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IBERO-AMERICAN CONGRESS
ON BIOTECHNOLOGY

3-6 SEPTEMBER 2024

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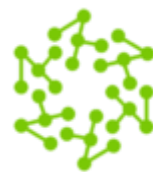
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Hello everyone. With great enthusiasm I welcome to all the participants in the version 2024 of the **International Conference BiolberoAmerica 2024**, a congress on Biotechnology and Biosciences held in Monterrey, Nuevo León, Mexico. I want to thank each co-organizers (personnel and institutions), sponsors, members of the organizing, executive and scientific committees.

On behalf of the executive and organizing committees, I gladly thank to the institutions and organizations involved in the organization of this scientific event, specially to:

Universidad Autónoma de Coahuila,
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The Mexican Association of Food Science (AMECA AC),
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FIAMBIOT, the International Federation of Biotechnology,
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Spanish Society of Biotechnology (Sebiot),
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It is a pleasure to give you the warmest welcome for the fourth international conference BiolberoAmerica 2024 Monterrey, Mexico, one of the most important events of biotechnology globally and that will last 4 days, starting today in face to face mode. Thank you for joining us. It is a pleasure for me to walk you through the conference, to inform you that we will have the participation of outstanding researchers in biotechnology and bioengineering, speakers, and delegates from several iberoamerican countries that have come today to share their knowledge and enhance our mental horizon to learn from the successful experiences they shared with us.

Our Institution, the Universidad Autonoma de Coahuila, is proud to have been selected as the venue to organize the 2024 edition of Biolberoamérica, the second event in Latin America organized by the FIAMBIOT. For this reason, we thank the members of the Fiambiot Central Council, the sponsors, the members of the international, national, and local scientific committees and the members of the organizing committee. Our team has made an important effort to provide the best of events. Enjoy it! Welcome to all.

Organizing Committee of Bioiberoamerica 2024



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PLENARY LECTURES



Integral approach of food industry byproducts. Circular Economy

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Abstract. The valorization of food byproducts has become a major subject of research nowadays. The new trends in the use of food byproducts in the development of healthy foods is a reality. However, research scientist and food technologist have to consider that food byproducts when used as ingredients in food innovation, interaction with food matrix must be well established. Therefore, the most important quality aspects to be considered are the integration in the food matrix, as well as the sensorial and nutritional properties after addition and processing. We have found promising results for their utilization as food additives for technological purposes, and as sources of bioactive compounds to enhance the health-promoting properties of food and beverage. We work with food technologists, nutritionists and sensory scientists to face the challenge of improving the palatability and consumer acceptance of these novel and sustainable foods. The circular economy (CE) systems approach is a sustainable model that encourages the continuous circulation of resources, and byproducts within a closed-loop system. This approach seeks to dissociate economic growth from resource consumption and environmental contamination. Under this system, products are designed and produced to be recyclable, reusable, and biodegradable, minimizing waste and pollution. We are collaborating very close with the food industry to reduce waste, lower costs, and promote sustainable development by adopting a CE systems approach. The possible use of tropical fruits byproducts as ingredients in the development of different foods will be discussed.

Keywords: food by-products, bioactive compounds, circular economy.



From Biowastes to Organic Recyclable Added Value Applications

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Plastic materials have become ubiquitous. They are widely used in many applications such as food and beverage, healthcare, cosmetics, consumer goods and home and garden industries. Most plastics start with hydrocarbons from crude oil. Our extensive collaborative work has turned that process on its head by developing biomass-based, biodegradable raw materials from among other sources food processing streams. We applied those natural products and biowastes to produce polyhydroxyalkanoate-based new organic recyclable materials that rival conventional petrochemical-based ones in technical characteristics and performance.

In this presentation, we will show several examples of new high performance structured and nanostructured materials where the raw chemicals derived from for instance food processing side streams are converted, via a low footprint biochemical processing, into a portfolio of bio-based biodegradable building blocks enabling the realisation of innovative biomedical, cosmetic, nutraceutical, pharmaceutical and packaging materials, including monolayers, biopapers, multilayer films, capsules, controlled release patches and antimicrobial yarns, to match or improve key functional requirements of commercial traditional benchmarks in terms of gas/liquid barrier properties, mechanical resistance, bioavailability, controlled release, biodegradability in the environment, elasticity, hot-tack and super-repellency among others, by tuning the functionalisation of base resins through biosynthesis and traditional and innovative processing routes such as high throughput electro-hydrodynamic processing.

Acknowledgements: This research was funded by the H2020 European Projects YPACK (ref. 773872) and USABLE PACKAGING (ref. H2020-BBI-JTI-2018), the Spanish Ministry of Science and Universities (project PID2021-128749OB-C31), Agencia Valenciana de Innovación (AVI), project INNEST/2022/25. The authors would like to acknowledge also the CSIC-PTIS Salud Global and Salud Global and the Conexion in Nanomedicina.

Key words: biomass upcycling, biomedical, pharma, cosmetic, packaging, controlled release, biomaterials.



Agricultural & Food Biotechnology

Advancing Biological and Biotechnological Systems Innovations for Food Systems in Sustainable Ecosystems

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Advancing systems-based scientific approaches are essential to drive agricultural and food systems and associated ecology-aligned life sciences to find solutions to interconnected challenges of agricultural sustainability, global food security, non-communicable disease/health challenges and all coupled to essential need of energy security with low carbon footprint. The sudden emergence of geo-political challenges from recent COVID-19 pandemic, breakdown in supply chains and on-going global conflicts calls for resilient diversified and localized agro-food systems innovations based on rigorous foundations in systems sciences to advance food security coupled to sustainable high value farming systems. Innate biological strengths of local food diversity with potential for better resilience to climate change due to robust ecological adaptations must be integrated with relevant indigenous ecological knowledge of natural and systems-based farming methods coupled to AI-based computing and information sciences. This is essential for addressing agricultural sustainability, aligned global food security challenges and associated public health burdens and how this can advance innovative value-added developmental strategies in diverse local ecologies globally. Additionally, current resilience of global food security is burdened with continuing challenges from inadequate macro and micronutrients leading to hunger in communities with extreme poverty. Furthermore, the current health challenges have shown that the larger burden of global food security with public health consequences is the rapidly growing burden of excess calories from hyper processed and low micronutrient diets. This is increasing non-communicable chronic disease (NCD) such as type 2 diabetes and its complications which further increases obesity burdened co-morbidity and failing immunity to sudden pandemics such as COVID-19. Innovations for improving food diversity with strengths of local ecosystems in Mexico and Latin American region must be integrated to rigorous biosystems driven scientific farming approaches aligning with higher intake of whole grains, legumes, fruits, and vegetables with high fiber to support beneficial microbiome and high key redox protective bioactive food systems. This allows integration of “food for health” and “food systems for climate resilience” paradigm to drive systems-based metabolic and microbiome biology of diverse indigenous foods of the region. This can advance practical solutions for climate resilient food systems and overall healthy food and nutritional security. These systems-driven scientific innovations are essential for advancing growth engines of regional development in Latin American region and can be supported with a range of new locally targeted AI-based computing and algorithm driven information strategies and data management methods.

Key words: Diverse Food Systems; Food Security; Healthy Foods; Systems Sciences; Localized Sustainability



Bioengineering & Bioprocesses

Bioprocesses and bioengineering: their impact on health

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During this presentation, Prof. Rito-Palomares will share his practical experiences in developing novel bioprocesses and bioengineering strategies with a clear impact on health. He will highlight his experience in the development of processes for the recovery of biomolecules with application in medicine, as well as the development of platforms for the detection of relevant molecules relevant for the diagnostic and monitoring of metabolic diseases. Additionally, Prof. Rito-Palomares will provide an overview of the research that is being conducted in the Institute for Obesity Research at Tecnológico de Monterrey, demonstrating how bioprocesses and bioengineering are essential to advancing medical science and improving public health.

Key words: Bioprocess, bioengineering, health.



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KEYNOTES



Agricultural & Food Biotechnology

Magnetic nanomaterial applications for surface adhesion fermentation, hyperthermic magnetic treatment, and magnetic extraction

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Introduction: The use of nanoparticles for the production and extraction of compounds of biotechnological interest is one of the most viable "promises" of nanotechnology. Despite this, relatively few successful studies have been reported to date. The goals of some studies developed by the Nanobioscience group are the use of magnetic nanomaterials for the production and/or extraction of bioactive compounds applied in biological control of phytopathogenic fungi and of carotenoids in a little-explored yeast *Rhodotorula toruloides*. **Methodology:** Chitosan-functionalized nanoparticles were obtained by hydrothermal treatment-assisted co-precipitation. Functionalization with protein molecules was performed using periodate. Surface adhesion fermentation was performed under appropriate conditions for *Streptomyces griseus* and *Rhodotorula toruloides*. Antifungal activity was measured from cell-free *Streptomyces griseus* extracts by inhibition of radial growth of *F. oxysporum*. The concentration of carotenes was evaluated spectrophotometrically. **Results:** The use of superparamagnetic manganese ferrite nanoparticles (NPs) coated with chitosan in fermentation processes led to an increase in the production of ethanol from banana peels, stimulating the production of compounds with antimicrobial activity in *Streptomyces griseus* and the production of carotenoids in *Rhodotorula toruloides*. The interaction between cells and NP varied depending on their functionalization and cell type. Using an external magnetic force allows easy separation between the immobilized biomass and the suspension, allowing the reuse of the biomass in biotechnological processes. Magnetic induction can be applied for hyperthermic treatment of microbial cells to extract intracellular compounds. Furthermore, the functionalization of NPs with affinity ligands allows for the development of enzymatic immobilization and magnetic extraction methods demonstrated for yeast invertase, fungal lipase, and carotenoids. **Conclusion:** The interaction between magnetic nanoparticles and biological systems allows the discovery of new phenomena and applications.

Keywords: Magnetic functionalized nanoparticles, surface adhesion fermentation, magnetic extraction.



Agriculture and Food Biotechnology

Climate Change ready rice: lessons from abiotic stresses

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Introduction: Rice is an important source of food and energy to more than a third of the world's population. In Brazil, rice is an important crop, being the State of Rio Grande do Sul responsible for ca 70% of its production. Climate changes are impacting agriculture in the form of a range of abiotic and biotic stresses. The understanding of plant response mechanisms to stresses is key to the development of resilient crops. Biotechnological tools can aid breeding programs to develop improved breeding lines. **Methodology:** Rice mutants were obtained by EMS (1.5%) and Gamma Rays (250Gy and 300 Gy). Genotypes were assessed by GBS using the 7K Infinium SNP genotyping platform (Illumina®) at the laboratory of genotyping services of the International Rice Research Institute - IRRI / Philippines and by RNASeq Analysis. **Results:** One chilling tolerant line FAEML 140 was developed. It tolerates colder temperatures during germination stage, which could help farmers to cultivate rice earlier in the spring. Also, five drought resistant lines developed from induced mutation on the cultivar BRS Pampeira and could have a potential to be cultivated in upland areas with less water availability. These lines have been characterized to understand the physiology and molecular mechanisms underneath their phenotypical changes. WRKY transcription factors were detected in RNASeq studies and their involvement in the stress response was characterized. **Conclusions:** Genotypes with resilience to abiotic stresses of chilling and drought were obtained and are being directed to commercial use.

Key words: abiotic stress, breeding, drought, chilling.



Agricultural & Food Biotechnology

Pineapple Side-Streams to Zero Waste: Overcoming Barriers in the First Scale-Up (Pep4Fish Project)

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Introduction: The sustainable valorization of agricultural by-products is crucial in reducing environmental impact and driving circular economies. AgroGrIN Tech under the Pep4Fish project seeks to address this by transforming not only fish waste and side-streams, but also to transform pineapple side-streams—waste materials from pineapple processing—into high-value products, particularly for fish feed, supporting the zero-waste objective. However, transitioning from lab-scale to the first industrial scale-up involves overcoming technical, economic, and logistical barriers. **Methodology:** The project involves extracting valuable compounds such as bromelain and fibers from pineapple waste through green extraction techniques (previously developed at lab scale) up to pre-industrial scale, followed by their integration into fish feed formulations. A multidisciplinary approach is used, combining food science, biotechnology, and process engineering. Pilot-scale trials were conducted to optimize extraction efficiency and product quality while adhering to sustainability criteria, followed by pilot unit implementation. **Results:** The scale-up successfully achieved a 75% conversion efficiency of pineapple waste into target products, with significant reductions in greenhouse gas emissions compared to traditional waste management practices. Economic analysis revealed a potential of increase earnings about 30% for pineapple processors by re-directing their waste and side-streams for AgroGrIN Tech system. Key barriers identified included initial capital investment, technological integration challenges, and regulatory hurdles, which have been tackled during this project. Strategies to overcome these barriers involved securing funding through public-private partnerships, establish key partnerships with potential customers, enhancing technology transfer through collaborative networks, and advocating for supportive policies. **Conclusion:** The Pep4Fish Project demonstrates the feasibility of converting pineapple side-streams into valuable products at a pilot scale, contributing to zero-waste goals and sustainable industrial practices. Overcoming identified barriers is critical for the successful commercialization and widespread adoption of this biorefinery approach. Future work will focus on further scaling up the process, enhancing product yields, and expanding the value chain to include additional by-products. The project underscores the potential of innovative waste valorization strategies in promoting environmental sustainability and economic resilience within the agricultural sector.

