/K1 (60%). However, the significantly highest ($p<0.001$) share of apoptotic cells was determined in cells treated with C/K1 and homozygotic milk HM2 fermented with K1 and K2 cultures. Treatment with the same three combinations of milk and starter cultures enhanced the expression of IL-8 ($p<001$), and CCL20, TSLP and MyD88 ($p<0.05$) in Caco-2 cells. Consequently, C/K1, MH2/K1 and HM2/K2 increased also the secretion of IL-8 and TNFα, and modulatory cytokine IL-6.

In conclusion, in the tested conditions, the starter culture K1 applied for fermentation of control heterozygotic milk (C) and homozygotic milk HM2 generated fermented products with the potential to enhance the immune response of the intestinal epithelium.

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Chlorantraniliprole, methomyl, metalaxyl and thiacloprid pesticides: *i silico* investigation and their determination in pickles using QuEChER methodology and HPLC-DAD

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Abstract

Chlorantraniliprole, methomyl, metalaxyl and thiacloprid are four frequently used pesticides approved as active substances in the formulations for pickles protection in the Republic of Serbia [1].

In silico analyses consisted of optimization of the structures of selected pesticides by conformational search and their molecular docking against acetylcholine esterase (AChE) of Mus musculus and Homo sapiens under Schrodinger 2022-3. Chlorantraniliprole has close contact with Thr 528 and His 381 from chain A through hydrogen bonding. Methomyl shows no hydrogen bonding with amino acids, but other types of interactions with several amino acids from chains A and B. Metalaxyl shows hydrogen bonding to Tyr 133 from chain A and plenty of other types of interactions with amino acids from chain A. Thiacloprid shows no hydrogen bonding with amino acids, but several other types of interactions with some amino acids from chain B.

Prepared extract of pickles using QuEChERS methodology and then analysed using HPLC-DAD. The calibration shows good linearity for all studied pesticides with correlation coefficients of 0.9913 - 0.9993. Metalaxyl was not detected. The concentration of chlorantraniliprole was 1.43 mg/kg, thiacloprid 0.77 mg/kg, and methomyl was 0.85 mg/kg. According to the calculated ADMET parameters, all selected pesticides are orally active.

This data are very important as a potential risk on human health using pickles as a food. The agricultural use of studied pesticides must be under control due to their toxicity, and their concentrations in consumed food must be within the recommended values set by international organizations.

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Session 3. M4SF Cluster

Impact of fermented foods on cardiometabolic health – an overview of the human studies within the Horizon Europe project Healthferm

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Abstract

The European Horizon project "Healthferm" aims to develop sustainable plant-based fermented foods and diets that deliver health benefits to consumers by design. The project involves five human intervention studies that have in common that they are performed in patients at risk to develop cardiometabolic disease and that they evaluate the impact of fermented foods on cardiometabolic risk factors including glucose homeostasis, lipid metabolism and inflammation. The studies vary from mechanistic studies that evaluate the impact of specific fermentation metabolites over studies that evaluate the impact of newly developed fermented food product to a more explorative portfolio diet study. This presentation will explain the aims, hypotheses and designs of the individual studies.

MICROBIOMES4SOY - Healthier diets and sustainable food/feed systems through employing microbiomes for soya production and further use

Angela Sessitsch & the MICROBIOMES4SOY Team

AIT Austrian Institute of Technology

Abstract

To better employ microbiomes for improving food systems, there is a need to better understand the interconnectedness of microbiomes across different parts of the food system and to explore how they connect. In MICROBIOMES4SOY, soya bean is the target plant to explore the microbiome pathway. Soya bean represents one of the alternative plant-based protein sources, it has a high protein content and ideal amino acid composition, and non-GM soya bean is increasingly cultivated in Europe. However, there is a need to increase the area of cultivation by addressing primarily climatic challenges, which currently limit soya bean cultivation in Europe, and to identify approaches improving the stress resilience of the plant. Furthermore, there is a need to reduce the input of fertilizers and pesticides, which is also one of the Green Deal aims. Here, the modulation of soil/plant microbiomes and microbial applications as plant strengtheners and fertilizers hold great potential. Furthermore, aquaculture is key for European and global food and nutrition security, and sustainable fisheries and aquaculture are primary food production systems that can substantially contribute to food and nutrition security by 2030. However, there is a need to make European aquaculture more competitive and sustainable, e.g., by replacing fish meal in aquafeeds with alternative protein sources. Ultimately, there is a need for healthy diets, which favour a healthy human gut microbiome, recognized as an important modifier of health and disease risk.

In a Multi-Actor Approach, MICROBIOMES4SOY aims at improving the food system based on soya bean, by providing microbiome-based understanding and innovations improving primary production and the nutritional value of soya seeds, by employing microbiomes to develop novel aquafeeds and by assessing the effect of protein dietary patterns on the human microbiome. The project will also develop transition pathways that are ready to answer food system actors' needs and deliver co-benefits relevant to the FOOD 2030 priorities.

Preservation of Microbial Consortia Associated with Fermented Foods: Apulian Table Olives as Case Studies

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Abstract

Microbial consortia associated with fermentation processes represent relevant resources in the agrifood sector. On the one hand, microbiomes selected through backslopping processes are crucial resources for important European artisanal, typical, traditional and Geographical Indication fermented productions [1]. On the other hand, complex microbial communities are considered bio-based resources for designing innovative and nature-inspired solutions in the food sector [2]. The scientific study and biotechnological valorization of microbial consortia of interest for food and beverage fermentations require the preservation guaranteed by microbial collections [3]. In this sense, innovation is needed to determine best practices to promote the conservation and revitalisation of complex microbial communities. Fermented table olives are products (plant-based and bio-processed) of considerable interest in the Mediterranean area,relevant from an economic, nutritional, functional and dietary microbe source point of view. The fermentation of these matrices is driven by complex consortia that include prokaryotic and eukaryotic microorganisms. All these aspects make table olives model foods for research activities in this sector. Here, we report original research data on the cryopreservation of microbiota associated with typical Apulian table olives, evaluating the effectiveness of protocols that include the use of glycerol and DMSO as cryoprotectants. The conservation has been assessed by using culture-dependent approaches, RNA-based metabarcoding analysis, and metabolic profiling evaluation by Biolog Ecoplate®.

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Traditional Fermented Foods as a source of beneficial microbes: a meta-analysis

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Abstract

Parmigiano Reggiano and Grana Padano are two of the world's best known Protected Designation of Origin (PDO) Italian cheeses, and are part of the Italy's cultural heritage. They are produced according to strict regulations which include a backslopping procedure, with the curd undergoing a fermentation in the autochthonous Natural Whey Culture (NWC). Each NWC shows its species- and strain-level biodiversity, strongly influencing the final sensory properties of the cheeses during the ripening. However, little is known about the potential health effects of the microorganisms (and their metabolites) inhabiting the cheeses.

In this work, we performed Whole Metagenome Sequencing on a total of 60 Parmigiano Reggiano, 42 Grana Padano and 18 Trentingrana cheeses. We performed bioinformatic analyses on the high-quality microbial reads to detect the presence of genes linked with health-promoting activities, and to unveil the microbial taxa involved.

Interestingly, we observed that all the cheeses harboured genes encoding for neuroactive compounds including γ-aminobutyric acid (GABA), propionate and conjugated linoleic acid (CLA), mainly linked to Non-Starter Lactic Acid Bacteria (NSLAB) such as Lactobacillus rhamnosus, Lactooccus lactis and Lentilactobacillus spp. Furthermore, the species found in the ripened cheeses carried genes encoding for potentially bioprotective compounds, such as Ribosomally synthesized and post-translationally modified peptides (RiPPs) including bacteriocins that could compete with spoilage/pathogenic species either in the food or after the ingestion.

Overall, our results pave the way for future research into the effects of traditionally ripened Italian cheeses on the gut-brain axis and on the overall human health.

Ackwnowledgements

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A genomic approach to non-conventional yeasts as bioresources for innovation in food and beverage fermentation: genus *Mrakia* as a case study

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Abstract

The genus Mrakia, a genus of non-conventional yeasts (NCY) in the Basidiomycota phylum, includes 20 environmental species, several with fermentative potential (De Francesco et al., 2018; Simões et al., 2023). Among these, Mrakia gelida has shown promise in beer prototype production, positioning this species as a valuable resource for innovation in beverage fermentation (Turchetti et al., 2023).

Exploring underutilized microbial species offers opportunities for innovation in fermentation, particularly in beverages, and advances in next-generation sequencing (NGS) technologies have revolutionized microbial research, enabling detailed genetic analyses and uncovering novel applications.

This study developed a genomic analysis pipeline for Mrakia and other NCYs. High-quality genomic data and advanced bioinformatic analyses are essential for comprehensive in silico evaluations of safety and industrial potential for species and strains.

We sequenced 13 Mrakia type strains from DBVPG and Westerdijk culture collections, using a hybrid approach combining Oxford Nanopore long reads and Illumina short reads to achieve high-quality genome assemblies. Repetitive sequences were masked, and average nucleotide identity analyses determined phylogenetic relationships. Gene prediction and annotation, supported by transcriptomic data, identified genetic markers linked to alcoholic fermentation, revealing Mrakia's molecular fermentative traits and emphasizing its biotechnological potential.

Future work will expand genomic and transcriptomic analyses to 7 additional Mrakia type species, improve gene annotation, and refine taxonomic classifications. This method could extend to other NCYs genera and species. A deep understanding of biodiversity, combined with a rational, genome-based approach to wet-lab characterization, can help unlock microbial bioresources' potential for safe, innovative applications in the food sector.

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Polyphasic characterisation of lactic acid bacteria diversity associated wit Subappennino Dauno sheep milk from ‘Gentile di Puglia’ breed: the design o multi-strains starter culture for Pecorino cheese and the design of a cross-ove cereal-based fermented beverage

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Abstract

Preserving and valorising Apulian agrobiodiversity is a key strategy to promote the innovation of agro-food systems associated with regional Marginal Areas (MAs), including a diverse range of animals, plants, and micro-organisms. Fermented foods, in particular, are defined as “those foods or beverages made through controlled microbial growth and enzymatic conversions of major and minor food components” [1]. Fermentation and fermented products represent interesting elements to ensure the transition of food systems toward greater environmental, economic, and social sustainability. ‘Gentile di Puglia’, literally “Gentle Apulian” in reason of the fine wool, 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) [2,3]. ‘Gentile di Puglia’, genetically adapted and resilient to the environmental conditions of marginal areas, is also recognised for cheese production. Cross-over fermentation has been defined by Dank *et al*. as “processes in which a microorganism from one traditional fermentation process is introduced onto a new substrate and/or to a new partner” [4]. 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).