Key words: Pineapple waste; Bromelain; Scale-up; Technology transfer.



Environmental Biotechnology

Application of nanomaterials on wastewater treatment processes

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Introduction: Intensive industrial activities have exacerbated the living conditions on Earth due to the discharge of highly polluted wastewaters, containing toxic contaminants, which represent a serious menace to warrant the prevalence of the next generations. Many of the released contaminants are recalcitrant and are not removed in conventional treatment systems; thus, new concepts and strategies are required for the efficient treatment of industrial wastewaters. In the present work, several case studies will be presented related to the application of nanomaterials (NMs) on the treatment of industrial wastewaters. **Methodology:** Carbon-based and metallic NMs were synthesized or recovered from industrial effluents, which were then characterized and applied in anaerobic treatment systems for improving the production of biogas as well as for enhancing the removal of persistent contaminants from wastewaters. **Results:** NMs applied in anaerobic treatment systems showed promising effects boosting the production of biogas from industrial wastewaters like those derived from slaughterhouses and piggery farms, as well as for enhancing the biodegradation of emerging contaminants, such as chlorophenols and pharmaceuticals. **Conclusion:** The application of NMs in anaerobic treatment systems could be a suitable strategy for improving the production of biogas and the removal of recalcitrant pollutants from industrial wastewaters.

Key words: nanobiotechnology; wastewater treatment; biogas; emerging contaminants.



Human Health and Biotechnology

One Health perspective on antimicrobials and the environment

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The interaction of biotic and abiotic factors in an environment is interconnected, and in order to achieve a balance, all the involved individuals must be taken into consideration. In this context, human health is closely related to animal health and is also affected by plants and the conditions of the environment. The idea is to consider the health of all living organisms on Earth as being related to one another, as the concept of One Health suggests. The World Health Organization has proposed the approach of One Health as a collaborative effort that aims to optimize the health of people, animals, and ecosystems. It is a transdisciplinary area with efforts at the local and global levels. Among the most pressing problems identified by the group of One Health experts, zoonotic and vector-borne diseases are included, food safety and foodborne diseases, and the crisis of antimicrobial resistance is included. The latter can be tackled from different areas, including the surveillance of antimicrobial-resistant strains from clinical settings, the presence of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARG) in the environment, or even the presence of residues from antibiotics. These efforts also include the use of extracts from medicinal plants as supplementary treatment for infectious diseases. The results of the analysis of ARB and ARG in soil sediments from Chihuahua rivers, Baja California mangroves, and the Cuatro Ciénegas protected site are presented. The antimicrobial capacity of essential oils against pathogenic multiresistant bacteria is also presented. Besides being a multidisciplinary approach, the One Health initiative should promote international collaboration, considering that many human health problems are present in undeveloped countries, and the research and novel information is usually found in developed countries.

Key words: Antimicrobial resistance, One Health, collaborative effort.

Microalgae based biorefineries: Engineering aspects for a sustainable source of biochemicals and biofuels

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Microalgae are interesting photosynthetic microorganisms due to their biomass rich in proteins, carbohydrates, lipids, phycocyanin and carotenoids. They have attracted significant industrial attention in the context of third generation (3G) biorefineries. Currently there are different cultivation processes: open ponds and closed photobioreactor systems, both show advantages and disadvantages in their operation. This work shows the design engineering aspects in indoor and outdoor microalgae cultivation systems, with emphasis on their application in terms of biorefinery concept. In addition, this work shows alternative strategies in culture media for the growth of microalgae endemic to Coahuila and *Spirulina platensis* to promote proteins, pigments and carbohydrates; extraction of compounds of interest by applying hydrothermal processes for the fractionation of microalgal biomass and conversion to bioethanol. These strategies encompassing technological developments in cultivation, extraction and processing are important as global interest in harnessing the valuable resources of microalgal biomass continues to grow.

Keywords: Circular bioeconomy, photobioreactor, Biomass, Bioprocess,



Environmental Biotechnology

Environmental Biotechnology: concern and goals for future generations

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Introduction: Technically, environmental biotechnology is defined as the use of biotechnological principles and techniques for the management, restoration, and enhancement of the natural environment. This discipline employs living organisms, biological systems, or their derivatives to develop processes that help address environmental problems. This includes the degradation of pollutants, wastewater treatment, the recovery of contaminated land (bioremediation), and the mitigation of climate change effects. **Methodology:** The analysis of the historical development of humanity and its relationship with the environment reveals key events that have spurred the adoption of environmental biotechnology to address problems we have created ourselves. **Results:** Environmental biotechnology has become a crucial tool in addressing the ecological issues facing our planet. As the world confronts challenges such as climate change, pollution, and biodiversity loss, biotechnology offers innovative solutions to mitigate negative impacts and restore damaged ecosystems. However, concerns also arise about the ethical implications and potential long-term effects on the environment and human health. **Conclusion:** To ensure that environmental biotechnology is used responsibly and beneficially, it is crucial to establish clear objectives for future generations. Development of Sustainable Technologies; Regulation and Oversight; Education and Public Awareness; Equity in Technology Access; Ongoing Research.

Key words: environmental, planet, concern, generations

Innovation and Tradition in Fermented Foods: Microorganisms in Latin American Artesanal Products

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Introduction: Fermentation is one of the oldest food transformation processes, originally developed as a preservation method. Currently, it is recognized that fermentation improves the nutritional value of foods, due to the metabolic changes made by microorganisms in food matrices. Although fermented foods are produced in various regions of the world, in Latin America the tradition has decreased over time, being limited mainly to the production of fermented beverages. To innovate in the production of fermented foods and enhance their consumption, it is necessary to recognize artisanal products and the microorganisms that participate in their fermentation processes. Therefore, the main objective of the research was to carry out a literature review to explore the importance of microorganisms in the production of artisanal fermented foods in Latin America. **Methodology:** For the development of the research, a systematic search was carried out in electronic databases such as Scopus and ScienceDirect, developing search equations that included the terms: "food fermentation", "Indigenous Fermented Foods", "Native Fermented Foods", among others. Documents such as research articles and review articles in English and Spanish are included. **Results:** Indigenous fermented foods are part of the cultural heritage of humanity and are recognized for their nutritional value. In Latin America, traditional fermented foods are elaborated with raw materials of greater consumption such as corn and cassava. The main microorganisms involved in fermentation processes are lactic acid bacteria, most of which are recognized as probiotic. **Conclusions:** Artisanal fermented foods in Latin America not only preserve cultural traditions, but also provide significant nutritional benefits thanks to the microorganisms involved in the process, so promoting and valuing these foods can encourage innovation in their production and increase their consumption in the region.

Key words: Food Biotechnology, Nutrition, Probiotics

Precision fermentation for the production of biobased chemicals in the context of biorefineries

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The rapid growth of the global population, climate change, and the depletion of fossil fuels necessitate the development of a resource-efficient and sustainable economy. Advanced biorefineries, which convert biomass from agriculture, forestry, marine environments, and waste into a diverse range of products and bioenergy, offer a promising solution. These biorefineries are essential to the circular economy, as they close resource loops and maximize output valorization. Their development relies on an interdisciplinary approach, with Biotechnology playing a crucial role. This talk will explore the current impacts, challenges, and future prospects of Biotechnology, emphasizing precision fermentation and synthetic biology in the context of biorefinery. A case study on the bioproduction of resveratrol, a high-value antioxidant polyphenolic compound, will be presented. Traditionally extracted from plants or synthesized chemically through complex and unsustainable processes, resveratrol can be biosynthesized through precision fermentation^{1,2,3}, providing a sustainable and economical alternative within the context of biorefineries^{4,5,6}.

Keywords: Precision fermentation, Biorefinery, Waste valorisation, Biobased chemicals; resveratrol

References:

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Topic: Agricultural & Food Biotechnology

Lactobionic acid as an antimicrobial agent against *Staphylococcus aureus* and *Escherichia coli* in food preservation. Combined effects with other protection techniques

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Introduction: The agri-food sector is plagued by the presence of microorganisms altering the quality and safety of food products. Although several tools have been developed to reduce contamination, it is difficult to apply a treatment without affecting the organoleptic qualities, hence the interest in combining two or more techniques at moderate intensities. The effectiveness of organic acids in food preservation has been well demonstrated. Lactobionic acid (LB) attracts a lot of attention for its several applications as a prebiotic and antioxidant agent, but its antimicrobial potential is relatively little studied. Thus, this work focuses on the evaluation of the antibacterial activity of LB, applied individually or in combination with UV- A and Ultrasounds (US) on *Staphylococcus aureus* and *Escherichia coli*, selected as models of foodborne bacteria.

Methodology: The effectiveness of the different treatments on the cited bacteria was performed with different techniques, namely Plate Count Agar for the enumeration of total viable cfu bacteria, flow cytometry with double staining CV6/PI as chromophores and scanning electron microscopy (SEM). Films-based on gelatin and LB was also developed and their effectiveness with the effect of UV was evaluated in culture medium and on cheese slices stored at refrigeration temperature.

Results: The values of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for LB vary from 12.5 to 25 mg/mL. The physiological analysis of the strains, performed with flow cytometry, indicates that the acid affects membrane impermeability and esterase activity. Likewise, scanning microscopy analysis revealed the deformation of cellular morphology. Singular treatment with UV-A for 45 min and 30 min with US showed an irreversible effect, while the co-treatment of each one with LB completely killed the bacteria, indicating a synergistic effect. **Conclusion:** Whatever the physical agent used, its combination with LB increases efficiency, while reducing treatment time and acid concentration. It is therefore desirable to apply this co-treatment for different food products.

Key words: Lactobionic Acid, Preservation, combination, UV rays, Ultrasounds

Industrial biotechnology

Application of ionic liquids and deep eutectic solvents in biocatalysis.

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Introduction: Ionic liquids (IL) and deep eutectic solvents (DES) have garnered significant interest for their application in biomolecule separation and biocatalysis, as promising alternatives to traditional solvents. Their unique properties, such as low volatility, high thermal stability, and tunable solubility, make them ideal for these purposes. These solvents can improve the efficiency and selectivity of biomolecule extraction in aqueous two-phase systems (ATPS), enhancing the sustainability and effectiveness of biocatalytic reactions. **Methodology:** IL and DES were characterized by density, dynamic viscosity, refractive index, FTIR, DSC, TGA and ¹H NMR. Binodal phase diagrams and tie lines were determined by the cloud point titration method, density and refractive index analysis. Lipase and protease activities were spectrophotometrically measured using *p*-nitrophenyl laurate and azocasein as substrates. **Results:** Several biocompatible IL and DES based on choline have been synthesized and characterized. Their behaviour as components of ATPS has been studied, obtaining the miscibility curves and tie lines at various temperatures. Their effect on the activity and stability of commercially relevant enzymes (i.e. lipases, proteases) has been evaluated. Their applicability to the separation of these enzymes and their activating effect in reactions such as lipase-catalyzed transesterification has been tested. **Conclusion:** The applicability of the studied ILs and DES for enzyme separation and their activation potential in lipase-catalyzed transesterification reactions has been confirmed.

Keywords: Ionic liquids, deep eutectic solvents, enzymes, separation.



Sustainable & Food Biotechnology & Product Development

Food Trends: Opportunities for Food Biotechnology & Product Development

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Introduction: The food system affects greenhouse gas emissions by almost 30%, being a sector that has shown limited progress towards achieving the goal of zero emissions by 2050. The food products that generate the most emissions are beef and dairy along with food waste and food losses. These concepts are identified with changes in consumer behavior and food preferences, where foods with high protein content, consistent with the well-being of the planet and of people and plants based. These trends are an opportunity for food biotechnology, with examples such as: large-scale production of animal proteins with lower emissions than current ones through cell culture and with a texture and taste similar to conventional animal-source proteins; production of animal proteins such as casein, collagen and others through precision fermentation; transformation and biological valorization of food waste under the concept of cellular economy.

Methodology: Pet food prototypes based on Black Soldier Fly larvae meal obtained through the bioconversion of residual organic waste by industry, generated by the aquaculture and agricultural industries are developed. The meal is combined with other protein sources for production of wet feed and pellets through extrusion. **Conclusions:** Alternative proteins production and evaluation are an opportunity for food biotechnology, due to its increasing demand, the production of pet foods based on Black Soldier Fly larvae is a good example of obtaining alternative proteins from the bioconversion of residual food waste.

Key words: Food Biotechnology, Alternative Proteins, Food Trends, sustainable Food Products

***Pichia pastoris*: From Molecular Engineering to Industrial-Scale Production of Recombinant Proteins**

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Pichia pastoris (*Komagataella pastoris*) has been a relevant expression system to produce recombinant proteins for both research and industrial applications for over 30 years. Its capacity to achieve high cell densities and perform eukaryotic post-translational modifications makes it an ideal host for producing complex proteins with proper folding and high yields. Advances in genomic technologies have further enhanced its utility, enabling codon optimization, development and discovery of new promoters and signal peptides, creation of synthetic genes, engineering of new strains, gene editing via CRISPR/Cas technologies and the use of innovative cloning tools like Modular Cloning Kits and GoldenPiCS.