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Harnessing Winery and Distillery By-Products with *Aspergillus oryzae*: Potential for development of innovative fungi-based fermented food and feed

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Abstract

Winery and distillery by-products, such as grape marc (GM), pre-distillation lees (PRE), and post-distillation lees (POST), pose environmental challenges due to their high chemical oxygen demand (COD). However, these by-products represent an underutilized resource with significant potential to contribute to sustainability. This study investigates the biovalorization of these by-products through the cultivation of the edible filamentous fungus *Aspergillus oryzae* to produce protein-rich fungal biomass via aerobic submerged fermentation. Hydrothermally pretreated and filtered substrates were used as liquid cultivation media at concentrations of 4% GM (w/v),50% PRE(v/v), and 10% POST (v/v). The effect of these substrates on fungal growth was assessed by analyzing protein, amino acids (AAs), mineral, and polyphenol content, alongside COD. The results showed that *A. oryzae* biomass had enhanced protein content (29.4–55.6%),approximately twice that of the substrates, following the order 50% PRE > 4% GM > 10% POST. Total AAs ranged from 10.9% to 28.5% dry weight, with leucine and lysine as the dominant essential AAs. The fungal biomass was rich in minerals such as phosphorus (132.4–175.4 mg/100 g),magnesium (13.4–14.1 mg/100 g), and iron (3.76–4.57 mg/100 g), and contained total polyphenols ranging from 7.54 to 36.2 mg gallic acid equivalent/g biomass. Additionally, the process achieved a COD reduction of 46–79%, mitigating the environmental impact of these by-products. This approach highlights the potential of transforming these by-products into high-quality protein, providing new opportunities for innovative applications in food and feed production, while promoting sustainable food systems within circular bioeconomy framework.

Keywords: *Aspergillus oryzae*, grape marc, wine lees, vinasse, protein rich fungi biomass.

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Exploration of lactic acid bacteria properties to design fermented flours enriched in prebiotic arabinoxylooligosaccharides from hemicellulosic agricultural by-products

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Abstract

By-products resulting from the processing of some cereals and vegetable of economic importance in EU are hemicellulose-rich materials and an interesting source of (arabino)xylooligosaccharides (A(XOS)), compounds associated with appealing bioactive properties, including prebiotics for human use. However, economic and environmentally viable valorization strategies for hemicellulose-rich materials through (A)XOS obtainment have not yet been thoroughly explored. The objective for this work was to design lactic acid bacteria fermentation processes of two agricultural by-products, brewer's spent grain (BSG) and artichoke by-products, as environmentally friendly strategies to obtain fermented flours enriched in oligosaccharides with prebiotic potential.

A collection comprising of 121 lactic acid bacteria isolates, including Pediococcus, Leuconostoc, Lactococcus, lactobacilli and Weissella species, previously recovered from spontaneous fermentations of BSG and artichoke by-products, was genotyped and screened phenotypically for traits related to metabolization of hemicellulose-derived carbohydrates, leading to selection of 18 strains for further analyses. Besides, Lactiplantibacillus pentosus DSM20314 and Levilactobacillus brevis DSM20056, described for their ability to ferment plant matrices, were also included in the investigation. Complete genome sequencing was achieved in the 20 strains and their CAZY functions (carbohydrate active enzymes) and metabolic complementarity were studied in silico. Likewise, its ability to ferment artichoke residues and BSG was examined. In all fermentations, pH dropped (between 0.15-2.35 points), which correlated with the microorganisms' growth (between 1.8-3.8 logarithmic units). The analysis of low molecular weight carbohydrates (LMWC) showed that in artichoke fermentations with three of the isolates (Lactobacillus kimchii, Lactocuccos lactis and Lactiplantibacillus plantarum) the xylose and arabinose content increased. In BSG fermentations, an increase in tri- and tetrasaccharides, arabinose and maltooligosaccharides was observed in fermentations with isolates of Levilactobacillus brevis, Lactobacillus paraplantarum and Lactococcus lactis.

With this information, fermentation conditions were designed for each byproduct, using consortia of microorganisms selected based on their metabolic capacities. Bacterial growth and formation of soluble LMWC were determined. The approach herein explored could open new avenues to develop strategies to obtain prebiotic enriched flours from hemicelluloses-rich by-products.

Sustainability and health as drivers of the rational design of fermented foods: brewer spent grain and pot ale as microbial resources to innovate in non-alcoholic beer brewing

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Abstract

Beer is the most widely consumed fermented beverage. However, as consumers are becoming increasingly aware of the detrimental effect of alcohol in their lives, the market of non-alcoholic beer has experienced a huge expansion in recent years. The removal of alcohol from beer can lead to challenges in relation to sustainability, since the high alcohol content in beer normally acts as a preservative and the high content of nutrients and oxygen in NAB can lead to microbial contamination (1). Moreover,current practices in the production of NAB, namely dealcoholisation and paused fermentation, are not currently streamlined. From the health perspective, NAB has great potential for diet improvement due to its high content in bioactive molecules (2),but the number of studies analysing its health effects is very limited. Therefore, the present project aims to address these sustainability and health challenges innovating in the microbial resources and approaches employed in NAB brewing, and by characterising its health benefits. Brewer spent grain (BSG) and pot ale (PA) are used as microbial resources for the isolation of microbes to be used during NAB brewing. Particularly, BSG from one brewery and one distillery along with PA from one distillery were used to isolate lactic acid bacteria, acetic acid bacteria and yeasts.

Isolates were identified by 16S sequencing and included species belonging to *Leuconostoc* ,*Lactococcus*, *Staphylococcus* and *Bacillus* genera. Future perspectives include full characterisation by whole genome sequencing and evaluation of GRAS traits. Lead candidates will be selected to assemble synthetic microbial consortia to be used during NAB brewing.

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Session 4. Health promoting effects of Fermented Foods

Plenary lecture

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Dietary microbes: Persistence in the gut and impact on health

Beneficial live microbes have been consumed in foods and beverages for thousands of years. More recently, observational and clinical studies have shown that consumption of these live microbes in fermented foods is associated with positive health outcomes. Nonetheless, most dietary microbes, if they indeed survive the trip, are transients and do not persist in the gastrointestinal tract. In this lecture, the dietary sources of live microbes will be reviewed, and evidence of their health properties will be presented. Strategies to enhance persistence of beneficial microbes will also be reviewed. An argument for including a recommended intake for live safe microbes in dietary guidelines will be proposed.

The impact of consumption of live dietary microbes on health: a scoping review

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Abstract

Humans consume live microbes as part of their diet, in particular through fermented foods. It has been postulated that there may be benefits associated with the consumption of live dietary microbes (1), regardless of their specific sources. However, for relevant interventions containing live microbes, the study outcomes, research end-points, methodologies, and quality of evidence in clinical trials is highly diverse. Although critical systematic reviews have previously addressed some specific health outcomes for certain dietary microbes (2), an overarching review of the evidence for health outcomes related to the more general consumption of dietary microbes was warranted. To this end, we systematically searched and collected literature from scientific databases to summarise and generate evidence maps contextualised through the research question: "Does consumption of live dietary microbes improve health in typical, non-patient, healthy populations?" (3). In the process, we also attempted address a possible adequate intake (AI) value for dietary microbes, similar to a recommended daily allowance (RDA) as suggested previously (4). Searches focused on probiotics and fermented foods as sources of live dietary microbes along with eight different health categories including gastrointestinal health, cardiovascular health and metabolic syndrome, and respiratory health, among others. The search yielded 228 relevant publications describing 282 experimental cases comprising 62 clinical end-points overall. A microbial dose of $\geq 2\times10^9$ CFU/day was associated with non-negative reported outcomes. Older population groups with a median age of 39 years were associated with positive outcomes. Additionally, the study highlighted the high levels of heterogeneity in live dietary microbe research in relation to health, associated study quality and the need to rectify them.

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Effect of Moderate Consumption of Different Phenolic-Content Beers on the Human Gut Microbiota Composition: A Randomized Crossover Trial

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The moderate consumption of beer has been associated with positive effects on health, and these benefits are driven, in part, by the antioxidant properties of phenolic compounds found in this beverage. However, the potential impact of beer polyphenols on the human gut microbiome and their consequences are yet to be elucidated. In this study, our aim was to evaluate the effect of three different phenolic-content beers on the gut microbiome and the potential role of the induced shifts in the antioxidant capacity of beer polyphenols. In total, 20 subjects (10 healthy volunteers and 10 individuals with metabolic syndrome) were randomly assigned in a crossover design to consume each of the different beers (alcohol-free, lager or dark beer) during a 2-week intervention. Significant changes in the relative abundance of Streptococcaceae and Streptococcus were found after beer consumption. An increased abundance of Streptococcaceae and Streptococcus was observed after the consumption of dark

beer, with no detected differences between baseline and alcohol-free/lager beer intervention. Moreover, some of the detected differences appeared to be related to the metabolic status. Finally, a decrease in porphyrin metabolism and heme biosynthesis was found after the intervention, especially after the consumption of dark beer. These results show that the antioxidant capacity of beer polyphenols may induce positive shifts in gut microbiota composition, and some of the observed changes may also boost the antioxidant capacity of these compounds.

The Effect of Fermented Vegetables on Human Health and Gut Microbiota

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Abstract

The impact of fermented foods on human health, particularly through gut microbiota, is a widely discussed topic. However, the number of nutritional studies demonstrating their health benefits remains limited. This study evaluated the effects of fermented vegetables (FVs) intake—including fermented baby carrots, kohlrabi, and kimchi—on human health parameters, primarily focusing on gut microbiota. In addition to microbiota analysis, we assessed anthropometric parameters, stool frequency, and data from nutritional diaries. A total of 66 volunteers were recruited, of whom 55 completed the study.

FVs are a valuable health product, combining dietary fibers and lactic acid bacteria, both essential for supporting healthy gut microbiota. To better understand the effects of FV consumption in different populations, participants were divided into three groups: healthy controls, individuals with constipation, and those recovering from an antibiotic course. Multiple analytical approaches were applied to evaluate: (1) the effects of FV consumption within the three focus groups, (2) the response of different gut enterotypes to a three-week FV consumption period based on taxonomic hierarchical analyses, and (3) the combined effects across the entire cohort. The choice of analyzed groups was crucial for interpreting the results, revealing varied effects depending on the context. Overall, the findings showed that consuming FVs modified gut microbiota composition, increasing the abundance of butyrate-producing and anti-inflammatory bacterial species. Additionally, phase angle—a key indicator of cellular health reflecting cell integrity and hydration—showed statistically significant improvement across the cohort, indicating better metabolic health after the intervention. Food diaries further revealed reduced sugar consumption among participants, highlighting an additional benefit of enriching diets with healthy fermented foods. These results demonstrate the clear positive impact of fermented vegetable consumption on human health, particularly through gut microbiota modulation and metabolic improvements.