The typical bioprocess for producing heterologous proteins with *P. pastoris* involves three-phases process: an initial batch phase for biomass accumulation, a fed-batch phase for further biomass increase, and an induction phase for gene expression, typically using methanol as the carbon source and inducer. Large-scale cultivations, highly aerobic, conducted at high cell densities, face challenges related to mass and heat transfer. These challenges necessitate careful monitoring and control of oxygen mass transfer, pH, substrate concentration and temperature. The kinetics of product formation, specific product formation rate (q_p), and specific growth rate (μ) are influenced by numerous physiological factors, making them critical to the bioprocess efficiency.

This GRAS organism has become widely used in the production of industrial enzymes, driven by the projected growth in the global enzyme market, which is expected to reach up to \$13.2 billion by 2030-2032. The yeast's ability to produce high levels of extracellular recombinant proteins while secreting minimal endogenous proteins facilitates purification and makes it a cost-effective option for industrial applications.

Successful examples from our work research with this yeast include the production of the phytase and tannase enzymes used in the feed or in the beverage industry. Through genetic redesign and protein engineering, we enhanced the thermal stability or specific activity of these enzymes, achieving titres of up to 6 g/L of culture for phytase and a specific activity 1.5 times higher than the native enzyme for tannase. Additional results and their discussion will be reviewed during the conference.

Conclusion: The genetic flexibility and bioprocess scalability with *Pichia pastoris* are crucial advantages for the proteins production for industrial applications.

Key words: *Pichia pastoris*, *Komagataella pastoris*, Industrial Enzymes, Tannase, Phytase.



Photonics biotechnology

Biotic Photonics: Manipulating Light at the Nanoscale Through Biotechnology

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Photonics, the manipulation of light at the nanoscale, has become a key enabling technology across nearly every research and technological field. Despite the strong historical links between photonics and semiconductor technologies, the application of photonic principles in biotechnology has become widespread, driven by the extraordinary possibilities offered by light as a means for detecting or manipulating biomaterials, even at the single-molecule scale. In this context, photonics is often used for specific goals in biotechnology, typically through the nanostructuring of well-known synthetic materials to control light interactions with biological systems.

On the other hand, the use of biotechnology to develop novel photonic systems has been overlooked until very recently, due to the belief that biological systems cannot produce high-quality photonic nanostructures. However, the last decade has demonstrated that living organisms do indeed grow very complex photonic nanostructures for a wide variety of biological functions. Examples range from complex three-dimensional protein-based structures for color production or thermal protection to specialized chloroplasts that use dynamic photonic structures to control light absorption during photosynthesis.

In this talk, we will provide an overview of some of the most relevant natural intracellular photonic structures present in photosynthetic organisms and the extraordinary roles they play for these organisms. We will also highlight the remarkable potential of these structures as alternative and sustainable nanomaterials to current synthetic photonic nanomaterials used in bio and semiconductor technologies. Finally, we will discuss our recent efforts to use biotechnology to tailor specific parameters (e.g. biosilica nanoporosity) in order to obtain suitable biotic materials for specific photonic applications, such as dye- and metal-free color production or biogenerated photonic chips.

Key words: photonics, natural photonics, biotic materials, morphogenesis

Interactions between phytopathogen consortia in agave diseases.

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Introduction: Although in nature microorganisms are part of complex multispecies consortia, the paradigm that infections are caused by a single species persisted, but next-generation sequencing methods revealed that human and animal diseases are the result of multi species interactions. However, reports on phytopathogen consortia and the interactions between the members are scarce. Mexico is the center of biological and cultural diversity of agaves, 75% of the known species are present in this country and play an important economic and social role, especially *Agave tequilana* Weber var blue (tequila), *A. salmiana* (pulque) and *A. angustifolia* (mezcal). The most common reported phytopathogens in agave are *Erwinia carotovora*, *Fusarium oxysporum* and *Colletotrichum* spp, but articles on the interaction of these pathogens with other microbial species or as a part of consortia are missing.

Methodology: A phytopathogen consortium was isolated from a gray spot lesion of *A. salmiana* leaves. Virulence of single strains and 62 different combinations of the consortium members (synthetic communities) were assessed in Braeburn apples measuring the lesion area. Induction of virulence was defined as the ratio of the lesion area of the synthetic consortium between that of the most virulence member. Interactions networks were constructed based on the lesion area. **Results:** The consortium was integrated by 6 fungi and two bacteria strains None of the consortium species belongs to the most common agave pathogens. Some single strains were not pathogens, but they increased the virulence of the synthetic communities when associated with other strain or strains, yet sometimes they decreased it. The interaction networks reveal that the interactions between strains depend on the specific members involved in the consortium, as well as the strength of those interactions. In addition, the same strain sometimes increases the lesion area and others decreases it depending on the members integrating the consortium. We also found that other agave diseases are caused by complex consortia. **Conclusion:** The fact that interspecies pathogenic consortia were detected represents a challenge for controlling diseases in agriculture, therefore understanding the fundamental mechanisms is important to stablish new management strategies.

Key words: agave-diseases, phytopathogen consortia, interaction networks



Bioengineering & Bioprocesses

Bioprocess development based on yeasts from alcoholic fermentation of agave

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Introduction: Mezcal production in Durango, Mexico, uses native yeasts to ferment cooked agave. These yeasts are phylogenetically independent from strains isolated in other regions of Latin America and Europe. They have also demonstrated fermentative characteristics, which make them attractive for developing biotechnological processes. This work aimed to study the yeasts involved in traditional mezcal fermentation in Durango, México, its phylogeny, and its biotechnological potential. **Methodology:** Some molecular techniques allowed us to identify isolated yeast strains and study their phylogeny. Two potential applications were examined. Three strains were selected to formulate an inoculant (*Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, and *Kluyveromyces marxianus*). The strains and their mixtures were physiologically characterized. Instead, isoamyl acetate (banana aroma) production by yeast began with strain and precursor selection. A simultaneous fermentation/extraction system was tested. The effect of the airflow and agitation on the isoamyl acetate production in liquid culture was also investigated. Banana aroma production was also assayed in solid-state fermentation using inert support. **Results:** Despite an initial high yeast diversity, *S. cerevisiae* was predominant at the end of fermentation in Region I. At the same time, *T. delbrueckii* predominated in Region II. Response surface analysis permitted us to identify three promising inoculants. *Pichia fermentans* and *K. marxianus* were the promising strains, while isoamyl alcohol was the best precursor for banana aroma production. A fermentation system well aerated and coupled to liquid-liquid in situ extraction with decane as the recovery solvent allowed the highest isoamyl acetate production (2.14 g/L). Isoamyl acetate production was demonstrated through solid-state fermentation, feeding the precursor in the gas phase. **Conclusion:** Mezcal made with the inoculant constituted by 75% *S. cerevisiae* and 25% *T. delbrueckii* was the best in terms of yield, richness of volatile compounds, and acceptability in sensory tests. A well-aerated liquid fermentation or solid-state fermentation on inert support are two promising systems for the bioconversion of isoamyl alcohol into isoamyl acetate.

Keywords: Native yeasts, Inoculant, isoamyl acetate.



Bioengineering and Bioprocess

Continuous Bioprocessing through Microfluidics: Innovations in Bioprocessing Technologies

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Integrated continuous bioprocessing is considered a key strategy for enhancing yield and cost-efficiency in biomolecule production, while also reducing material usage, equipment size, and carbon footprint. However, scaling up these processes is often costly due to the expensive infrastructure and high material consumption, necessitating the development of innovative, cost-effective screening and process development methods. Microfluidic devices have gained popularity as tools for accelerating the creation of new biomanufacturing techniques, thanks to their capability to rapidly test a wide range of variables with minimal reagent use. Most existing literature has focused on using microfluidic devices for optimizing individual unit operations rather than adopting a comprehensive optimization approach that evaluates the entire biomanufacturing process. To address this, an integrated microfluidic platform has been developed to simulate the bioprocessing of a target protein, facilitating process design and optimization. The integrated chip includes a production module for the model protein Green Fluorescence Protein (GFP), a lysis module for releasing the intracellular protein through chemical lysis, and an Aqueous Two-Phase Extraction (ATPE) module for concentrating the target protein in one phase via liquid-liquid extraction. By combining multiple unit operations into a single device, it is possible not only to optimize conditions for each operation independently but also to assess their combined effects on the overall process.

M.A. Wahab, C. Domingues, A.M. Azevedo, V. Chu, J.P. Conde, M.R. Aires-Barros, An integrated microfluidic device for continuous bioprocessing, *Separation and Purification Technology*, 332 (2024) 125702

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Topic (Times New Roman, 10p)

ANTIMICROBIAL PEPTIDE DIGITAL OPTIMIZATION ENABLES BIOINSPIRED PRODUCT GENERATION.

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Antimicrobial peptides (AMPs) have attracted considerable attention because of their multiple and complex mechanisms of action toward resistant bacteria. However, reports have increasingly highlighted how bacteria can escape AMP administration. In response to this challenge, we have developed a novel computational approach that utilizes genetic and Joker algorithms, as well as an artificial intelligence strategy, to design bioinspired AMPs derived from bacteria, plants, and animals. This approach has yielded different peptide classes, including those with an unusually high proportion of cationic residues and hydrophobic counterparts. At least dozens of peptides emerged as prototype AMP among natural analogs screened for their activity against an engineered luminescent *Pseudomonas* strain. Peptides were further characterized in structure, activity, mechanism of action, and biotechnological potential for developing new compounds beneficial for human and animal health. Most novel peptides were unstructured in water and underwent a coil-to-helix transition in hydrophobic environments. This conformation was corroborated by NMR analysis in dodecylphosphocholine micelles, which revealed an α -helical structure. The generated Peptides caused a bactericidal effect at low micromolar concentrations on several resistant bacteria, causing membrane disruption without triggering depolarization but rather hyperpolarization. Finally, the large-scale production strategies used to prepare such peptides for the market were also discussed. In summary, the present work presents an innovative computational approach to explore natural products to design short and potent peptide antibiotics that could be used against resistant bacteria, offering a promising solution to the global challenge of antibiotic resistance.

Keywords: antimicrobial peptides, bacterial resistance, drug design, artificial intelligence, membranes



Agricultural & Food Biotechnology

Polyembryony in maize: Applications and perspectives

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Maize is considered a very important plant in the culture and diet of many Latin American countries, since it provides a large amount of energy due to its sugar, protein and starch content. In addition, this plant has a great adaptation to various climates and soils, which is why it is also an important crop worldwide, being one of the most consumed along with rice and wheat. Among the different mutations that have been identified in this plant is polyembryony, which is a condition in which a single seed can generate more than two individuals. This phenomenon is of great importance for commercial production and multiplication, and can reduce the number of seeds for sowing and costs, for both, storage and seed transportation. It has been found that polyembryony is a phenomenon that promotes an increase in the nutritional quality of the grain, mainly in the tryptophan and lysine portion, thereby increasing the protein quality. In addition, an increase in the fat content has been observed in the kernel, where a higher content of oleic and linoleic acids is observed, components which may be of interest for current diets. In this conference, advances in genetics, cytogenetics, nutritional composition, oleic acid and applications of polyembryonic maize for sprouts will be discussed.

Key words: Zea mays, genetics, cytogenetics, composition, oil

Agricultural & Food Biotechnology

Design of a sustainable probiotic whey-based beverages using *Lactobacillus rhamnosus* GG

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Introduction. The addition of value to whey is still a challenge for dairy industries aiming towards a circular bio economy. This presentation will bring a practical solution for the production of a probiotic beverage that includes the valorization of whey and Costa Rican guava fruit pulp and other examples in this area that can evaluate the production of probiotic beverages.

Methodology and results. First an optimized whey media was supplemented with yeast extract and fermented in a bioreactor, reaching a maximum growth rate of 0.32 h⁻¹ after 48 hours of fermentation, for a later evaluation of the survival kinetics of *Lactobacillus rhamnosus* GG (LGG) in the formulated drink during refrigerated storage. The survival kinetics of LGG in the formulated drink was not affected by the addition of CRG pulp (P>0.05). The shelf-life of the inoculated beverage surpassed 40 days with a minimum population of 10⁶ CFU/mL accomplishing the legal requirements for probiotic labeled food products. For sensory evaluation stage, three different formulas of the beverage with different whey content were compared. The highest acceptability was achieved for the one with 50% whey. **Conclusion.** The use of Costa Rican guava for the production of probiotic whey-based beverages is a promising application.

Key words: *Lactobacillus rhamnosus* GG, fermentation, Costa Rican guava fruit, microbial growth, microbial survival.