Acknowledgements

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Development of a fermented food with validated beneficial impact on health: a case study with a cheese including a Lacticaseibacillus casei capable to enhance muscle insulin sensitivity.

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Abstract

Functional decline and sarcopenia are inversely correlated with quality of life during aging, especially in population with low appetite. Nutritional strategies based on adapted energy and protein intake are insufficient to limit sarcopenia, explained by a lower efficiency in using nutrients. The atypical gut microbiota from intestine-resected patients (i.e., 90% Lactobacilli), when transferred to axenic rats, can improve the use of dietary nutrients and metabolism1. We hypothesized that bacteria strains selected from this specific microbiota could be a source of probiotics capable to improve nutrients handling and limit sarcopenia in older adults2.

The strategy employed was: Bacteria were selected on their capacity to preserve Caenorhabditis elegans longevity and muscle integrity (Lacticaseibacillus casei CNCM I-5663 (L63) selected). One month supplementation of L63 was tested on old rats subjected to 25% food restriction vs ad libitum (AL). Food restriction induced a 10% decrease in muscle mass (R vs AL $p < 0.05$), an effect tempered to -2% with concomitant daily supply of 109 CFU L63 (R+L63 vs AL $p<0.05$). In muscle, the activation of the Akt/mTOR/S6 pathway was observed in R+L63 vs R ($p<0.05$). Therefore, L63 can preserve muscle mass via a stimulation of protein synthesis3. To allow validation in humans, the strain (obtained from humans) was tested for its capacity to be incorporated in the production of a lactic soft cheese, with preliminary results on sensory analyses. Current work aims at testing the safety of L63 and resistance to gut environment (low pH and bile acids) before testing in humans.

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A new strategy for the development of functional fermented foods to support human health

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Abstract

Fermented foods have a variety of positive effects on human health, which are not only attributed to the presence of bacteria but also to microbial metabolites that can otherwise also be produced by the human gut microbiota.

In an *in-silico* study we compared the genomes of 600 strains derived from the Liebefeld Culture Collection, which comprises >15,000 isolates mainly derived from the cheese environment, 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 thus hypothesized that fermented foods can be produced that complement the metabolic activity of dysbiotic gut microbiota. Consequently, we have enhanced the production of selected bioactive compounds in fermented milk (vitamins, amino acids derivatives) by selecting strains based on the presence of genes encoding enzymes in the targeted metabolic pathways.

The functionality of the fermented milks was then evaluated in mice 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.

We have finally 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 with powerful bioinformatic tools improves our capability to develop functional fermented foods.

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A multi-omics approach to study different Greek PDO fermented foods and wines

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Abstract

Greece has a long tradition producing fermented foods and wines some of which have been awarded with protected designation of origin (PDO) status. While some of these products have been under rigorous research, others have not yet been systematically studied for their overall properties. Here we present a multi-omics approach to rapidly determine aspects of the biodiversity and technological properties of the microbiome of certain PDO products, including Sfela white brined cheese, Kalamata natural black table olives and two wines (red Agiorgitiko of Nemea and white Moschofilero of Mantinia). We first developed methods for shotgun metagenomics analysis in the absence of interfering host DNA, especially for table olives and wines. The microbiome profiles revealed the presence of both lactic acid bacteria and yeast with one group often dominating depending on the product. In certain instances, culture-based analysis followed by the identification of microbial isolates with MALDI-TOF MS did not fully corroborate metagenomic findings, indicating the presence of dead cells or viable but non-culturable cells (VBNC) in the samples analyzed. Furthermore, the rapid volatilomics analysis of the products with GC-IMS identified several compounds that in almost all cases were specific for the samples of each producer. In several instances, the volatilomics fingerprints could be correlated to certain microbial taxa and metabolic pathways of metagenome-assembled genomes (MAGS). Overall, our approach produced important amounts of new information about the products under investigation.

In vitro human gastrointestinal digestibility and colonic fermentation of whey, casein and edible yeast-based proteins

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Abstract

The growing global demand for protein has led to the exploration of sustainable and healthier alternative protein sources, like yeast-based proteins (YBP). YBP are considered an environmentally-friendly,fermentation-based solution with high nutritional quality and bioavailability. While in vitro studies
cannot fully replicate the complexity of in vivo digestion,they serve as useful alternatives to animal models for assessing protein digestibility.

This study aimed to evaluate the digestibility profile, amino acid bio-accessibility, and the impact on gut microbiota of YBP compared to whey and casein as reference proteins. A 5-hour TIM-1 digestion model was used to assess the digestibility and amino acid bio-accessibility of three separate YBP production batches, whey, and casein. Dialysate and ileal effluent samples were collected hourly, and total nitrogen and free amino acids were quantified.

To evaluate the microbial impact, the YBP digestate was subjected to a 48-hour Colon-on-a-Plate batch fermentation. The microbial composition was analysed by shotgun sequencing, and the microbial activity was assessed through the quantification of short-chain fatty acids(SCFAs),branched-chain fatty acids (BCFAs), and untargeted metabolomics using LA-REIMS,using faecal samples from 11 healthy donors.

The results showed that YBP, casein, and whey were comparable in terms of digestibility.

Although YBP exhibited a different metabolomic fingerprint compared to casein and whey, the microbial activity, as measured by SCFA quantification, was significantly increased for all protein sources compared to the blank, but not significantly different between the protein sources. Whey and casein resulted in a higher abundance of certain bacterial taxa compared to YBP.

Keywords: Alternative protein, digestibility, fermentation, yeast-based protein, microbiota,GLP-1

Grana Padano and Parmigiano Reggiano cheeses microbiomes exhibit neuroactive potential and bioprotective activities

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Abstract

Parmigiano Reggiano and Grana Padano are two of the world's best known Protected Designation of Origin (PDO) Italian cheeses, and are part of the Italy's cultural heritage. They are produced according to strict regulations which include a backslopping procedure, with the curd undergoing a fermentation in the autochthonous Natural Whey Culture (NWC). Each NWC shows its species- and strain-level biodiversity, strongly influencing the final sensory properties of the cheeses during the ripening. However, little is known about the potential health effects of the microorganisms (and their metabolites) inhabiting the cheeses.

In this work, we performed Whole Metagenome Sequencing on a total of 60 Parmigiano Reggiano, 42 Grana Padano and 18 Trentingrana cheeses. We performed bioinformatic analyses on the high-quality microbial reads to detect the presence of genes linked with health-promoting activities, and to unveil the microbial taxa involved.

Interestingly, we observed that all the cheeses harboured genes encoding for neuroactive compounds including γ-aminobutyric acid (GABA), propionate and conjugated linoleic acid (CLA), mainly linked to Non-Starter Lactic Acid Bacteria (NSLAB) such as *Lactobacillus rhamnosus*, *Lactooccus lactis* and *Lentilactobacillus* spp. Furthermore, the species found in the ripened cheeses carried genes encoding for potentially bioprotective compounds, such as Ribosomally synthesized and post-translationally modified peptides (RiPPs) including bacteriocins that could compete with spoilage/pathogenic species either in the food or after the ingestion.

Overall, our results pave the way for future research into the effects of traditionally ripened Italian cheeses on the gut-brain axis and on the overall human health.

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Enrichment of sourdough with probiotic strains: impact on microbiological an physicochemical properties

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Abstract

Sourdough is a traditional fermentation process that has been used for centuries to produce a variety of baked goods. The unique flavor and texture of sourdough bread results from complex microbial interactions between lactic acid bacteria (LAB) and yeasts. This study aimed to enrich sourdough with probiotic strains of LAB, yeasts, or co-cultures, and to evaluate the resulting prototypes microbiologically and physicochemically. LAB and yeast strains were isolated from naturally fermented sourdough to ensure the selection of robust,adaptable microorganisms. These strains were used to develop prototype formulations tested both in monoculture and co-culture to assess their individual and synergistic effects on sourdough properties.

The prototypes were inoculated with *Lactiplantibacillus plantarum* MMES4 and *Maudiozyma humilis* T80.10. Flowcharts outlining the preparation and fermentation processes were provided to facilitate future scaling and standardization in industrial applications. The physicochemical properties,including pH, moisture content, and texture, were thoroughly analyzed to understand the impact of microbial enrichment. Additionally, microbiological evaluations assessed the viability and activity of the probiotic strains during fermentation and baking processes.

The results demonstrated that enriching sourdough with selected probiotic LAB and yeast strains,whether in monoculture or co-culture, significantly improved the physicochemical properties compared to the control. These findings highlight the potential of incorporating potential pr0obiotics into sourdough to enhance its microbiologically and physicochemically qualities while meeting consumer demands for functional, health-promoting baked goods.

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We are grateful for the financial support and the collaboration of all institutions and researchers involved, which have made this study possible.

The Antimicrobial Properties of Different Strains of Yeasts Isolated from Commercial and Homemade Kefir from North Macedonia and Their Characterization as Potential Probiotics

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Abstract

Kefir is a fermented milk beverage produced by the action of bacteria and yeasts contained in kefir grains, with a unique taste and unique properties. They typically contain water (89-90%w/w), lipids (0.2% w/w), proteins (3.0% w/w), sugars (6.0% w/w), and ash (0.7% w/w), and vary in size from 0.3 to 3.5 cm in diameter. Kefir yeasts are less studied than kefir bacteria, and therefore this work focused on the isolation and *in vitro* characterization of yeasts obtained from commercial and homemade kefir from North Macedonia and their characterization as potential probiotics, in comparison with the commercial probiotic *Saccharomyces boulardii*.

Resistance to acid and bile salts, thermotolerance test, salt tolerance test, as well as cell adhesion test were investigated. A safety assessment was also carried out to confirm the non-pathogenic nature of the isolates, with hemolysis assay and resistance to antifungal drugs. The yeast strains isolated in this work also showed antimicrobial activity against enteric pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* and against fungi *Aspergillus flavus*, *Aspergillus niger*, *Penicillium expansum* and *Penicillium commune*.