4th **BIO IBEROAMERICA**

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AGRICULTURAL & FOOD BIOTECHNOLOGY

Agricultural & Food Biotechnology

Development and bioactive potential of films from plant residue enriched with eucalyptus extract and oregano essential oil

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Introduction: Traditional plastic food packaging is typically non-renewable and non-biodegradable, creating a need for new eco-friendly and sustainable alternatives, such as plant-based films. Plant agro-industrial wastes have been explored as sources of polymeric matrices in film development. Additionally, natural compounds from plants, like plant extracts (PEs) and essential oils (EOs) can provide these films with antimicrobial and antioxidant activities. So, this work aimed to produce polymeric films with licorice-based polymers incorporated with eucalyptus extract and oregano EO. **Methodology:** Licorice residue was characterized using high-performance liquid chromatography (HPLC) and used to develop films with alginate and glycerol. Films were produced with oregano EO (2%), eucalyptus extract (1%), and a combination of both (1% each). The antimicrobial activity was tested against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) using the viable cell method. Antioxidant activity was assessed by 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-difenil-1-picrilhidrazil (DPPH) scavenging assays. **Results:** The results showed that the residue consisted mostly of insoluble fibers from approximately 74%, with lignin being the main constituent from approximately 33%. The residue was, then, used to produce films incorporated with eucalyptus extract and oregano EO as bioactive agents. The antimicrobial activity test showed that the films with licorice residue incorporated with eucalyptus extract and oregano oil was able to completely inhibit *E. coli* after 2 h of exposure; Besides that, the film incorporated with eucalyptus extract was able to completely inhibit *S. aureus* after 4 h of exposure and the film incorporated with eucalyptus extract and oregano oil inhibited this bacterium after 2 h. Regarding the antioxidant potential of the films, the film with licorice residue (no extract nor oil) was the one with the least antioxidant activity (ABST: 226,7428 Trolox equivalents (μM)/mg film; DPPH: 162,2766 Trolox equivalents (μM)/mg film). The incorporation of eucalyptus extract and oregano EO to the films significantly increased its antioxidant activity. **Conclusion:** Polymeric films made from licorice residues and incorporated with EOs and PEs, due to their antimicrobial and antioxidant activities, can constitute an alternative to conventional food packaging.

Keywords: plant residue; plant extract; essential oil; polymeric film; bioactive potential.

Agricultural & Food Biotechnology

Antimicrobial activity of potential essential oils to replace potassium sorbate in fruit preparations

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Introduction: In recent years, the food industry has faced the challenge of producing high-quality products using natural preservative alternatives instead of synthetic ones. Essential oils (EO), naturally present in various plants, have potential as alternatives due to their antimicrobial activity and high food safety (Generally Recognized as Safe, GRAS). This study aimed to investigate the antimicrobial activity of EO against common spoilage and pathogenic microorganisms in fruit preparations, to use as a substitute for potassium sorbate (PS). **Methodology:** The antimicrobial activity of lemongrass and lemon EO was tested using the microdilution method in 96-well plates against *Lactobacillus plantarum*, *Escherichia coli*; *Candida intermedia*, *Pichia fermentans*, *Aspergillus niger* and *Penicillium glabrum*. EO were tested at concentrations ranging from 1.56 to 50% and PS at 0.03 to 4%. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. **Results:** The MICs of lemongrass and lemon EO for *E. coli* were 3.12 and 12.5%, respectively, while for *L. plantarum*, they were 1.56 and 6.25% respectively. Regarding the yeasts, the MIC of lemongrass and lemon EO for *C. intermedia* were 1.56 and 3.12%, respectively, and for *P. fermentans*, they were 1.56 and 1.56%, respectively. Lemongrass EO showed the same MICs value of 6.25% against *A. niger*, and *P. glabrum*. Lemon EO also displayed the same MICs values of 25% for *A. niger*, and *P. glabrum* representing the highest MICs values observed. The MBCs values was similar the MICs values to all microorganisms except to *L. plantarum* that were 6.25 and 25% to lemongrass and lemon EO, respectively. PS showed MICs values of 1 and 2% for *E. coli* and *L. plantarum*, respectively, with MBCs values of 2% for both. For yeasts, the MIC was 0.06% for both with MBC values of 0.06% for *E. coli* and 0.5% for *L. plantarum*. The MIC and MBC values were the same of 0.06% for both fungi. **Conclusion:** The EO tested showed significant antimicrobial potential at low concentrations, particularly lemongrass EO. Future studies will incorporate this oil as substitutes for PS to verify their ability to maintain antimicrobial effectiveness over time when incorporated in fruit preparations.

Keywords: antibacterial activity, antifungal activity, essential oil, food preservative, potassium sorbate.

***In vitro* antifungal evaluation of lactic acid bacteria culture extracts of against
Colletotrichum gleosporioides and *Fusarium oxysporum*.**

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Abstract

The development of post-harvest diseases in different crops generates large economic losses, in addition to a large generation of waste. Currently the use of microorganisms as a strategy for biological control has increased considerably, because unlike synthetic fungicides, these do not cause harm to human health or the environment. In this context, lactic acid bacteria (LAB) are a promising source, because due to their "GRAS" status generally recognized as safe, they can be used in different food matrices, as they have the ability to generate secondary metabolites with biological activity, providing protection against unwanted microorganisms. The objective of this work was to evaluate the *in vitro* antifungal activity of seven LAB extracts, against two important fungi in avocado and tomato crops, *Colletotrichum gleosporioides* and *Fusarium oxysporum*. The results showed a greater antagonistic effect by the 11C4 strain of *Lactobacillus plantarum*, obtaining 41.07% and 32.04% inhibition against *F. oxysporum* and *C. gleosporioides* respectively, therefore, *L. plantarum* offers an attractive alternative strategy as biocontrol against postharvest phytopathogens of interest.

Key words: Biocontrol, antifungal activity, lactic acid bacteria, postharvest diseases, *C. gleosporioides*, *F. oxysporum*.

Oxidation inhibition of canola oil by ellagitannins encapsulated in ethylcellulose: application in a frying process

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ABSTRACT.

Introduction: The utilization of pomegranate peel polyphenols as natural antioxidants has been a subject of interest in the context of edible oils, given the oils' susceptibility to oxidation. The polymer ethylcellulose is used generally for pharmaceutical formulations as encapsulation agent of bioactive molecules. **Objective:** The objective of the present work was to encapsulate ellagitannins in ethylcellulose and evaluate the antioxidant capacity in oil submitted to deep-frying. **Methodology:** Ellagitannins were extracted from pomegranate husk and purified by liquid chromatography using Amberlite XAD-16 as stationary phase. Ethanolic fraction was recovered and concentrated by rotary evaporation. Ellagitannins were incorporated into ethylcellulose 20 cP at ratios of 1:1.25, 1:2.5, and 1:5 (ellagitannins:ethylcellulose). Encapsulated ellagitannins were submitted to heating for 90 min into 10 g of commercial canola oil at temperatures of 145, 160, and 190 °C. The peroxide index was evaluated according to AOCS Cd 8-53 method. Then, 5 g of encapsulates were mixed with 1 L of canola oil and used for the elaboration of fries. The process was repeated during 5 frying cycles. Peroxide index was quantified after every frying cycle. **Results:** Encapsulation ratios of 1:5 and 1:2.5 reached 99 % of encapsulation efficiency, while the ratio of 1:1.25 reached efficiency of 64 %. The production of peroxides in edible oil went 1.92 ± 0.39 , 0.85 ± 0.27 , and 0.72 ± 0.20 mEq/Kg at temperatures of 145, 160, and 190 °C, respectively. It represents 3.7 times lesser peroxides compared to control. The use of encapsulated ellagitannins in the frying process decreased the peroxide index showing the following results (mEq/Kg; cycles 1-5): 1.47 ± 0.00 ; 2.44 ± 0.06 ; 3.26 ± 0.26 ; 3.40 ± 0.04 ; 4.00 ± 0.02 . On the other hand, treatments without ellagitannins reached the following values (mEq/Kg; cycles 1-5): 4.05 ± 0.11 ; 4.20 ± 0.37 ; 4.51 ± 0.78 ; 4.62 ± 0.23 ; 5.66 ± 0.21 . **Conclusion:** Ellagitannins encapsulated in ethylcellulose inhibited the oxidation of edible canola oil used in deep-frying process.

Palabras Clave: Edible oil, peroxide index, antioxidant, punicalagin, punicalin.



Agricultural & Food Biotechnology

Solid-State Fermentation with *Saccharomyces cerevisiae* and Its Effect on Phenolic Content and Antioxidant Capacity of Blue Corn

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Introduction: Corn (*Zea mays* L.) is the most harvested cereal worldwide, accounting for 40% of global cereal production. Pigmented corn varieties are rich in bioactive phenolic compounds like carotenoids and flavonoids, including anthocyanins, giving grain colors ranging from light red to deep purple. Solid-state fermentation (SSF) is a biotechnological process that involves the conversion of a substrate through the metabolic activity of a microorganism to obtain a molecule of interest. SSF improves the matrix's appearance, taste, color, nutritional composition, and functionality. This work aimed to determine the phenolic compound content and antioxidant capacity of blue corn extracts under SSF using *Saccharomyces cerevisiae*. **Methodology:** The grain was soaked in water (77 °C) for 4 h to adjust to 70% humidity and pH 5.0. Subsequently, 495 g of grain were mixed with 250 mL of culture medium (g/L: peptone 20, yeast extract 5, and NaCl 0.23) previously inoculated with *S. cerevisiae* (1×10^6 cells/gdm). The wet mixture (15 g) was packed in tray bioreactors and incubated at 30 °C for 120 h, sampling every 12 h. Extracts were obtained with methanol-water-lactic acid (80:19:1) and total phenolic content (Folin-Ciocalteu) as well as antioxidant capacity against DPPH and ABTS radicals were determined. **Results:** *Saccharomyces cerevisiae* was able to grow using blue corn as a substrate, enhancing the extraction of phenolic compounds, reaching the highest value at 60 h of culture (2.07 mg GAE/gdm), with antioxidant capacity against DPPH and ABTS radicals of 0.16 and 1.69 mgTE/g, respectively. Compared to the unfermented (control), the developed bioprocess increased the phenolic compound content by 12.07 %, and the antioxidant capacity by up to 23.07% and 50.53%. **Conclusion:** This is the first study of SSF using yeast on blue corn as a substrate, demonstrating the effectiveness of the bioprocess in increasing the phenolic compound content and antioxidant capacity of the grain.

Keywords: bioprocess, bioactive phenolic compounds, fermented pigmented maize, *Saccharomyces cerevisiae*.

A COMPARATIVE INVESTIGATION ON MICROWAVE-ASSISTED EXTRACTION OF PECTINS FROM *TILIACORA* SP. LEAF AND *BETA VULGARIS* PULP

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Abstract

Introduction: Pectins are non-starchy, heteropolysaccharides composed of α -1,4-D-galacturonic acid chains with diverse applications as gelling, stabilizer, emulsifier, edible coating, drug delivery, and wound healing agents. Increasing research has proposed new sources for pectin and improved extraction techniques, thus, this study compared the yield and antioxidative potentials of microwave-assisted extracted pectin from Vietnamese leaf, VL (*Tiliacora* sp.) and sugar beet pulp, SBP (*Beta vulgaris*). **Methodology:** A 10 g of the test materials (1:20 w/v) and 2% citric acid were treated with a pressurized microwave autoclave (2.67 Bar) at 134.8°C for 15 min and pectin extracts were recovered by filtration, precipitation, and ethanol washing. Water extraction without microwave treatment was also carried out. Yields, characteristics, and antioxidative potentials of test samples were obtained using standard methods. **Results:** Pectin yield (% dry basis) was significantly higher in the microwave-assisted treated VL, (28.6 ± 0.38) than the SBP (18.8 ± 0.08), while the water extract of SBP (14.3 ± 0.01) is higher than that of VL (10.2 ± 0.05). FTIR analysis indicated that the raw materials contain uronic acid as a pectin index, while the pectin (water and microwave-assisted extracts) are composed of high methoxyl pectins. The antioxidative inhibition of pectins against DPPH radicals ranged from 84.88 % (VL microwave-assisted extract) to 40.39 % (SBP water extract) after 6 h of reaction. **Conclusión:** Pectin yields and antioxidative potential of test samples were improved by the microwave-assisted process and this can greatly inform the utilization of *Tiliacora* sp. and *B. vulgaris* as pectin sources in food and non-food applications.

Keywords: Innovative techniques, extraction, pectic substance, cell wall.

Agricultural & Food Biotechnology

Fungal cell wall degrading enzymes from *Trichoderma* strains isolated from the central-southern region of the State of Chihuahua

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Introduction: *Trichoderma* is a cosmopolitan fungus that colonizes cellulosic material and the rhizosphere of plants. *Trichoderma* exerts biocontrol mechanisms on Ascomycetes, Basidiomycetes and Oomycetes that cause diseases in plants; these mechanisms consist of competition for space and nutrients, antibiosis and mycoparasitism. Mycoparasitism starts with identifying the pathogen. *Trichoderma* attaches and coils on the pathogen, producing cell wall degrading enzymes that cause lysis or cell death of the pathogen. Chitinases are cell wall degrading enzymes that hydrolyze the chitin chain bonds present in the fungal cell wall. Chili wilt is one of the main diseases affecting the production of jalapeño peppers, and it is crucial to search for ecological alternatives such as biocontrol to eradicate the pathogens causing wilt. The general objective of this work was to determine the production of fungal cell wall degrading enzymes of *Trichoderma* strains isolated in the central-southern region of the state of Chihuahua.