This study found that homemade kefir contains yeasts that have better probiotic properties than yeasts isolated from commercial kefir. This suggests that homemade kefir may be a useful source of various probiotic yeasts that can improve human health and well-being. Yeasts are particularly promising because they are not affected by antibacterial agents, and this property is relevant since some therapies combine the administration of probiotics with antibiotics in the treatment of gastrointestinal infections.

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A review of the current evidence base for the health benefits of the cultured dairy food cottage cheese

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Abstract

Cottage cheese (CC) is a member of the "fresh cheese" family of cheeses, and a cultured dairy product, depending on production methodology (1). CC is widely consumed (2) due to its culinary versatility and some perceived health benefits, yet the evidence of direct health effects is not well established. We performed a review describing the production and nutritional characteristics of CC, before exploring the current evidence of health effects from human intervention, *in vitro*, and *in vivo* models. Despite widespread consumption and advocated health benefits, there is a lack of evidence from high-quality human randomised controlled trials. To date, a limited number of human intervention models with CC have explored nutrient bioavailability, metabolic health, and appetite regulation, in small, niche study populations. Findings with *in vitro* and *in vivo* models suggest that CC may be an efficacious vehicle for bioactive compounds. In conclusion, CC may be an impactful cultured dairy product that can impose a myriad of benefits across health outcomes including cardiometabolic, gastrointestinal, body composition, appetite regulation, and nutrient status. However, there is a need for high-quality human randomised controlled trials to develop a substantiated evidence base relating to the full potential of CC to impact human health.

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Comparative phenolic composition of the fermented pomegranate juic by lactic acid bacteria and their antioxidant capacity

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Abstract

Pomegranate (*Punica granatum*) is a fruit rich in polyphenols, known for its high antioxidant capacity and health benefits (Laurindo et al., 2024). Recently, a functional fermented pomegranate juice (FPJ) was formulated using lactic acid bacteria (LAB) isolated from fruits and vegetables with high biological benefits (Isas et al., 2023). However, changes in the polyphenol profile due to lactic fermentation have not been thoroughly evaluated. This study assessed the impact of fermentation with LAB strains, *Lactiplantibacillus paraplantarum* CRL2051 and *Lactiplantibacillus plantarum CRL2030*, on the polyphenol profile of pomegranate juice (PJ) using LC-ESI-QToF. Fermentation was carried out at 30 ºC for 48 h, resulting in fermented pomegranate juices (FPJ-CRL2051 and FPJ-CRL2030). Their phenolic profile and antioxidant capacities were compared with those of unfermented juice (PJ) at 0 h and 48 h. A total of seventy-one polyphenols were identified, with anthocyanins being the most abundant (62.5–69.2%), followed by tannins (18.3–25.3%), hydroxycinnamic acids (4.8–5.6%), flavonols (3.4-3.9%), flavones (1.2-1.5%), flavanols (1.0%), other phenolic acids (0.4-0.6%), and flavanones (0.2-0.3%). Fermentation did not alter the polyphenol profile of any formulated juice. Quantification by LC-ESI-QToF revealed higher polyphenol levels in FPJ-CRL2051 (988±69mg/L) and PJ-0h (966±73mg/L) compared to FPJ-CRL2030 (906±195mg/L) and PJ-48h (875±135mg/L), although differences there were not statistically significant. Antioxidant capacity, evaluated using FRAP and ABTS assays, remained consisted across samples. These results indicate that LAB fermentation preserves the phenolic composition and antioxidant activity of pomegranate juice, supporting its potential as a functional beverage with health-promoting properties.

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Sourdough Enhances Functional and Technological Properties of Gluten-Free Sorghum Bread

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Abstract

Sourdough technology is an ancient biotechnological practice integral to producing cereal-based fermented foods. Lactic acid bacteria (LAB) in sourdough contribute significantly to food safety,texture, aroma, and bioactive properties. This study investigated the impact of sourdough addition on the technological and functional properties of gluten-free sorghum bread. Red sorghum flour was processed into bread supplemented with sourdough at varying levels (10%, 20%, and 30%),using *Lactobacillus casei* and *Lactobacillus acidophilus* as starter cultures. Functional properties,including antioxidant activity and total phenolic content, along with rheological (dough hardness and stickiness) and textural (hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness) parameters, were evaluated. Bread fermented with 30% *L. casei* sourdough exhibited the lowest hardness (374.73 g), gumminess (247.70), and chewiness (236.14) values, with springiness and cohesiveness remaining similar to the control. FTIR analysis revealed molecular changes in protein structure due to sourdough addition. The total phenolic content ranged from 70.42 to 76.44 mg GAE/100 g DW. Notably, bread with 10% *L. casei* sourdough displayed the highest radical scavenging activity (37%) compared to the control (33%). These results suggested that sourdough sorghum breads were rich in phytochemicals and suitable for individuals with celiac disease, offering enhanced functionality and safety while maintaining desirable sensory attributes.

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Effects of fermentation and age on postprandial responses to milk and yogurt in young and older men

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Abstract

Fermentation of foods is believed to have beneficial effects on metabolic health. This study assessed 6-h postprandial responses of young adults (YA) and older adults (OA) to milk and yogurt intakes[1]. In a randomized crossover design, 28 men (14 YA, 20-35 years; 14 OA, 65-80 years) consumed 600 mL of whole milk or yogurt after an overnight fast, with a 1-week washout. Triglycerides, glucose, and insulin were measured in both study arms, while free fatty acids (FFAs) were measured in the yogurt arm only. Responses were analyzed using a 2×2 factorial design. At baseline, BMI and metabolic parameters were similar across age and product groups ($p > 0.05$). Compared to milk, yogurt intake resulted in lower postprandial glucose (incremental area under the curve [iAUC] = -1.04 vs -1.18 mmol·6h/L) and insulin (iAUC = 135.2 vs 115.1 pmol·6h/L) responses (p-product and p-product*time <0.05), with no differences observed for triglycerides. However, triglyceride responses were lower in YA compared to OA (iAUC = 0.37 vs 1.18 mmol·6h/L, p-age and p-time*age <0.001). A similar age effect was observed for six FFAs (FDR-age and FDR-time*age <0.05). These FFA responses strongly correlated with triglycerides in OA (rm = 0.382 to 0.754), whereas only one significant correlation was observed in YA (rm =0.342). The findings suggest that dairy fermentation may modulate postprandial metabolic responses, supporting its role in promoting metabolic health. However, the distinct responses in OA underscore the need for tailored dietary strategies addressing age-related metabolic changes to support healthier aging.

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Benefits of unfermented and fermented pomegranate juice on redo status in human colonic cells

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Abstract

Pomegranate (*Punica granatum*) is a fruit rich in polyphenols that has demonstrated antioxidant activity and health benefits, including chronic bowel diseases (Laurindo et al., 2024; Toschi et al., 2022). Recently, a functional fermented pomegranate juice (FPJ) was formulated using lactic acid bacteria (LAB) isolated from fruits and vegetables, demonstrating biological benefits (Isas et al., 2023). This study aims to evaluate the effect of a pomegranate juice fermented with *Lactiplantibacillus paraplantarum* CRL2051 (30ºC for 48h, FPJ-CRL2051) on the redox status in colonic Caco-2 cells under physiological conditions (juice isolated) and after tert-butyl hydroperoxide (*t*-BOOH)-induced oxidative stress (400µM, 3h). Redox status was compared with that of the unfermented juice at 0h (PJ0) and 48h (PJ48). Under physiological conditions, Caco-2 cells were incubated for 24h, and any juice induced irreversible cell damage (viability remained unchanged up to 100µg/mL). At realistic concentrations (0.01-10µL/mL), PJ0 and PJ48 decreased reactive oxygen species (ROS) generation and glutathione peroxidase (GPx) activity, increased the glutathione (GSH) content, and did not modify glutathione reductase activity (GR), whereas FPJ-CRL2051 reduced GSH levels and enhanced GPx at the highest concentration tested. Additionally, cell pre-treatment with PJ0, PJ48 and FPJ-CRL2051 for 21h ameliorated the reduction in cell viability induced by *t*-BOOH. For PJ0 and PJ48, this protective effect was associated with decreased ROS and protein oxidation, and increased GPx and GR activities in comparison to t-BOOH-stimulated cells, but oxidative stress was not ameliorated by FPJ-CRL2051 incubation. Overall, these results highlight the potent antioxidant activity of pomegranate juice and its potential as a health-promoting beverage.

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Valorisation of legumes using the fungal solid-state fermentation

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Abstract

Lower filamentous fungi belonging to group Zygomycetes (*Mortierella*, *Actinomucor*, *Umbelopsis*, etc.) are well known as excellent producers of polyunsaturated fatty acids such as gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid etc., sterols (ergosterol, desmosterol etc.), carotenoid pigments, coenzyme Q, enzymes (e.g. amylases, proteases, lipases) and amino-polysaccharides (chitin, chitosan). One of the promising technology for production of these metabolites are based on fungal solid-state fermentations (SSF). SSF is fermentation technique employing microorganisms grown on solid substrate with absence of free water. Since these conditions represent the natural habitat of fungal strains, the production of important nutrients mentioned above (from human point of view) represents promising way of natural enrichment of solid matrixes that are ready-to-use in food industry.

Our current work focuses on employment of three different fungal strain (*Mortierella alpina*, *Actinomucor elegans*, *Umbelopsis isabellina*) in solid-state fermentation process, where four different types of legumes (white bean, red lentil, yellow pea, green pea) serves as solid matrix. Each of obtained fermented products were enriched with the polyunsaturated fatty acids – gamma-linolenic or arachidonic acid, coenzyme Q10, carotenoid pigments. Furthermore, the antioxidant potential of prepared fermented products has increased.

Thus the application of lower filamentous fungi represents very intriguing method for increasing the nutritional value of legumes.

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An optimised procedure to prepare pre-digested (fermented) foods fo studying their impact on gut microbiota during *in vitro* colon fermentation.

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Abstract

Fermented foods are suggested to positively influence gut health[1]. In baked fermented foods (e.g. sourdough bread) that no longer contain living microorganisms, this might result from the production of fermentation metabolites and modifications in the food matrix, such as changes in the fibre composition and structure[2]. *In vitro* batch colon fermentation (IVCF) is emerging as a valuable tool to investigate the mechanisms responsible for the effect of fermented food on gut microbiota[3]. Such procedures require a sample pre-treatment that removes digestible components and retains indigestible (both soluble and insoluble) components to resemble upper gastro-intestinal digestion. However, the importance of these sample pre-treatments (e.g. pre-digestion, dialysis) is often overlooked and previous studies that have considered it, used different protocols[3]. Therefore, this study aimed to understand the impact of different methods to obtain the *undigested fraction* on the outcomes of an IVCF experiment. Oats were selected as raw material because of their high soluble fibre content and INFOGEST was used to mimic digestion. Oat flour, the digested pellet of oat flour, the digested pellet of oat flour with soluble fibres (obtained through dialysis of the digested supernatant), and the digested pellet of oat flour with 10% of the digested supernatant were fed into an anaerobic IVCF. SCFA composition was determined after 24 and 48 h. The results highlight the impact of *in vitro* pre-digestion and the collection method on the composition (i.e. arabinoxylan, β-glucan, starch, protein) of the *undigested fraction* and outcome of the IVCF.

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Novel whey fermented beverage enriched with a mixture of juice concentrates: evaluation of antimicrobial, antioxidant and ACE activities before and after simulated gastrointestinal digestion

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Abstract

This study was conducted to develop and evaluate the functional properties of a novel whey-based fermented beverage enriched with juice concentrates and health-promoting ingredients, during cold storage for 28 days. Whey produed during cheesemaking, generates huge volumes of a waste product that must be valorised in order to minimise the environmental impact and at the same time utilise the nutritional value of the whey's ingredients. The formulation included whey protein isolate (5%), juice concentrates (10% apple, raspberry, and cranberry), and inulin (4 %). Fermentation was carried out with the following probiotic strains: Lacticaseibacillus rhamnosus (LGG), Lacticaseibacillus casei (431), and Lactobacillus helveticus (R0052) at a concentration of 2%. Antimicrobial activity was evaluated against food borne pathogens including Listeria monocytogenes (strains 33423 and 33413), Staphylococcus aureus (113 and Newman), Bacillus cereus (DPC 6089), Escherichia coli (NCTC 9001), and Salmonella enteritidis (NCTC 6676). Antioxidant capacity was measured using DPPH and FRAP assays, and angiotensin-converting enzyme (ACE) inhibitory activity was assessed before and after in vitro digestion during the whole storage period. Results showed a significant increase in antioxidant, antimicrobial and ACE-inhibitory activity following simulated gastrointestinal digestion. Our findings demonstrate significant potential for developing a novel functional fermented whey product.

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Exploring the probiotic potential of Boza, a traditional fermented drink

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Abstract

Boza is the traditional fermented drink of Central Asia, dating back 8,000–9,000 years, and had spread throughout cultures including the Balkans and Eastern Europe [1]. Based on fermentation of cereals like millet, bulgur, corn, and barley by lactic acid bacteria and yeasts, boza has much more nutritious, phenolic acids, and antioxidants, thus, it confers many digestive, nutritional,and disease-preventive benefits [2].

Fermentation was induced by adding starter cultures, which were incubated for three days, into previously boiled and strained cereal bases [3]. Isolation of the microorganisms was done on selective media, yielding 46 bacterial and 12 yeast isolates. Morphological analysis revealed 95%of Gram-positive cocci and 5% of Gram-positive bacilli among bacterial isolates and oval to spherical yeast cells.

Physiological and biochemical characterization showed high probiotic potential, including tolerance to low pH (4 isolates at pH 2, 13 isolates at pH 3), bile salts, and different concentrations of NaCl. Besides, most of the isolates presented good sugar fermentation profiles and catalase-negative activity. Health safety evaluations depicted all 16 isolates as being γ-hemolytic *in vitro*, most showed multiantibiotic-resistant potential. All these 16 isolates demonstrated high antimicrobial activities against various human pathogenic microbes including *Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa*. In contrast,no antimicrobial effect was shown on *Candida albicans*.

These results show the potential of boza as a probiotic beverage with prominent health-promoting and disease-preventing features, thus requiring further investigation into the possibility of different biotechnological applications.

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Development of a health fermented beverage from oat suitable fo individuals with celiac disease

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Abstract

Consumer demand for healthier gluten-free foods has notably increased in recent years. Oat is a gluten-free cereal with unique nutritional and health-promoting characteristics. However, it has been scarcely used as ingredient in gluten-free foods due to the controversy regarding oats safety for celiac individuals[1]. This study was focused on the development of a gluten-free fermented oat beverage through technological processes including germination and fermentation by *Lactiplantibacillus plantarum* WCFS1. The nutritional and bioactive composition, as well as the safety and health-promoting properties of the beverage were explored by *in vitro* assays and in celiac individuals through a nutritional intervention study. Results showed that the oat beverage was a good source of protein, thiamine, riboflavin, β-glucan, and phenolic compounds. This beverage exhibited anti-inflammatory activity in LPS-stimulated RAW 264.7 murine macrophages. Furthermore, it reduced cholesterol levels and improved gut microbiota composition in celiac-suffering volunteers. This study shows the beneficial health effects derived from fermented oat products for celiac population.

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Comparative microbial and functional characterization of kombucha from different starter SCOBYs

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Abstract

The scientific community is showing a growing interest in fermented foods, with attention to the beneficial potential of the microorganisms present in these products. My doctoral project focuses on kombucha, a fermented beverage of Chinese origin produced through the symbiotic fermentation of yeasts and bacteria in sweetened tea [1]. This process is driven by a microorganism mixed culture known as SCOBY ("Symbiotic Culture of Bacteria and Yeasts"), which forms a cellulose film produced by bacteria and acts as a fermentation starter. Kombucha's microbial composition is highly variable and can include numerous genera of yeasts, lactic acid bacteria, and acetic acid bacteria [2]. This variability calls for in-depth studies of SCOBY's microbial biodiversity to better understand the fermentation process and the properties of kombucha. The project aims to examine intra - and inter-SCOBY diversity to characterize microbial communities for their fermentation performance and for their potential health benefits. In addition, the impact of repeated SCOBY use on kombucha biodiversity is evaluated. Two SCOBYs and the derived kombucha were analyzed for their physiological and functional features by assessing the viable cell count, determining nitrogen content to evaluate nutrient availability, and measuring specific enzymatic activities. Additionally, a metabolite profiling was conducted *via* HPLC and GC-MS in order to highlight specific differences between the two different SCOBYs. Finally, kombucha's safety and functional properties have been assayed on cell lines

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Probiotic survival and impact on gut microbiota and metabolites during *in vitr* fermentation

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Abstract

Mechanistic studies are essential to fully understand how food-derived bacteria can modulate the gut environment and confer health benefits. In this study, we aimed to explore the survival of the probiotic *Lactiplantibacillus plantarum* Tensia and a potential probiotic *Lacticaseibacillus paracasei* A11 in a gut model system, their interactions with the gut microbiota, and metabolite formation.

The strains have been shown to survive the harsh digestive conditions (Infogest) when incorporated into a cheese matrix. They were subjected to *in vitro* batch fermentations (10^7 CFU/ml) alongside undigested cheese constituents and human fecal bacteria. High survival of A11 was observed over three days of fermentation, as confirmed by agar plating and MALDI-TOF MS identification, whereas a decline was noted for Tensia. This trend was corroborated by 16S rRNA microbiota sequencing, although both strains significantly impacted the overall gut microbiota composition, including an increase in *Bifidobacterium*, compared to the control (undigested cheese without the strains). Enhanced short-chain fatty acid (SCFA) production was observed with the inclusion of either Tensia or A11. Ongoing NMR metabolomics studies aim to elucidate the temporal interactions between microbiota and metabolites, providing further insights into the impact of these "probiotics" on the gut environment.

We believe this experimental approach offers a promising supplement to the studies that primarily focus on survival in the upper digestive system.

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Lactofermentation of agricultural waste (broccoli leaves) as a strategy for the extraction of phytochemicals with health-promoting bioactivities

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Abstract

Broccoli (Brassica oleracea var. italica) is valued for its health benefits due to its high content of sulphur-containing compounds, polyphenols, vitamins and minerals. However, only 15% of the crops, mainly the florets, are used, leaving a substantial biomass, such as stems and leaves, rich in bioactive compounds. Fermentation offers a promising approach to valorise these by-products by extracting their phytochemicals and transforming them into functional foods and food ingredients with health benefits (Salas-Millan et al 2022). This study investigates the anticancer potential of a lactofermented broccoli leaf extract (LFB) by assessing its effects on cell viability, oxidative stress and molecular pathways in human intestinal epithelial HT-29 cells. The study aims to correlate the bioactive composition of LFB with its cytotoxic mechanisms.

LFB treatment significantly reduced HT-29 cell viability, with effects associated with several bioactive compounds, particularly isothiocyanates. Proteomic analysis showed that LFB induced cytosolic calcium elevation, mitochondrial stress, decreased membrane potential and increased reactive oxygen species (ROS). This pro-oxidant environment triggered ferroptosis, endoplasmic reticulum (ER) stress via lipid oxidation and the unfolded protein response. Inhibition of key pathways implicated ER stress as the primary driver of cytotoxicity, autophagy as a resistance mechanism and necroptosis as an early cell death pathway.

These findings highlight the role of agro-industrial by-product valorisation in the development of innovative health-promoting foods within a circular economy framework, using lactofermentation as an assisted extraction of phytochemicals.

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Enhancing Functional Health Properties of Blueberry Juice via Probiotic Fermentation

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Abstract

Several new plant-based fermented foods have entered the market in recent years, due to the global revival of interest in fermented dairy alternatives and for the purported health claims1. In this framework, fermented blueberries may hold a special place: often marketed as a superfood, blueberries include several health-promoting phytochemicals (vitamins, antioxidants, beneficial plant compounds) that could be maximized through fermentation2.

The present study aims at characterising the functional profile of a fermented blueberry juice by native Leuconostoc mesenteroides and industrial probiotic Lactiplantibacillus plantarum strains combining both ex vivo and in vitro approaches (impact on microbiota diversity through an ex vivo colon model, evaluation of cytotoxicity, barrier integrity and antioxidant activity on CaCo-2 cell lines).

The health impacts of the fermented juice were assessed using the micro-Matrix™ bioreactor platform as an ex vivo colon fermentation system, simulating colonic conditions (controlled pH, temperature, and oxygen levels) with pooled faecal inoculum. The results revealed a significant modulatory effect on the gut microbiota, notably reducing Clostridium perfringens and Escherichia spp. after 24 hours. In vitro analyses on CaCo-2 cell lines further validated the health benefits: the fermented blueberry juice was non-cytotoxic, it protected the epithelial barrier integrity under lipopolysaccharide (LPS)-induced stress, and exhibited high antioxidant activity, though slightly lower than that of the unfermented commercial blueberry juice.