Methodology: From a crude protein extract of 10 *Trichoderma* strains grown on synthetic medium measured on days 3, 5 and 10 of incubation, the total protein content was determined by the Pierce technique, followed by the evaluation of enzymatic activity of two exochitinase (chitobiosidase and N-acetylglucosaminidase). **Results:** Total protein content varied from 801.50 to 134.97 µg/mL on day 3, 575.52 to 79.41 µg/mL on day 5, and 831.08 to 165.25 µg/mL on day 10. The chitobiosidase production on the third day was from 0.02 to 1.06 U/mL, on the fifth day from 0.02 to 1.90 U/mL and the tenth day from 0.05 to 1.11 U/mL. N-acetylglucosaminidase production on

day 1 ranged from 0.01 to 0.36 U/mL, on day 5 from 0.01 to 1.80 U/mL and on day 10 from 0.04 to 1.91 U/mL. **Conclusion:** Based on the results, five native strains of *Trichoderma* produced enzymes (chitinases) with a greater capacity to degrade the cell wall, which has the promising potential as biocontrol agents against phytopathogenic fungi.

Keywords: *Trichoderma*, Proteins, Enzymes, Exochitinases.



Agricultural & Food Biotechnology

Extraction of bioactive compounds from plants of the mexican semidesert and their application in the food industry

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Introduction: The semi-desert region located in northern Mexico is the home to endemic plants that have been part of traditional medicine since ancient times. They have recently been studied due to their sustainable applications in the food and pharmaceutical area, bringing economic and social benefits. The aim of this work is to extract and characterize bioactive compounds from semi-desert plants: *Parthenium hysterophorus*, *Ephedra antisiphilitica*, *Heterotheca inuloides* and *Parthenium incanum* through ultrasound-assisted extraction (UAE).

Methodology: The optimization of the extraction by UAE, was evaluated using a Box Behnken design with two factors (Mass/solvent ratio and solvent concentration) at three levels (1:10, 1:15, 1:20, 50, 60 and 70%). The antioxidant activity of the extracts was determined by DPPH, ABTS and FRAP. Additionally, inhibition tests were carried out with *E. coli* and *S. aureus*. Finally, the inhibitory effect of the α -amylase enzyme was determined. **Results:** The extraction process evaluated by Box Behnken design favored treatment number three for each species, at the same time, the mass/solvent ratio significantly influenced the three tests (DPPH, ABTS and FRAP). On the other hand, the extracts that inhibited *E. coli* were those of treatment three and six for Hierba amargosa and Arnica, as well as treatment three for Pitorreal and Mariola, in the case of *S. aureus* only the extracts belonging to treatment three of Mariola and Arnica showed inhibition. Lastly, in the tests with the α -amylase enzyme, Arnica was the only plant that showed inhibition. **Conclusions:** The Box Behnken design allowed the selection of the best positioned treatment for the four plants, which must undergo cytotoxicity tests and subsequently the addition to a food matrix.

Keywords: Semi-desert species, extraction process, ultrasound, antioxidant activity, antimicrobial activity.



Agricultural & Food Biotechnology

Encapsulation and Evaluation of Physicochemical Properties of Bioactive Compounds from *Citrus sinensis* and *Vitis vinifera* by-products

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Introduction: The by-products of the fruit industry represent a global environmental problem due to their inadequate disposal. As a result, research on their utilization and minimizing their effects has gained importance in recent years. In this regard, orange (*Citrus sinensis*) residues and grape pomace (*Vitis vinifera*) from juice and wine production are rich in various bioactive compounds, such as flavonoids, anthocyanins, and catechins. Although these compounds have potential for application in the agri-biotechnological industry, their application in pure form can cause structural changes, leading to degradation. Therefore, maintaining their stability is vital, with encapsulation being one of the ideal options, allowing for extended stability, controlled release, and improved solubility and bioavailability. **Methodology:** The encapsulation of the bioactive compounds from *C. sinensis* and *V. vinifera* residues was carried out using a BUCHI Mini Spray Dryer B-290. The physicochemical properties of the encapsulates (moisture, solubility, water activity (Aw), hygroscopicity, wettability) were also determined, and finally, a confirmatory encapsulation test was conducted using FTIR. **Results:** The results indicate that the use of agavins allowed for a recovery of over 50% of the encapsulated bioactive compounds. In evaluating the physicochemical properties, both samples of encapsulates were within the ideal ranges reported in various literature sources. Finally, the FTIR analysis of functional groups identified the functional groups of the agavins, pure bioactive compounds, and encapsulated bioactive compounds, demonstrating that the encapsulation process was successful. **Conclusion:** The spray drying process yielded encapsulates with acceptable physicochemical properties, showing potential for application in various areas of the agri-biotechnological industry. The FTIR analysis demonstrated that the encapsulation and protection process of the bioactive compounds was effective.

Keywords: *Vitis vinifera*, *Citrus sinensis*, Agavins, Spray drying, Encapsulation



Agricultural & Food Biotechnology

DEVELOPMENT OF AN ASSAY FOR THE DETECTION OF THE BACILLUS CEREUS CERULIDE TOXIN IN FOOD RICH IN STARCH.

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Introduction: *Bacillus cereus*, a sporulated Gram positive bacillus, found in dry and high-starch food, is responsible for 20% of the annual foodborne illnesses in the world. Certain strains cause emetic syndrome, an intoxication characterized by nausea and vomit with a rapid onset, caused by the production of the cereulide toxin, a 1,2 kDa cyclic dodecadeptide, which acts as a potassium ionophore, and it is stable to a wide range of pH, elevated temperatures and enzyme treatments with trypsin or pepsin. The aim of the work is to perform a qualitative assay for the detection of the toxin in food rich in starch with benefits over current assays. **Methodology:** *B. cereus* *Ces* positive strains from the Sanitary Microbiology Laboratory and previously well-characterized were grown on nutrient broth containing starch and centrifuge to discard the cellular package. A standard KCl solution was added to the supernatant, and the remaining potassium was determined by the addition of a Sodium tetraborate solution and measured at 578 nm. Different concentrations of Valinomycin were used as a positive control. **Results:** The *Ces* positive cultures showed a decreased absorbance compared to the one obtained from the KCl standard. The qualitative procedure is based on the decrease of the potassium concentration in solution due to the toxin ability to specifically bind to potassium cations, shown by the product of the ion exchange of the remanent potassium with Sodium tetraborate, a precipitate measured in spectrophotometer. For the semi-quantification of the method a curve that correlates absorbance with different concentrations of the Valinomycin was obtained due to the structural and functional similarity of the Valinomycin with Cereulide. **Conclusion:** A reliable and profit assay was developed for the detection of the toxin directly from grown cultures through the determination of the remaining potassium.

Keywords: Cereulide, food starch rich, potassium, *Bacillus cereus*.



Agricultural & Food Biotechnology

Development of a Microbial Consortium for Lactic Acid Production Using Sugarcane

Bagasse

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In response to contemporary environmental challenges, the pursuit of sustainable and eco-friendly technologies has reached unprecedented levels. In this context, second-generation biorefineries emerge as an innovative solution, using microbial bioconversion processes, employing bacteria and yeast, for the efficient conversion of high-value-added compounds and biofuels. The objective of this work was to use the sugarcane bagasse in the production of lactic acid. This work was based on second-generation biorefineries using sugarcane bagasse and evaluating a microbial consortium of *Lactobacillus pentosus* ATCC 8041 and *Saccharomyces cerevisiae* PE-2. The process involved several stages: biomass characterization, hydrothermal, enzymatic saccharification with, and fermentation with a bacteria/yeast consortium. Hydrothermal pretreatments were performed under different conditions at 180° C for 30 minutes to evaluate biomass fractionation. Subsequently, enzymatic hydrolysis was conducted under different slurry conditions: 1% (w/v) of total pretreated solids with 5% (v/v) total pretreated liquids and 5% (w/v) of pretreated solids and 25% (v/v) total pretreated liquids with cellulases/ hemicellulose: 1:2 (v/v) and enzyme loading: 15 FPU/g pretreated solids and fermentation was evaluated using a strategy of pre-simultaneous saccharification and fermentation with the microbial consortium and samples were taken during 24, 48, 72, 96 and 120 h. Lactic acid was obtained with a production of 19.74 g with low solid loading and 39.32 g with high solid loading, both quantified at 120h of fermentation. This study highlights the feasibility of utilizing agro-industrial waste in second-generation biorefineries in the production of lactic acid.

Keywords: Hydrothermal processing, Lignocellulosic material, Enzymatic hydrolysis, Biorefinery

Unlocking microalgae residues potential: novel substrates for fungal solid-state fermentation towards functional and bioactive compounds development

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Introduction: In recent years, blue biotechnology has triggered the search for new sources of bioactive and nutritional compounds such as microalgae biomasses, being up today recognized as novel and attractive sources. As a result, numerous investigations have developed processes for extracting natural and value-added molecules (polyphenols, protein, pigments, polysaccharides among others) but on the other hand generating a spent biomass after processing that comprises a by-product with still nutritional value, which can be used for the development of fungal solid-state fermentation (SSF) bioprocessing technology. The objective of the present study is to evaluate the potential of 3 spent microalgae biomasses (*Spirulina platensis*, yellow and white *Chorella vulgaris*) from protein obtention using a GRAS microorganism (*Aspergillus oryzae*). **Methodology:** The evaluation englobed the potential of using microalgae residue as SSF substrate (pH, water absorption index, critical point of humidity), as well as the compatibility of the microorganism (radial growth), in addition, the analysis of soluble protein content (Bradford assay) and the antioxidant properties [1]. **Results:** The results have shown varied initial pH values (pH 2.5 to 5.8), being not limiting values for fungal development on the biomasses. The growth of the microorganism has shown variations with respect to the type of biomass implemented, ranging from 0.27 ± 0.06 to 0.99 ± 0.19 cm/day. These findings could be explained due to their nutritional diversity between biomasses [2], the recovered extracts showed a decrease in the soluble protein after 120 h of incubation from 9.62% to 42% and exerted antioxidant activity (FRAP and DPPH methods) these values could be the result of the fungal metabolism. **Conclusion:** The sea by-products (microalgae) comprise a potential substrate for SSF technology, that could be used under the new blue bioeconomy trend. However, further studies on optimization processes are required to guarantee a successful implementation of SSF into the food value chain.

Key words: Alternative protein, microalgae, antioxidant activity, antimicrobial potential

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Production of *Trichoderma harzianum* Spores Via Solid-State Culture Using Peanut Shell as Substrate

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Introduction: *Trichoderma harzianum* is an important biological control agent. Their conidia produced via solid-state culture are the active principle of many commercial products applied in agriculture. *T. harzianum* produces high concentrations of cellulases and xylanases. Therefore, their conidia could be produced on lignocellulosic waste. Thus, this study evaluated peanut shell (PS) as solid substrate-support for the conidia production of *T. harzianum*. It was used different media impregnated in PS. **Methodology:** Two particle sizes of PS (1-2 cm and 2 mm) were impregnated with distilled water, or Spezieller Nährstoffarmer Agar (SNA) (moist up to 60%). The impregnated PS was inoculated with conidia suspension of 1×10^6 conidia/g and was incubated at 30 °C for 7 d in tray bioreactor (4 x 4 x 4 cm). The conidia production was evaluated every 24 h using a Neubauer hemacytometer. **Results:** In all treatments, maxima conidia production was reached after 6 d of culture. The conidia production reached in trays packed with 2 mm PS particles and impregnated with SNA (9.70×10^8 conidia/g) was significantly higher than those obtained in the other treatments (Tukey, $\alpha=0.05$). Fourfold higher conidia production can be reached using SNA to impregnate the PS. **Conclusion:** A production of conidia comparable to that reported in first-generation substrates can be obtained using PS as a substrate. Production yields can be improved by decreasing the particle size and adding SNA medium.

Key words: Conidia, biological control, waste valorization, bioprocess, sustainable agriculture.

Agricultural & Food Biotechnology

Extraction of flavonoids from grape waste with potential antihyperglycemic activity *in silico*

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Introduction: Utilization, recovery, and efficient agro-industrial waste management are vital to minimizing environmental impact and initiating a more sustainable food and agricultural system. Grapes waste presents different compounds of interest to the food industry, highlighting phenolic compounds. Due to their chemical structure, flavonoids present bioactivities and physicochemical properties with potential applications. Microwave-assisted extraction is an alternative method for obtaining bioactive compounds, preserving their chemical composition and bioactivity. This work aimed to obtain flavonoids with potential antihyperglycemic activity from grape waste as an alternative use of waste derived from food processing. **Methodology:** The extraction factors studied were time (300 and 150 s) and power (800 and 400 W). HPLC-MS carried out a phenolic profile for identification. The antihyperglycemic activity was analyzed by *in silico* molecular docking of pancreatic α -amylase (ID: 1HNY) on Autodock Vina. Receptor-ligand molecular interactions and visualization were analyzed using PyMOL. **Results:** Flavonoids identified were myricetin, kaempferol, quercetin, and catechin; they presented binding affinity and molecular interaction *in silico* of less than 3.5 Å with the catalytic amino acids (Asp197, Glu233, and Asp300) of pancreatic α -amylase with binding energy greater than -7.0 kcal/mol. α -Amylase is an enzyme directly related to the digestion of soluble carbohydrates and the associated metabolism of glucose. **Conclusion:** Flavonoids from grape waste can be applied as a functional food additive that can reduce the rate of postprandial glucose absorption.