Our results suggest that probiotic-fermented blueberry juice can be a promising functional product that need to be further validated through a targeted clinical trial. This research highlights the potential for fruit- based fermented beverages to serve not only as refreshing drinks but also as health-promoting products in an increasingly health-conscious market.

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Health effects of Ethnic Fermented Foods

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Abstract

Food science and technology have evolved to enhance food availability, nutrition, safety, and marketability, addressing global challenges such as hunger and malnutrition. Projections indicate that by 2030, over 670 million people may face hunger, emphasizing the need for innovative and sustainable solutions. Ethnic fermented foods (EFF), originating from the heritage and culture of ethnic groups who use their ethnomicrobiological knowledge of fermentation with local plant or animal-based ingredients, provide a useful means of promoting a healthier and more inclusive global food system. These foods are produced using traditional techniques, combining local ingredients with artisanal practices. However, urbanization and changing food habits threaten their preservation, along with the diversity of native microorganisms essential for their production.

This research systematically examines the health effects of EFF. Despite the global diversity of EFF, such as natto, kimchi, and tempeh, originating from Asia, many remain understudied. A comprehensive review aims to identify the health benefits and potential risks associated with these foods. A systematic search in PubMed, Scopus, and the Cochrane Library Central resulted in 3,488 entries, which were narrowed to 423 abstracts and 187 full texts after screening by two independent reviewers. CADIMA software was used to select references. Data synthesis will focus on clinical indications and primary outcomes, offering valuable insights of health benefits of EFF.

This systematic narrative review will highlight the importance of EFF in improving health, emphasizing their role in sustainable food systems, and underscoring the need to preserve traditional practices.

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Nutritional changes during fermentation of phycobiliprotein-rich extracts from *Arthrospira platensis*

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Abstract

Arthrospira platensis, known as spirulina, is a protein-rich cyanobacterium with numerous applications in the food and nutraceutical sectors [1]. While fermentation is an ancient preservation technique that enhances food materials' digestibility and nutritional value, its impact on microalgae biomass or its fractions is poorly understood [2].

This study aimed to evaluate the physicochemical changes occurring during the fermentation of spirulina phycobiliprotein-rich extract. The isolated protein-rich extract was fermented with three lactic acid bacteria: *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, and *Levilactobacillus brevis* under anaerobic conditions and glucose supplementation. Samples were collected and analysed at three time points from 0 to 48 hours. Microbial counts indicated significant growth for all strains ranging from 8.8 to 10.1 log CFU/mL with a significant pH reduction. The maximum pH drop was observed in the sample fermented with *L. plantarum*(3.84 ± 0.03) after 24 hours. The pH reduction was directly associated with lactic acid production, as verified by liquid chromatography. Moreover, the pigments phycocyanin and allophycocyanin were decreased over time due to sensitivity to pH and proteolytic activity during fermentation. HPLC-MS/MS verified the release of free essential and non-essential amino acids. Moreover, the same technique verified the presence of B complex water-soluble vitamins, with pyridoxine (B6) produced by fermenting microorganisms during fermentation. Lastly the antimicrobial properties of the obtained fermentates have been revealed.

On the other hand, LC-MS/MS analysis showed that biogenic amines (primarily tyramine and histamine) are also produced during fermentation, though at safe levels. These results suggest that spirulina protein may be a suitable fermentation substrate, highlighting the fermentation's impact on its physicochemical properties. Nevertheless, further studies are necessary to evaluate the bioactivity and safety of the fermented extracts.

Acknowledgements

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The use of infusions from mulberry, raspberry, nettle and rooibos leaves to obtain fermented kombucha-type beverages

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Abstract

The health-promoting properties of kombucha (e.g. aiding digestion, detoxifying the body, boosting immunity, and antibacterial properties) are causing an increase in consumption of this beverage worldwide. The most commonly known are kombuchas, obtained by fermenting teas, including black and green. However, there are many valuable plant species with high bioactive potential whose leaves can be used for infusion and inoculation with SCOBY culture. Nettle leaves have anti-inflammatory, antibacterial properties and support the urinary system. Mulberry leaves-derived products in the form of powders, extracts and capsules are commercially available as functional foods and dietary supplements for controlling body weight and blood glucose. Raspberry leaf infusions relieve symptoms of colds, diarrhea, and colic pain. Rooibos is caffeine-free and low in tannins, supporting the circulatory and digestive systems. The infusions were prepared by pouring 200 mL of boiling water over 2 g of mulberry/raspberry/nettle/rooibos leaves. Sucrose was used as a source of sugars at a dose of 10 g/200 mL. The brewing process lasted 5 minutes. The cooled (±25°C) beverages were centrifuged, and then the SCOBY culture was added. The fermentation process lasted 7 days at a temperature of 25°C.
In beverages the concentrations of organic acids, sugars and ethanol were determined by HPLC. The antioxidant activity of non-fermented beverages and kombuchas was assessed using FRAP and ABTS methods. Total polyphenol content was analyzed using the Folin-Ciocalteau method. The aroma profile of kombuchas was verified using an electronic nose.

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Insights into the impact of sourdough starter culture and breadmaking process on wholemeal bread properties linked with the starch digestion

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Abstract

Interest in healthy, sustainable diets[1] drives research into how fermentation affects wholemeal bread quality. The literature suggests a slower starch digestion rate upon consuming sourdough-type bread compared to yeast-leavened bread because of chemical changes during fermentation[2]. However, *in vivo* trials show conflicting results of the sourdough's effect on blood glucose response after bread consumption[3]. The relation between the sourdough consortium and the breadmaking process, and their impact on bread properties associated with the starch digestion rate upon consumption remains to be elucidated[4]. The present study dealt with how sourdough use and fermentation time during breadmaking impacted wholemeal bread properties by using three wholemeal wheat sourdoughs started with *Fructilactobacillus sanfranciscensis* and *Maudiozyma humilis*; *Pediococcus pentosaceus* and *M. humilis*; or *Companilactobacillus crustorum* and *Wickerhamomyces anomalus.* Baker's yeast was used as a reference and wholemeal bread loaves were prepared using 100 or 530 min of fermentation during breadmaking. The results highlighted that the sourdough consortium and breadmaking process directed the specific bread volume (1.57 ± 0.03 to 2.90 ± 0.08 mL/g), pH (6.2 ± 0.1 to 4.1 ± 0.1), total titratable acidity (3.6 ± 0.1 to 11.9 ± 0.2 mL), and water-extractable arabinoxylan content (0.64 ± 0.01 to 1.23 ± 0.04 %dm). However, limited variation was found in the *in vitro* starch digestion rate and resistant starch levels. This study highlighted that a long breadmaking process and sourdough started with *C. crustorum* and *W. anomalus* acidified and solubilised arabinoxylan to the greatest extent, which may considered promising to lower the starch digestion rate.

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Impact of lactic acid fermentation and *in vitro* gastrointestinal digestion on th phytochemical profile and antioxidant capacity of rice, tiger nut, and carob plant-based beverages

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Abstract

Fermented plant-based beverages represent a promising alternative for functional food development[1]. The impact of lactic acid fermentation on tiger nut (TB), carob (CB), and rice (RB) beverages was evaluated in fermented (F) and unfermented (UF) beverages before and after INFOGEST2.0 *in vitro* digestion. Phytochemical profiling was determined using UPLC-ESI-QTOF- MS/MS and antioxidant capacity employing ORAC, TEAC and total polyphenols (TP)[2-3]. Metabolomic analysis identified 18, 44 and 15 phytochemicals in TB, CB and RB, respectively, and revealed fermentation-induced matrix-specific changes: (i) F-TB maintained TP while increasing homovanillic acid (78.9%) and producing hydroxyoleic acid isomer b; (ii) F-CB exhibited higher TP and new compounds, like phloroglucinol, cynaroside A and gallotannins; and (iii) F-RB showed increased TP and unique ethyl vanillin generation. Digestion affected TP bioaccessibility differently: F-TB retained <5%, F- and UF-CB showed minimal retention (<1%), while F- and UF-RB maintained 22-33%. Regarding specific compounds, F-TB preserved homovanillic acid (19.9%) and sinapoyl alcohol (78.3%); F-CB improved citric and 3-propylmalic acid retention (32.4% and 64.4% vs 8.5% and 10.3% in UF-CB); and F-RB showed contrasting dihydroxystearic acid isomer b behavior after digestion(87.2% decrease in F vs 262.3% increase in UF). Antioxidant capacity reflected phytochemical changes: F-TB maintained significant ORAC values post-digestion (77.8%) correlating with its preserved homovanillic acid and sinapoyl alcohol, F- and UF-CB showed reduced ORAC and TEAC values matching its significant loss of phenolic compounds and gallotannins post-digestion, while F-RB displayed the highest TEAC increase post-digestion despite moderate ORAC changes. These findings reveal matrix-specific patterns crucial for optimized functional fermented beverages.

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Kombucha beverages from white, green, red, and black teas-physicochemical characterization and antioxidant potential

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Abstract

Kombucha is a fermented drink prepared from flowers or leaves, mainly tea. It is valued for its health-promoting properties, which it gains during fermentation thanks to the use of Symbiotic Culture of Bacteria and Yeast (SCOBY). In the present study, four variants of kombucha were prepared, differing in the type of tea used: red 'Oolong', white 'Pai Mu Tan', black 'Assam' and green 'Sencha'. Symbiotic Culture of Bacteria (*Acetobacter*, *Gluconobacter*) and Yeast (*Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Dekkera*, *Candida*, *Torulospora*, *Koleckera*, *Pichia*, *Mycotorula* and *Mycoderma*) were used for fermentation.

Depending on the type of tea, water was used for brewing at a temperature of 70°C to 96°C, and the brewing time was from 2 to 5 minutes. The brewed teas were filtered into a glass container, sucrose was added (50 g/L) and left to cool at room temperature for 2 hours. When the tea reached a temperature of 20-25°C, SCOBY was added, the container was covered with gauze and the samples were placed in a place without direct access to sunlight. Fermentation was carried out at a temperature of 25°C for about 7 days.

The concentrations of sugars, ethanol and organic acids were determined by HPLC (Knauer Berlin, Germany; DAD and RI detectors). Total polyphenol content was analyzed using the Folin-Ciocalteu method. The antioxidant potential of teas and kombuchas was assessed using ABTS method. Changes in catechin content were determined using infrared and Raman spectroscopy methods. Additionally, changes in beverage viscosity and colour parameters (CIE *L*a*b**) were monitored.

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Session 5. Booster and hurdles for innovation in FF

The challenge of microbiome preservation and storage: the case o fermented sausages

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Abstract

Fermented foods is probably the oldest known field of application of microorganisms and while spontaneous fermentations are still widely used in fermented foods production, the knowledge build around microbiome represent a new area of strategic development for the fermented foods industry. While the biobanking of microorganisms has experienced a big advancement and rules to be followed for the proper safeguard of microbial resources have been now established, there is a new territory that researchers are exploring which is the preservation of entire microbiomes. This aspect poses several challenges, not only related to the maintenance of the microbial vitality and the taxonomical equilibrium of the ecosystem, but also for the microbial functions. In the frame of the SUS-MIRRI.it project (Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy) we investigated the stability of fermented sausages microbiome stored at -80°C per a period up to 12 months. Samples were stored after different preparation (sausage as such; 1:10 dilution; pellet obtained from the 1:10 dilution) and every 3 months they were defrosted and analyzed by viable counts and 16S rRNA gene sequencing. The results obtained underlined that microbial ecology is maintained both at the level of the vitality and of the taxonomy, however when microbiomes were subjected to propagation for an eventual use as inoculum for the fermented sausage production, difficulties were found due to changes in microbial proportions with respect to the original microbiome stored. More specifically, when the microbial diversity in the sausage was higher, more complicated was to propagate the microbiome.

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Impact of fermentation of oats with varying endogenous enzyme activity levels on the extractability of its proteins and dietary fibres

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Abstract

Oat-based liquid and semi-solid foods typically have low protein and dietary fibre contents, limiting the functional and nutritional benefits of these constituents in such products [1]. This is largely due to the low extractability of oat proteins and dietary fibres in aqueous media [2]. Food fermentation offers a possible solution by activating oat-associated enzymes that can modify the molecular structure of these constituents. In this study, suspensions of wholemeal flour made from both non-kilned and kilned (heat-treated) oat, with and without enzyme activity, respectively, were fermented with a commercial *Lactiplantibacillus plantarum* strain at 30°C for 24 h. The predominance of this strain over the background microorganisms was verified through 16S rRNA gene sequencing. Following fermentation, the suspensions were centrifuged, and the supernatants analysed for protein, β-glucan, and arabinoxylan concentrations. Fermentation increased the β-glucan extractability in kilned oat suspensions from 23% to 64%, which was attributed to the hydration of cell walls. No such effect was found for arabinoxylans in kilned oats. For non-kilned oat suspensions, the β-glucan and arabinoxylan extractabilities increased to 39% and 27%, respectively, which could be ascribed to oat-associated β-glucanase and xylanase activities. The lower β-glucan extractability in fermented non-kilned compared to kilned oat suspensions was the result of extensive β-glucan degradation to glucose in the former case. Despite proteolysis in non-kilned oats, the protein extractability remained low (16%) after fermentation. This study showed that, while inoculation of oat suspensions with *Lacp. plantarum* effectively enhanced oat dietary fibre extractability, its impact on oat protein remained limited.

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Direct Injection Mass Spectrometry for the Real-Time Volatilomics in Food System Microbiomes: the Potential of Providing Temporal Dimension in Multi-Omics Studies

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Abstract

Fermentation represents a sustainable innovation in food systems, leveraging microbial diversity to improve product quality and safety while maintaining a low environmental footprint. Additionally, fermentation processes serve as exemplary models for microbiological research and the development of biotechnological tools. Advancing our understanding and optimization of fermentation through 'omics' technologies, particularly metabolomics, is essential. Metabolomics provides detailed insights into the metabolic states of microorganisms and the dynamics of their pathways [1]. Among these metabolites, Microbial Volatile Organic Compounds (mVOCs) play a critical role in shaping sensory attributes and influencing consumer preferences. Profiling VOCs during fermentation yields valuable information about microbial ecosystems, identifying markers to monitor fermentation dynamics and optimize product outcomes in terms of yield, quality, and safety. Proton Transfer Reaction-Time-of-Flight Mass Spectrometry (PTR-ToF-MS), a Direct-Injection Mass Spectrometric (DIMS) technology, offers non-destructive, real-time analysis with high sensitivity and automation [2]. This technique has proven highly effective for studying microbial volatilomes in various agro-industrial fermentation processes [3]. Here, we present case studies illustrating the potential of PTR-ToF-MS for volatilomics applications in food microbiology and microbiome research. These examples underscore the importance of integrating temporal dynamics into metabolomics, highlighting its relevance for multi-omics studies of dynamic systems such as food-associated microbiomes.

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Fermented vegetables: The path to increased demand and innovation in Norway (FermGrønt)

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Abstract

Fermentation is one of the oldest methods humans have leveraged to preserve food and is considered a natural process. Globally, there is a growing interest in fermented foods. In addition to preservation, fermentation can contribute to improving the nutritional content with potential health benefits, as well as modifying the sensory and functional properties of food products. Another important aspect is that, through extending food shelf life, fermentation can potentially reduce food waste and increase food security and sustainability. Vegetable fermentation opens innovation possibilities and can contribute to a diverse range of healthy and durable Norwegian vegetable products.

Several Norwegian actors have set the focus on increasing the consumption of vegetables. Despite the positive properties and opportunities, fermented vegetable products are not as widespread in Norway as in other European countries. The barriers that explain the lower demand of fermented vegetables in Norway are unknown, and there is a need for more research studies on the topic that are adapted to the Norwegian context.

With the project "FermGrønt," we will map opportunities and barriers to expand the market for Norwegian fermented vegetables through four sub-goals: 1) gather insight into consumers' knowledge and attitudes, 2) map food producers' interest, 3) gather knowledge about relevant vegetables, and 4) map the opportunity space and identify further research needs. The expected result is to compile sufficient preliminary knowledge to shape a subsequent project where the goal will be to increase both the demand and the market for new fermented food products based on Norwegian vegetables.

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Exploring the microbial dynamics of Gluten free teff sourdough starter culture

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Abstract

Commercialization of gluten free foods is increasing globally due to the increase in celiac disease and diabetes. Therefore, there is a pressing need to develop gluten free nutrient-rich foods1. The objective was to characterize microbial differences in the sourdough starter cultures of white and brown-seeded teff flour, an emerging gluten free crop2. Microbiomic approaches were used to characterise changes over the sourdough starter fermentation time. DNA from starter cultures collected at 0h, 6h, 12h, 24h, 36h, 48h, 72h and 96h was used to establish bacterial and fungal populations by 16S rRNA and Internal Spacer Unit (ITS) sequencing. Alpha and beta diversity indicated differences between white and brown teff starters. Greater diversity was observed in brown compared to white teff starters. Genera Gp15 and Streptophyta were abundant in both starters but Clostridium sensu stricto was more predominant in brown starter. Fungal diversity was greater in brown starter and suggested the predominance of Udeniomyces and Naganisha in brown and Epicoccum and Microidium in white starters. Fermentation time had a significant impact on bacterial and fungal abundance. After an initial 24h there was a rapid reduction in fungal abundance in both teff starters and bacterial abundance increased over time. We conclude that after 24h, wild yeasts were replaced by an increase in bacterial genera which could metabolise carbohydrates to produce acids and thereby increasing “sourness” to sourdough. This study suggests that selecting an early sourdough starter would be best to minimize sourness and improving the flavour of gluten free sourdough teff based foods.

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Fermented oils – the sustainable novel ingredient assessment on lipi characterization platform

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Abstract

The use of oleaginous microorganisms (capable of accumulating lipids exceeding 20% w/w of their dry cell mass) as sources of oils and fats, known as fermented oils or single cell oils, holds significant industrial potential for large-scale applications. While the production cost of fermented oils is higher than that of conventional oils and fats, their production is appealing due to its sustainability and ability to utilize various industrial by-products as feedstock for fermentation[1].

ÄIO is a deep tech startup that produces fermented oils by upcycling low value timber industry side-streams like sawdust. The goal is to use the oils in various food applications to substitute palm oil, coconut fat and animal-based lipids.

However, there is too limited information for fermented oil characterization and therefore together with TFTAK the novel oil physical-chemical characterization platform will be designed to assess the quality and safety of these novel ingredients. During the project both the oily biomass and fermented oil batches will be evaluated chemically (total protein, lipid, fiber, volatile composition, etc.,) and physically (solubility, viscosity, water and oil holding capacity, emulsifying effects, foaming capacity, gelling formation, moisture, water activity, melting and freezing point, volatile composition, shelf-life studies, rheology (viscosity), etc.,). The sensorial profile (taste, aroma and texture) will be evaluated by a professional panel.

The chemical, physical and sensorial aspects will give a valuable insight and provide data for assessment of the safety and quality of novel fermented ingredients.

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Repeatability of the online Fermented Foods Frequency Questionnaire (3FQ) for the assessment of fermented foods consumption patterns across European regions. Preliminary results

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Abstract

Fermented foods are consumed worldwide and are characterized by numerous variations regionally. Fermented food consumption using specific tools remains undocumented and hence lack specific consumption recommendations.
This project, under the framework of the PIMENTO COST Action (CA20128), aimed to assess the repeatability of a tool specifically created to quantitatively estimate frequency of fermented food intake across the four European Regions.

Participants (18+ years) from 4 European Regions were recruited to respond to an online questionnaire, using a GDPR certified platform. The repeatability of the online Fermented Food Frequency Questionnaire (3FQ) was assessed after consent to respond a second time no more than 6 weeks following their first response. Spearman's correlation coefficient and Cohen's Kappa statistic were used.

A representative sample to assess repeatability within each region was achieved for all regions other to Northern, for a total of 1254 individuals (372 Central Eastern; 338 Western; 424 Southern; 115 Northern). Preliminary results regarding the questionnaire's repeatability showed strong correlations for all main food groups (rho>0.65 for fermented dairy products, beers, wine, etc.) (Table). However, for less globally known fermented foods which varied by European region, such as fermented chickpeas and fermented teas, the correlation was medium to low (rho:0.2-0.5). Results provide evidence of a strong repeatability of the 3FQ suggesting a useful tool for epidemiological research to help address current gaps in fermented food intakes. An additional database for a sub-group of the total sample per European region includes 24-hour recall data and will be used for a complete validation of the 3FQ tool.