Keywords: molecular-docking, α -amylase, metabolism-glucose, functional-additive.

DNA-Free Genome Editing in Soybean using CRISPR/Cas9 Ribonucleoprotein Delivery

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Introduction: Soybean (*Glycine max* [(L.) Merrill]) is one of the most economically relevant crops in the world, serving as a major source of vegetable protein and feedstock for biodiesel production. CRISPR/Cas9 technology has been used to improve soybean productivity and quality by enabling rapid and precise genetic modifications. In many countries, plants developed by DNA-free gene editing methods, which utilize ribonucleoproteins (RNPs) or mRNAs, are not regulated as genetically modified organisms, hence reducing the cost and time required to release new cultivars to the market. However, DNA-free methods are still relatively underexplored for soybean molecular breeding. In this study, a DNA-free genome editing protocol was developed by bombarding soybean embryonic axes with RNPs carried by gold particles. **Methodology:** The expression optimization of the Cas9 nuclease from *Staphylococcus aureus* (SaCas9) was carried out in *E. coli* BL21(DE3) pLysS. sgRNAs were designed to target the phytoene desaturase gene (*GmPDS*). RNP activity was validated through *in vitro* cleavage assays prior to complexation with gold particles (60 µm) and embryonic axis bombardment of *G. max* var. Williams 82. Plants were regenerated *in vitro* and pre-selected based on a chlorotic phenotype, potentially caused by *GmPDS* knockout. Three sets of experiments, named A.1, A.2, and A.3 were conducted with variations in shot pressure, number of shots, and embryo sonication time. **Results:** The model used to optimize the heterologous expression of SaCas9 resulted in a maximum yield of 25 mg/L of expression. *In vitro* cleavage tests to validate the RNPs indicated an editing efficiency of nearly 90%, varying according to the ratios of SaCas9, sgRNA, and DNA template. In total, 365 embryos were bombarded. Sanger sequencing and alignment analyses identified seven individuals with indels in the sgRNA binding site, of which six were from A.3 experiment, resulting in an *in vivo* editing efficiency of 8%. **Conclusion:** This protocol presents a viable alternative for accelerating the generation of edited soybean cultivars with new agronomic traits.

Key words: SaCas9, Transgene-free, *Glycine max*, RNP

Herbicidal activity of micro and nano encapsulated microbial metabolites from phytopathogenic organisms and plant extracts on the growth of *Sorghum bicolor* and *Phaseolus vulgaris* and their potential in weed control.

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Introduction: Agricultural production is affected by weeds that compete with crops for resources, decreasing yield. Therefore, developing organic alternatives such as microbial metabolites and plant extracts is necessary. Moreover, incorporating these alternatives into biopolymeric matrices may enhance their efficacy. The objective of this study was to assess the herbicidal activity of metabolites from *Alternaria* spp., *Colletotrichum* spp., and *Macrophomina* spp., as well as extracts from *Piper nigrum* and *Solanum rostratum*, both in micro and nano-encapsulated formulation (NPs) with biopolymers, on the development of *Sorghum bicolor* and *Phaseolus vulgaris* as indicator plants. Additionally, the study aimed to identify the compounds present in these metabolites and extracts. **Methodology:** The metabolites were obtained through liquid fermentation, and extracts were provided by GreenCorp Biorganiks de Mexico S.A. de C.V. Compound characterization was conducted using HPLC-MS, and NPs were produced via ionotropic pre-gelation. Subsequently, these NPs were evaluated on seedlings of *S. bicolor* and *P. vulgaris* under greenhouse conditions. The experimental design was randomized with three replicates, and data were analyzed using analysis of variance followed by Tukey's mean comparison ($p \leq 0.05$). **Results:** The highest phytotoxicity on *S. bicolor* plants was observed with NPs loaded with metabolites from *Alternaria* spp. and extracts from *P. nigrum* and *S. rostratum*, in addition to the unencapsulated extract of *S. rostratum*. Regarding *P. vulgaris* plants, phytotoxicity was lower compared to *S. bicolor* plants, with once again NPs loaded with metabolites from *Alternaria* spp. and extracts causing the most phytotoxicity. It was also observed that unencapsulated metabolites from *Alternaria* spp. had phytotoxic effects. **Conclusion:** Metabolites from *Alternaria* spp. and *Macrophomina* spp., as well as extracts from *P. nigrum* and *S. rostratum*, hold potential for weed control in post-emergence; however, their effectiveness may increase when formulated as NPs. Nevertheless, further research is necessary to enhance the efficacy of NPs loaded metabolites and plant extracts.

Key words: Bioherbicides, plant extracts, nanobiotechnology, organic agriculture



Agricultural & Food Biotechnology

Breakfast Cereals: Insights into Market Variety and Potential Innovation

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Introduction: More than half of the Portuguese population consumes breakfast cereals (BC), which have high sugar, saturated fatty acids, and salt contents while being low in fiber. **Methodology:** To address these shortcomings, the nutritional facts of 178 BC available on the Portuguese market were analyzed, alongside a literature revision to identify improvement strategies. **Results:** Only 26% of BC were classified as having good nutritional quality (A or B Nutri-Scores). The unflavoured expanded BC had the highest percentage of A or B Nutri-Scores, followed by the unflavoured extruded, cornflakes, and petals. The flavoured and co-extruded BC had the lowest percentage. All BC elaborated from single-pseudocereals, and combinations of cereals and legumes were of good nutritional quality, followed by those elaborated from cereals and pseudocereals, single-cereal, and multi-cereals. The highest percentage of low fat, low saturated fat, source of protein, low sugars, sugars-free, low salt, very low salt, and salt-free claims are found in the unflavoured cornflakes and expanded BC, and flavoured extruded BC. The fat-free, saturated fat-free, source of fiber, vitamins, and minerals, and high fiber, vitamins, and mineral claims were mainly found in the flavoured extruded and both flavoured and unflavoured petals. Regarding formulation, the fat-, protein-, sugar-, and salt-family claims were more present in percentage terms in the single-cereal BC while fiber-, vitamins- and minerals-family claims were more present in the multi-cereal ones. Only 5% of the BC analyzed reported health claims, which referred to vitamins and minerals. The literature suggests that the usage of sprouted wheat whole meal, red and black rice, purple corn, biofortified maize, legumes, fruit by-products, or other materials such as algae, silkworm pupae powder, and butterfly pea flower can improve the fiber and protein contents of BC, enhance its physicochemical and sensorial properties, and increase both antioxidant activity and phytochemical contents. **Conclusion:** In this way, it is possible to overcome nutritional gaps and increase the consumption of deficient food groups with new and more differentiated BC with underused raw materials and better acceptance by the consumer. These strategies highlight the need for nutritional improvement in BC to meet consumer health trends and market needs.

Key words: Breakfast cereals, Nutri-Scores, claims, nutritional quality, improvement.



Antimicrobial effect of *Capsicum annuum* on pathogens causing foodborne diseases

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Introduction: Nutritional quality and food safety are two aspects that have a direct impact on the health and life quality of populations, therefore foodborne diseases (FBD) are a problem that is considered in a technological, economic and social environment, this leads us to seek new ways for food to have a longer shelf life without compromising the quality, composition and characteristics of products. The use of extracts from different sources such as plants, fruits or vegetables is a great option to substitute chemical additives used in food. That is the case of plants belonging to the *Capsicum* genus, which have been reported to have antimicrobial activity when extracts of different species such as *C. chinense*, *C. frutescens*, *C. pubescens*, among others, are used; however, there are still few studies on *C. annuum* (bell pepper) regarding its biotechnological use. **Methodology:** The *Capsicum annuum* extract was made by macerating the bell pepper sample in isopropyl alcohol and then evaporating it with a modified Soxhlet and finally concentrating it with a rotary evaporator. The inhibition test of *Capsicum annuum* extract was performed by the modified Kirby Bauer method using *Salmonella Typhi*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Cronobacter sakazakii*. **Results:** A yield of 1.6% was obtained from the bell pepper extract. Inhibition halos with a diameter between 7 and 13 mm were recorded in the strain corresponding to *Bacillus cereus*. **Conclusion:** *Capsicum annuum* extract has inhibitory action against *Bacillus cereus*.

Key words: *Capsicum annuum*, extract, inhibition, *Bacillus cereus*.

Bioactive compounds and antioxidant activity of *Myrtillocactus geometrizans* extracts in postharvest quality of berries

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Introduction. The application of edible coatings (EC) based on different natural matrices (e.g. chia mucilage) has shown favorable effects to extend the shelf life of fruits and vegetables, in addition to being an appropriate vehicle for the incorporation of bioactive compounds. Extracts obtained from plants of arid zones of Mexico have different properties as antioxidants, antimicrobials, etc., which in synergy, potentiate their protective action. The objective of the present study was to evaluate the effect of EC based on chia (*Salvia hispanica* L.) functionalized with extracts of *Myrtillocactus geometrizans* (G) on the postharvest quality of blackberry. **Methodology.** Two extracts were obtained: hydroalcoholic extracts (HA) and aqueous extract (EA), characterized in Total phenolic content (TPC), antioxidants (ABTS and DPPH) and a shelf life at 85% of HR were tested using a edible coating (EC) of chia mucilage. **Results.** EHA presented a higher total phenols content (TPC) with values of 9.9 ± 0.3 g of gallic acid (GA) kg^{-1} of extract, as well as better antioxidant potential, expressed as the concentration of extract required to cause 50% oxidation inhibition ($\text{CI}_{50} = 5.6 \pm 0.5$ mg mL^{-1}). Considering the TPC and antioxidant potential results, EHA was selected as the source of natural bioactive compounds to be incorporated into a chia-based edible coating (EC+G). The application of EC+G served as a protective barrier against weight loss and color retention, with weight loss values of 6.74% in fruits coated with R+G, while the control fruits (uncoated fruits) had a weight loss of 8.62% during storage. Moreover, the fruits with EC+G retained color and maintained better appearance during the 15 days of storage compared to the control fruits. **Conclusion.** This research seeks to highlight the importance and scope of developing new technologies to extend the shelf life of blackberries, a crop of significant commercial relevance in Mexico.

Key words: *Myrtillocactus geometrizans*, shelf life, edible coating.



Agricultural & Food Biotechnology

Production of single-cell protein from agroindustrial wastes

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Introduction: Protein intake in the daily diet contributes not only to physical development, but also to a strong immune system and overall health balance. They play a fundamental role in the nutritional aspect, as they are involved in various bodily functions such as the building of muscles, bones, skin and organs. They are also vital for the production of enzymes, hormones and antibodies. Current forms of protein consumption are based on animal and vegetable sources, the lack of protein has created the need to search for new sources. The single cell protein can help balance these needs. Agroindustrial wastes are excellent substrates for obtaining value added compounds, such as unicellular protein. In the present research project, submerged fermentation was employed as a bioprocess for the production of *Candida utilis* yeast biomass from orange peel, corn cob, avocado peels and seeds.

Methodology: The chemical composition of the agroindustrial wastes was evaluated, the three media composed of 10 % (g/L) of agroindustrial wastes as carbon source, with the addition of mineral salts and an inoculum of 1×10^6 cells/mL at 150 rpm at 30 °C for 120 h were compared. The optimization evaluation was carried out with a Box Benken 3^3 design with a total of 15 treatments, where independent variables were: Nitrogen source content, corn cob substrate content and temperature. The highest substrate consumption was obtained with corn cob wastes and a production of 8.65 ± 0.39 g/L and $12.6 \% \pm 0.12$ of biomass and total protein, respectively.

Conclusion: The design showed that both substrate and nitrogen concentration show positive significant differences, indicating that as the amount of $(\text{NH}_4)_2\text{SO}_4$ and substrate increases, higher biomass production is expected. These nutrients are critical for the growth of the *Candida utilis* strain and thus for the production of unicellular protein.

Keywords: Submerged fermentation, yeast, optimization, single cell protein.



Agricultural and food biotechnology

Impact of Cold Plasma Treatment on the Physical Properties of Grapefruit Juice

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Introduction: Cold plasma treatment is an innovative technique used to modify the physicochemical properties of food, offering potential benefits to the food industry. This study investigates the effect of plasma treatment on the physicochemical properties of grapefruit juice, including pH, temperature, electrical conductivity, salinity, soluble solids, and color.

Methodology: Samples of 50 ml of fresh grapefruit juice were subjected to plasma treatment using a device positioned 2 cm above the liquid. The agitation was maintained at a constant 2 rpm, and air was used as the working gas. The operational conditions included a power of 30 watts, with variations in time (2, 4, 6, 8, and 10 minutes) and frequency (400, 600, 1200, and 1600 Hz).