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Table 1. Socio-demographic & Anthropometric characteristics in the study population by repetition of the 3FQ

	1st 3FQ n	1st 3FQ (n=12.646)^2			2nd 3FQ n	2nd 3FQ (n=1.248)^2			Spearman Correlation n n	Spearman Correlation Coefficient^3
Sex^4 (females)	**8,588**	**67.92%**			**856**	**68.59%**			**1247**	**0.970****
Age	**12,583**	**41.11**	±	**14.73**	**1,243**	**42.42**	±	**13.95**	**1240**	**0.972****
Height	**11,122**	**170.45**	±	**10.82**	**1,089**	**170.03**	±	**8.93**	**979**	**0.974****
Weight	**11,075**	**71.39**	±	**17.93**	**1,091**	**70.57**	±	**16.04**	**984**	**0.964****
BMI^5	**10,349**	**24.53**	±	**5.26**	**1,019**	**24.43**	±	**4.85**	**877**	**0.940****
Marital status	**12,566**				**1,243**				**1234**	**0.901****
Married/ living with a spouse	7,085	56.38%			747	60.10%				
Single	4,118	32.77%			398	32.02%				
Divorced/separated/widowed	819	6.52%			76	6.11%				
Prefer not to answer	544	4.33%			22	1.77%				
Educational level	**12,584**				**1,245**				**1243**	**0.862****
University diploma	4,145	32.94%			371	29.80%				
9-12 years of study	2,899	23.04%			198	15.90%				
MSc	2,780	22.09%			329	26.43%				
PhD	1,996	15.86%			271	21.77%				
6-9 years of study	325	2.58%			29	2.33%				
<6 years of study (primary education, elementary school)	244	1.94%			42	3.37%				
Prefer not to answer	195	1.55%			5	0.40%				
Relevant scientific background (Yes)	**3,873**	**30.74%**			**476**	**38.17%**			**1243**	**0.838****
Employment status (employed)	**9,099**	**73.93%**			**949**	**77.03%**			**1222**	**0.911****
Smoking status	**12,567**				**1,242**				**1237**	**0.911****
Non-smoker	8,695	69.19%			884	71.18%				
Smoker	2,584	20.56%			201	16.18%				
Ex-smoker	1,288	10.25%			157	12.64%				
Food allergies-intolerances (Yes)	**1,983**	**15.68%**			**184**	**14.74%**			**1247**	**0.764****
EU region	**12,646**				**1,248**				**1247**	**1.000****
Central-Eastern Europe	5,185	41.00%			372	29.81%				
Western Europe	2,577	20.38%			338	27.08%				
Southern Europe	2,569	20.31%			424	33.97%				
Northern Europe	2,315	18.31%			114	9.13%				

*Correlation is significant at the 0.05 level (2-tailed),**Correlation is significant at the 0.01 level (2-tailed).

[1] 3FQ, Ferrmented Food Frequency Questionnaire

[2] n: study sample population.

[3] Spearman's correlation coefficient and Cohen's Kappa statistic (only for frequencies of consumption) were used to determine the level of correlation and itterrater agrrement between the 2 repetitions of the 3FQ. Spearman's correlation coefficient is interpreted as follows 0.1 - 0.3: weak correlation, 0.3-0.5: medium correlation and 0.5-1.0 strong correlation). Cohen's Kappa is interpreted as follows: values ≤ 0 indicates no agreement, 0.01– 0.20 none to slight, 0.21–0.40 fair, 0.41– 0.60 moderate, 0.61–0.80 substantial and 0.81–1.00 almost perfect agreement[1].

[5] Variables are presented as percentage assigned to each category except for age, height, weight and BMI that are presented as mean ± sd due to their normal distribution.

[6] BMI: Body Mass Index, calculated as weight (kg) divided by height squared (cm^2).

Table 2. Spearman's correlation coefficients and Cohen's kappa statistic to check Repeatability of the main 3FQ[1] food groups by repetition of the 3FQ[1] for the whole sample of participants

	1st 3FQ n	1st 3FQ (n=12.646)^2	2nd 3FQ n	2nd 3FQ (n=1.248)^2	Spearman Correlation n n	Spearman Correlation Coefficient^3	Cohen's kappa statistic^3
plant based meat and/or dairy alternatives^4	6,604	52.22%	696	55.72%	1247	0.632**	63.05 **
Fermented dairy products	11,999	94.88%	1,192	95.44%	1247	0.668**	66.74% **
Fermented pulses and legumes	8,414	66.53%	825	66.05%	1247	0.532**	53.19% **
Fermented meat and/or fish products	9,721	76.87%	937	75.02%	1247	0.607**	60.68% **
Fermented vegetable products	11,283	89.22%	1,137	91.03%	1247	0.583**	58.20% **
Fermented cereal products	11,636	92.01%	1,183	94.72%	1247	0.523**	52.02% **
Chocolate	11,760	92.99%	1,167	93.43%	1247	0.588**	58.75% **
Fermented non-alcoholic beverage products	3,644	28.82%	331	26.50%	1247	0.723**	72.33% **
Vinegar	8,585	67.89%	916	73.34%	1247	0.675**	67.54% **
Coffee	10,362	81.94%	1,025	82.07%	1247	0.865**	86.54% **
Fermented tea	5,196	41.09%	489	39.15%	1247	0.542**	54.19% **
Cocoa beverages	5,804	45.90%	597	47.80%	1247	0.692**	69.20% **
Beer or cider	7,681	60.74%	780	62.45%	1247	0.837**	83.72% **
Wine	8,197	64.82%	832	66.61%	1247	0.877**	87.75% **
Strong spirits	6,093	48.18%	604	48.36%	1247	0.782**	78.22% **

*Correlation is significant at the 0.05 level (2-tailed),**Correlation is significant at the 0.01 level (2-tailed).

[1] 3FQ, Ferrmented Food Frequency Questionnaire

[2] n: study sample population.

[3] Spearman's correlation coefficient and Cohen's Kappa statistic (only for frequencies of consumption) were used to determine the level of correlation and itterrater agrrement between the 2 repetitions of the 3FQ. Spearman's correlation coefficient is interpreted as follows 0.1 - 0.3: weak correlation, 0.3-0.5: medium correlation and 0.5-1.0 strong correlation). Cohen's Kappa is interpreted as follows: values ≤ 0 indicates no agreement, 0.01– 0.20 none to slight, 0.21–0.40 fair, 0.41– 0.60 moderate, 0.61–0.80 substantial and 0.81–1.00 almost perfect agreement.

[4] Intakes are presented as percentage of positive answer regarding the consumption of each food group irrespective frequency or portion size.

Metagenomic and volatilomic approaches to monitor bacterial community and volatilome changes in brine during the spontaneous fermentation of Spanish style cv. Chalkidiki green table olives

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Abstract

The spontaneous fermentation of table olives is driven by a complex indigenous microbiota, primarily lactic acid bacteria (LAB) and yeasts, which influence the final product's quality [1]. Metagenomic and volatilomic approaches can link microbial composition to phenotypic traits, offering insights into fermentation mechanisms [2]. This study examined the relationships between bacterial taxa and volatile organic compounds (VOCs) during the industrial-scale spontaneous fermentation of Spanish style cv. Chalkidiki green table olives (1, 2, and 4 months). Microbial genomic DNA was extracted directly from the brine samples and subjected to sequencing of the V3-V4 region of the 16S rRNA gene. VOCs were determined by headspace SPME-GC-MS. Spearman's correlations were performed using SPSS software (v. 29.0, SPSS Inc., Chicago, USA). Significant Spearman's correlations ($p<0.05$) were found between 7 bacterial taxa and 44 VOCs, visualized in a heatmap diagram. The dominant bacterial taxa identified were correlated with 12 VOCs (6 for *Lactiplantibacillus* and 9 for *Secundilactobacillus*), especially alcohols (4), esters (4), acids (2) and phenols (2). Higher number of correlations were observed for the subdominant bacterial taxa, such as *Vibrio* and *Marinilactibacillus* showcasing the diverse microbial impact on the volatilome. Among the 90 bacterial taxa-VOCs pairs found in total, 8 exclusive positive correlations were recorded, e.g., methyl 3-methylbutanoate-*Shewanella*, 1-heptanol-*Vibrio*, linalool oxide-*Shewanella*, decanal-*Marinilactibacillus* and benzyl acetate-*Lentilactobacillus*. Enrichment of datasets (e.g., yeasts/fungi targeted amplicon metagenomics or shotgun metagenomic sequencing) could enable a more holistic investigation and provide a deeper understanding of the microbiota's role in contributing to the fermentation process and quality of the final product.

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Growth responses of *Lacticaseibacillus rhamnosus GG* in oat-based mil alternative as a function of temperature

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Abstract

Lacticaseibacillus rhamnosus GG, previously known as *Lactobacillus rhamnosus*, is often the dominant *Lactobacillus* spp. and exhibits many desirable characteristics for a good probiotic strain. Furthermore, it has several applications in the food industry and has been used as a starter culture in various food fermentation processes (Sun et al. 2019; Xavier-Santos et al. 2022). Despite its broad interest and the application within the dairy practice, data on the growth of *L. rhamnosus* GG in alternative substrates to milk are not generally available. Therefore, in our study, growth responses of *L. rhamnosus* GG as affected by incubation temperature ranging from 8 ºC to 44 ºC in oat-based milk alternative were investigated. The growth dynamics of studied strain was positively determined by the incubation temperature leading to an increased growth intensity in the exponential phase (except for marginal temperatures). Final counts in stationary phase have reached concentrations of 8.3 log CFU.ml^{-1} (V = 4.2 %) from an initial 2.7 log CFU.ml^{-1} (V = 3.9 %). During growth, pH levels decreased by about 1.98-2.91 units, with final values ranging from 3.73 to 4.73 in the stationary phase.

Cardinal temperatures using cardinal temperature model with inflection (CTMI) were evaluated as follows: T_{opt} = 40.7 °C, T_{min} = 5.4 °C, T_{max} = 45.6 °C with maximum specific growth rate ($_{max}$) of 0.819 h^{-1} at T_{opt}. The results obtained may help optimize fermentation processes in dairy-free practice to provide reliable carriers of probiotic lactic acid bacteria for consumers.

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Application of the probiotic strain *Levilactobacillus brevis* ACA-DC 1705 in sour beer production

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Abstract

The development of functional alcoholic beverages to promote human health is an emerging field for both academia and industry. In particular, the use of beer as a vehicle for probiotic bacteria is a major challenge due to its hostile environment 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) on the basis of 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. 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 mono-culture (control) brew samples, while sensory analysis revealed no significant differences to a commercial sour beer. The results suggest that *L. brevis* ACA-DC 1705 is a promissing candidate for probiotic sour beer production under appropriate brewing and storage conditions.

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