Results: The results showed that the treatment at 400 Hz had a more notable impact on increasing the pH of the juice, while higher frequencies tended to stabilize the pH levels, keeping them closer to the initial value of 3.29. In particular, the 10-400 Hz treatment reduced the pH to 3.17, while the other frequencies gradually recovered to higher levels. Changes in temperature were minor, with a slight maximum increase of 5.7 °C, ensuring the preservation of nutritional quality.

Conclusion: Cold plasma treatment offers an effective alternative for preserving grapefruit juice by achieving controlled modifications in its physicochemical properties. This study provides evidence of the potential of cold plasma technology for application in the food industry, promoting fresher and healthier products without compromising essential nutritional properties.

Key words: Cold plasma, Physicochemical properties, Grapefruit juice, Food treatment, Food preservation.

Encapsulation of amaranth protein hydrolysates with ACE-I inhibitory activity: characterization, stability, and influence on bioactive properties

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Introduction: Hypertension is the chronic elevation of blood pressure. One of its causes is the activity of angiotensin converting enzyme-I (ACE-I), therefore the aim is to inhibit it. Protein hydrolysates released from amaranth protein, have this effect. However, they may eventually lose this bioactivity due to gastrointestinal conditions. It has been reported that strategies such as encapsulation in edible biopolymers can solve these problems. **Methodology:** Protein hydrolysates with ACE-I inhibitory activity obtained previously through fermentation with *Enterococcus faecium*-LR9 strain were encapsulated. For this, a 1% solution was made with the biopolymers alginate (A) and pectin (P) at different ratios: 100% A, 75% A 25% P, 50% A 50% P, 25% A 75% P, 100% P. Protein hydrolysate was added at 1%. This solution was dripped into a 0.1 M CaCl₂ solution. The encapsulates obtained were evaluated in wet and dry state. These samples were characterized by determining their size, degree of roundness and encapsulation efficiency. The selected samples were subjected to *in vitro* digestion, according to the standardized INFOGEST protocol, the peptide release and ACE-I inhibitory activity of the digests were determined. **Results and discussion:** The size analysis of the wet and dry encapsulates showed that as pectin was added the size increased, even in the 100% P treatment there was a total deformation of the encapsulates, so it was not possible to measure their size. This is because pectin has a faster gelation and egg-crate structures are formed in "stippling mode", which causes non-uniform pores and large sizes. For this reason, the degree of roundness is also affected. Treatment 100 % A obtained the highest encapsulation efficiency (95.57 % ± 0.49), which is associated with the properties of alginate and its interaction with the protein hydrolysates. The encapsulates manage to retain the ACE-I inhibitory activity after the encapsulation process, as compared to the unencapsulated protein hydrolysate which loses all this bioactivity. The 75% A 25% P treatment achieved the best results during this process. **Conclusions:** 100% A and 75% A 25% P amaranth protein hydrolysates encapsulates retain ACE-I inhibitory activity even after *in vitro* digestion.

Key words: Protein hydrolysates, encapsulated, pectin, alginate, digestion

Hemp Protein Isolate: Evaluation of its potential applicability in food matrices through functional characterization and *in vitro* digestion.

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Introduction: Hemp cake is an agro-industrial waste obtained from oil production and that usually ends up in landfills. Although hemp seed is mostly known for its oil nutritional quality, its protein content is also a noteworthy attribute. Hemp protein isolate (HPI) is reported to be rich in essential amino acids and is a promising food ingredient, contributing to sustainability and human health. The objective of this research was to evaluate the techno-functional properties (TFP) (solubility, emulsifying capacity, zeta-potential) and *in vitro* digestion of HPI. **Methodology:** Hemp Protein was isolated from defatted seed cake by isoelectric precipitation, 84.6% protein content was obtained. Water- and oil-holding capacity were measured and compared with Soy Protein Isolate (SPI). SPI and HPI zeta-potential and solubility were measured as pH-dependent parameters. Pseudoternary graph was used to evaluate HPI and SPI emulsifying capacity and emulsion stability. The protein digestion was evaluated by INFOGEST *in vitro* model, protein hydrolysis degree was measured by OPA, and structural changes were observed by Confocal Microscopy. **Results:** TFP of SPI were significantly higher than HPI. However, HPI has shown a better oil-trapping capacity; while its water-holding capacity was, both compared to SPI. With respect to zeta-potential and solubility, both protein isolates have shown similar behaviour, being highly stable at pH 2, 3, and 7; because of the ionization of amino acids the solubility was highest for pHs further isoelectric point. HPI showed emulsifying capacity, however, SPI shown a capacity to trap higher amounts of sunflower oil under the same conditions. *In vitro* digestibility assay of HPI compared to SPI shown no significant differences in amino acids concentration at the end of the gastric and intestinal phases, structural changes were observed on CLSM images. **Conclusions:** Due to its suitable TFP, HPI is a promising protein for application as a food ingredient. Moreover, the *in vitro* digestibility of HPI is comparable to that of SPI, revealing not only its potency as a techno-functional ingredient. Applications in food matrices such as apple or grape juice and cold meat are suggested due to their physicochemical characteristics and may even increase the nutritional value of these foods.

Key words: Hemp Protein Isolate, Functional Properties, *In vitro* digestion, INFOGEST.



Agricultural & Food Biotechnology

Evaluation of Different Carbon Sources on the Growth Kinetics of *Bacillus subtilis* as a Biofertilizer

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Introduction: *Bacillus subtilis* emerges as a sustainable and environmentally friendly alternative to agrochemicals, beneficial for both the environment and human health. It acts as a growth promoter and biocontrol agent against pathogens. Optimizing its growth is crucial for the creation of bioproducts aimed at enhancing agricultural yields, reducing costs, and minimizing losses.

Methodology: This study aimed to evaluate different carbon sources as nutritional factors through microbial growth kinetics. Six carbon sources were analyzed, including naturally derived sugars: sucrose, fructose, lactose, dextrose, honey, molasses, and piloncillo. Microbial growth kinetics were assessed using McFarland turbidity to quantitatively determine cell concentration per mL. Absorbance readings were taken every 12 hours for 72 hours.

Results: Statistical analyses revealed significant differences among the various evaluated factors. The highest growth rates were observed in media supplemented with molasses and piloncillo, highlighting these carbon sources as the most favorable for the growth of *B. subtilis*.

Conclusion: The results provide valuable insights into the conditions necessary to optimize the cultivation of *B. subtilis*, with potential applications in the production of biofertilizers and biocontrol agents that are environmentally friendly and safe for human health.

Keywords: carbon sources, natural sugars, *B. subtilis*, microbial kinetics



Agriculture & Food Biotechnology

Monitoring *cis* configurations in canola oil (*Brassica napus* L.) when bleached using high-power ultrasound

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Introduction: Canola oil is one of the most important vegetable oils in Mexico, because it is widely used in food preparation. However, the most commonly used methodology to bleach it is about a 180 min process in combination with a high temperature (100 °C) and a high clay percentage (3% w/w). Despite this methodology, which is well known in the industry, the high temperature and the long process could cause changes in *cis* configurations, which give an unhealthy final product. Therefore, the main aim of this study was to develop an emergent bleaching process using high-power ultrasound with the intention of reducing this parameter and, in this way, avoiding the changes in *cis* configurations and the appearance of *trans* configurations in the entire bleaching process, which was analysed using spectroscopies FTIR, and Raman.

Methodology: An ultrasonic bleaching system was built using three clay percentages (1, 2, and 3% diatomaceous earth) with a three-time process (60, 75, and 90 min) at 60 °C. On the other hand, a conventional method was applied to an oil sample and was considered as control. To analyze the samples once the bleaching treatments were carried out, we used the mid-infrared (400–4000 cm⁻¹) and Raman spectra (300–3300 cm⁻¹). **Results:** Both infrared and Raman spectra showed that no ultrasonic treatment changed or encouraged *trans* configuration appearance on oil samples by not observing changes in the 720, 1650, and 3010 cm⁻¹ bands, as shown by the infrared spectra. Regarding the Raman analysis, important groups in the oil constitution were identified, such as the configuration of *cis* groups at 1280 cm⁻¹ and 1660 cm⁻¹, the ester group at 1750 cm⁻¹, and the methyl group at 2970 and 3015 cm⁻¹. **Conclusions:** These results indicate that the treatments do not modify their oil molecular configuration give us a healthy product. High-power ultrasound show be a viable option with the assistance of the conventional method to obtain a stable product.

Keywords: Canola oil, spectroscopy FTIR and Raman, *cis* configurations.

Agricultural & Food Biotechnology

Influence of Different NaCl Concentrations on β -Mannanase Activity.

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Introduction: β -Mannanase is an enzyme that randomly cleaves the internal 1,4- β -mannosidic glycosidic bonds present in the structure of mannan/heteromannan. Assessing the impact of NaCl on its activity is essential for optimizing industrial applications. This study aimed to analyze the effects of different NaCl concentrations on β -mannanase activity. **Methodology:** The enzyme was produced through solid-state fermentation in sterile 125 mL Erlenmeyer flasks, using a mixture of components (73.27% cocoa bean shell, 17.64% ora-pro-nóbis, and 9.09% tamarind shell). The fungus used was *Penicillium roqueforti* ATCC 10110. The sterile substrate was inoculated with 10^7 spores per gram of substrate, with 69% moisture, and incubated at 22 °C for 7 days. Different concentrations of NaCl (0.01 M, 0.05 M, 1 M, 2 M, 3 M, 4 M, 5 M, and 6 M) were used to assess the tolerance of the β -mannanase from *P. roqueforti* ATCC 10110 to salt. The activity of β -mannanase with NaCl was determined using the DNS method and expressed as relative activity (%). **Results:** An increase of up to 18% in β -mannanase activity was observed with the addition of NaCl compared to the control (without salt). The enzyme remained active at all tested concentrations, including 6 M, where the residual activity exceeded 100%. Halotolerant enzymes have a surface rich in acidic residues and few hydrophobic residues. The carboxyl groups of the acidic residues interact with water molecules, creating a solvation layer that facilitates hydration. **Conclusion:** The data show that the β -mannanase from *P. roqueforti* ATCC 10110 is tolerant to high salt concentrations, exceeding that of seawater (0.46 mol L^{-1}), and can be applied in processes that replace freshwater with seawater, such as in the saccharification of lignocellulosic biomass or in high-salinity processes.

Keywords: Hemicellulase, enzyme, lignocellulosic biomass, *Penicillium roqueforti*, solid state fermentation.



Agricultural & Food Biotechnology

Evaluation of the Hydrothermal Processing on Wheat Straw for Xylooligosaccharides Production

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Introduction: Xylooligosaccharides (XOS) can be obtained through the partial hydrolysis of lignocellulosic materials rich in xylan, such as agro-industrial residues. Hydrothermal processing (autohydrolysis) is a common method for producing XOS, involving hydrothermal treatment of the material with pressurized hot water, without external catalysts. The study aimed to evaluate the temperature and time conditions for hydrothermal processing to maximize hemicellulose solubilization for xylooligosaccharides production. **Methodology:** In this study, wheat straw with 0.5 mm particle size was characterized for ash, extractives, moisture, and Klason lignin content, expressed as percentages. Hydrothermal processing was performed in a 190 mL reactor using a central composite design with 11 trials and a triplicate at the central point. Operational conditions varied from 150 to 180°C and 10 to 30 minutes, with a solid-to-liquid ratio of 1:10. After pretreatment, the pH of the liquid fraction was measured, and the LogR_o (severity factor) was calculated using Polymath software 6.0. **Results:** The results showed that the wheat straw contained 27.36 ± 0.26% Klason lignin, 14.02 ± 0.45% ash, 9.63 ± 0.43% moisture, and 8.33 ± 0.31% extractives. The LogR_o ranged from 3.58 to 3.97, being highest under the most severe conditions (180°C/30 minutes), correlating with the lowest pH (4.96). This indicates greater xylan removal and increased H⁺ ion production due to water autoionization and acetyl group breakdown in hemicellulose at high temperatures. A darker color in the pretreated solid fraction was also observed, likely due to sugar caramelization. **Conclusion:** It is concluded that the condition of 180°C/30 minutes may be associated with increased XOS production due to more effective hemicellulose solubilization. Further analyses will be conducted to deepen the research.

Keywords: Hemicellulose, autohydrolysis, lignocellulosic biomass.

Agricultural & Food Biotechnology

**Insecticidal Effect of *Pseudomonas entomophila* on the Model Insect *Galleria mellonella*
(Order: Lepidoptera)**

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Introduction: Pests pose a significant problem in crop maintenance and have had negative effects on global food supply and economy. While chemical insecticides have been used to control pests, their use has a considerable negative impact on ecosystems and human health. Biological control agents offer a viable and effective alternative for the control of pest insects from various orders, including the bacterium *Pseudomonas entomophila*. This bacterium produces toxins harmful to insects of the order *Lepidoptera*, disrupting their growth and even causing death, thus playing a key role in its ability to control insect pests. **Methodology:** The bacterium *P. entomophila* was provided by the company Greencorp, and it was reactivated on a plate before being transferred to tryptic soy broth at 30°C, 24 hours, and 120 rpm to obtain the pre-inoculum. Three culture media were evaluated: tryptic soy broth (TSA), King B (KB), and Luria Bertani (LB), which were inoculated at an initial concentration of 1x10⁶ cells/ml, 30°C, 120 rpm. Two samples were taken, one at 24 hours and the other at 48 hours. These samples were adjusted to a concentration of 1x10⁷ cells/ml, and an insecticidal bioassay was conducted against *Galleria mellonella* larvae at the L3 stage, applying the treatment by ingestion according to IRAC methodology, where the response variable was the percentage of mortality over 7 days. **Results:** It was determined that the best culture medium was LB with a fermentation time of 24 hours, showing the highest mortality rate of 36.67% at 168 hours of evaluation on the model larvae (Tukey p=0.05). The other treatments were inferior. This mortality is attributed to the toxins generated by the bacterium *P. entomophila*. **Conclusion:** The use of biological agents like *P. entomophila* reduces the dependence on chemical pesticides, benefiting both the environment and human health. This makes *P. entomophila* a cost-effective bacterium, generating solutions and benefiting the soil of Mexico and the world.

Keywords: *Pseudomonas entomophila*, Entomopathogen, Lepidoptera

Agricultural and Food Biotechnology.

Effect of polyphenols extracted by maceration of *Larrea tridentata* and walnut shell (*Carya illinoensis*) against *Alternaria solani*.

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Introduction: Potato and tomato crops are affected by pathogenic fungi such as *Alternaria solani* which causes the disease known as early blight. Gobernadora (*Larrea tridentata*) and nutshell (*Carya illinoensis*) contain large amounts of phytochemicals including polyphenols which have been shown to have antifungal effects. In this study, the effect of polyphenols in vitro of ethanolic extracts of *Larrea tridentata* and *Carya illinoensis* obtained by maceration against the fungus *Alternaria solani* is evaluated. **Methodology:** Phytochemicals macerated from *Larrea tridentata* leaves and nutshell talc were characterized by hydrolyzable phenols, total flavonoids at different maceration temperatures and different ethanol:water concentrations and subsequently evaluated for DPPH and ABTS antioxidant activity. In addition, their antifungal activity against the fungus *Alternaria solani* was evaluated using the simple diffusion confrontation technique. **Results:** For characterization, 903.2 mgQE/g of flavonoids were obtained at a temperature of 60°C with an ethanol concentration of 70%, and 123.6 mgQE/g of hydrolyzable polyphenols were obtained at a temperature of 60°C with an ethanol concentration of 70%. 6 mgQE/g at a temperature of 50°C with 100% ethanol in the case of *Larrea tridentata*, and in the case of walnut shells, 372.7 mgQE/g of flavonoids were obtained at a temperature of 60°C with an ethanol concentration of 50% and 144.5 mgQE/g of polyphenols at a temperature of 60°C with an ethanol concentration of 50%. Greater antioxidant activity was obtained at 100% ethanol in the case of *Larrea tridentata*, and in the case of walnut shells, greater DPPH activity was obtained at 60% ethanol and ABTS at 100% ethanol. *Larrea tridentata* had a higher percentage of inhibition against the fungus *Alternaria solani*, with 64.30% at 50% ethanol. **Conclusion:** *Larrea tridentata* showed higher amounts of hydrolyzable polyphenols and total flavonoids and antioxidant activity, as well as higher percentage of inhibition against *Alternaria solani* than walnut shells.

Key words: *Larrea tridentata*, *Carya illinoensis*, *Alternaria solani*, polyphenols, flavonoids



Agricultural & Food Biotechnology

Cold frequency plasma pretreatment for tomato (*Solanum lycopersicum* L.) seed germination

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Introduction: Improvement in seed germination has a direct effect on crop production, and seed germination of various food crops can be enhanced by various physical and chemical treatment methods, such as ultraviolet light, hot water soaking, and the use of fungicides and hormones. Although earlier described methods improve seed germination, these are labor-intensive or not eco-friendly methods. Recently, cold plasma has been successfully used for treating plant seeds, and can effectively improve the germination of seeds, seedling growth, and crop yield under a pollution-free controlled environment by increasing the permeability of the testa by modifying its structures. The aim of this work was to evaluate different times and frequencies of a cold plasma system and its effect on tomato seed germination. **Methodology:** Germination assays were carried out on tomato seeds pretreated with cold frequency plasma, where different times (30, 150 and 300 s) and frequencies (500, 750 Hz) were evaluated, giving a total of 6 treatments, to observe the effect of these factors on some germination parameters such as percentage, rate and mean time. 6 seeds per treatment were placed by triplicate, at 28 °C with periods of light and darkness of 16:8 h respectively for 12 days. **Results:** The results showed that treatments 2 (150 s and 500 Hz) and 4 (30 s and 750 Hz) showed the best results in percentage (83.3 %) and rate (0.41 seeds/day) of germination compared to a control (75 % and 0.37 seeds/day), and treatments 3 (300 s and 500 Hz) and 6 (300 s and 750 Hz), which have the longest exposure time obtained the lowest values of percentage and germination rate (0 and 16 % respectively). **Conclusion:** The results obtained show that for both frequencies, short times of exposure to plasma provide the best results (30 and 150 s), and increasing these times shows a decrease in germination parameters, however, these results indicate that the use of a cold frequency plasma equipment as a pretreatment for tomato seeds promotes germination depending on the time of exposure.

Key words: Seed germination, cold plasma, tomato seed.



Mathematical models to study the influence of imbibition temperature on the sugar extraction at sugar mill factory.

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ABSTRACT

The imbibition temperature impacts on the operational performance of the cane juice extraction stage remains inconclusive or unsupported by industry experimental data. This research analyzes the effect of imbibition temperature on the mill train juice temperature, bagasse moisture and sucrose composition, and the mill train juice starch composition. By using mathematical models, as a useful tool to control the extraction stage of the raw sugar manufacturing process, is quickly and accurately to predict the output variables of the extraction stage. The results indicated the imbibition temperature does not influence the temperature of the mixed juice and not allow reaching the minimum temperature recommended for asepsis (65 °C). It is established that when the imbibition temperature decreases from 85°C to 40°C, bagasse moisture increases up to 1.8 units, while sucrose extraction is reduced by 12%. On the other hand, if the imbibition temperature is increased from 40°C to 85°C, the starch composition of the mixed juice increases by 15%. The multiple linear regression mathematical model developed in this work was validated for imbibition water temperature between 40-80°C in more than one sugar mill, demonstrating an adequate predictive capability.

Keywords: Imbibition temperature, sugar extraction.



Agricultural y food biotechnology

Trapping of zinc nanoparticles and *Lactobacillus* sp. in bacterial nanocellulose

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Probiotics are affected by several factors such as pH, oxygen, and temperature, which can significantly impact their viability and survival rate during processing, storage, and passage through the gastrointestinal tract. Microencapsulation using bacterial nanocellulose is a promising option to address these challenges, as it is biodegradable and biocompatible with humans. Therefore, the objective of this work is the encapsulation of *Lactobacillus* sp. in bacterial nanocellulose films loaded with zinc nanoparticles (ZnONP), synthesized through a green process in the presence of an extract rich in polyphenols (*Buddleja scordioides*). The growth kinetics of *Lactobacillus* sp. was studied to identify the exponential and the stationary phases. Additionally, the trapping of ZnONP and *Lactobacillus* sp, during fermentation, in bacterial nanocellulose (NCB) was performed in HS medium with a concentration of 100 ppm ZnONP and 1 ml of *Lactobacillus* sp. under static conditions at 37 ± 1 °C for 3 days. The results shown that the stationary phase begins 20 hours after inoculation. This time was used for preparing the inoculum for entrapment. FTIR analysis confirmed the entrapment of pure ZnONP and *Lactobacillus* sp. in the bacterial nanocellulose networks. The presence of characteristic bands of NCB were observed, the strong and broad peak around 3300 cm^{-1} , associated with the stretching vibration of the hydrogen bond from hydroxyl groups in NCB, as well as the peak at 1030 cm^{-1} , characteristic of the C-O single bond, a stretching vibration belonging to the polysaccharides in cellulose. Microbial kinetics indicate that the optimal stage for microorganism recovery is the stationary phase, as it is the growth phase where the population continues to increase. The entrapment of ZnONP in nanocellulose networks is primarily due to the presence of OH groups in the cellulose structure, which generates a partially electronegative layer on the surface and allows the adsorption of metal cations.

Key words: Lactobacillus, Nanocellulose, Encapsulation



Agricultural & Food Biotechnology

Comparative Analysis of Sugarcane Bagasse Delignification via Organosolv and Combined Autohydrolysis-Organosolv Methods

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Introduction: Sugarcane bagasse is a lignocellulosic biomass, composed of cellulose, hemicellulose and lignin with potential for be used in wide range of industries. Lignin has been approached in industrial and biotechnological applications due to its properties as versatility, antioxidant activity and ability to improve the strength and permeability of various materials. For this reason, the objective of this work was to extract and characterize lignin from sugarcane bagasse through organosolv and hydrothermal-organosolv processes. **Methodology:** For organosolv process the biomass was subjected to 170, 180 and 190°C for 20 min using ethanol (40 %, v/v) and NaOH (0.1 %, w/v) in a high-pressure reactor. For hydrothermal–organosolv sequential process the bagasse was pre-treated at 180°C for 20 minutes followed by organosolv process at 170,180 and 190°C for 20 minutes. The recovered liquid and solid phases of both processes were separated by filtration and the lignin was recovered by precipitation with hydrochloric acid, then was centrifugated, water washed and dried. Recovery yield lignin, FTIR analysis, TGA analysis, antioxidant and antimicrobial activity on lignin were evaluated. **Results:** The lignin subjected to sequential hydrothermal–organosolv process with 180°C for 20 minutes shows the highest recovery yield of 12% these results were better than the ones obtained with organosolv process with the highest yield of 8%. FTIR analysis shows functional groups correspondent to lignin, specifically in the region's bands of 3300-2700 cm⁻¹, 2850-2600 cm⁻¹ and 1740 cm⁻¹. Lignin recovered from sequential process shows antimicrobial activity and higher antioxidant activity by DPPH (60% inhibition) than the extracted by organosolv process (40% inhibition). **Conclusions:** Sugarcane bagasse is a good source to obtain lignin, specially recovered through sequential process of autohydrolysis-organosolv, maintaining good properties that can promote a relevant property for application in food industries as films.

Key words: Biorefinery; hydrothermal process, Biomass, Lignin

Unfolding the bioactive potential of microalgae biomasses through the development of an integrative processing model

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Introduction: Microalgae biomasses have been defined as novel protein sources, however, their complex cell wall limits the potential application of microalgae protein [1]. Thus, novel trends on disruptive, extraction and processing methods, using green and eco-friendly agents for valuable molecules obtention have raised up. The objective of the present study is to evaluate the potential of *Spirulina platensis*, *Tetraselmis striata*, *Chlorococcum Sp*, *Tisochrysis lutea* and two *Chlorella vulgaris* (Yellow and White) for protein extraction using conventional and novel sustainable techniques. **Methodology:** The evaluation englobed the evaluation of integral sequential extraction technique using acid-hot treatment (HCl, citric and acetic acid), ultrasound and enzymatic assisted extraction (Cellulase). The recovered extracts were subjected to a protein quantification (Bradford assay), antioxidant (ABTS and FRAP methods) and antimicrobial evaluation (against *E. coli* and *Salmonella*). Also, the molecular weight distribution of peptides and proteins was evaluated using a FPLC equipment. **Result:** The extraction of protein was different according to the analysed biomass, additionally, in all the biomass the higher protein concentration was obtained using HCl+UAE+Cellulase ($4.82 \pm 0.06\text{g}/100\text{g}$) and AA+UAE+Cellulase ($4.53 \pm 0.032\text{g}/100\text{g}$). Similarly, the antioxidant activity increased up to 14 times for ABTS and 6.5 times for FRAP in contrast to the non-hydrolysed biomass (control), the antimicrobial activity showed an inhibitory effect at higher concentration (5% w/v) using *Spirulina spp* extract, otherwise, *Chlorella spp* extract showed inhibition in all tested concentrations (5%, 2.5% and 1.75% w/v). Finally, the FPLC analysis showed that the molecular weight (MW) of recovered proteins englobes a low molecular weight profile between 1 to 5 kDa. This molecular distribution has been related with higher bioactive properties including antioxidant and antimicrobial [2]. **Conclusion:** The combination of HCl or AA + UAE showed to be effective to hydrolyse microalgae biomasses. The extract showed a higher antioxidant activity and relevant antimicrobial activity against food pathogens with the higher protein treatment, additionally, the protein profile elucidated the molecular weight of the molecules, and all the extract englobes molecules in the range of bioactive hydrolysed proteins.

Key words: Sustainable protein extraction, Novel microalgae, Bioactive properties, Low peptides

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Agricultural & Food Biotechnology

Extraction of Bioactive Compounds Present in Black Beans (*Phaseolus vulgaris* L.) Using Solid-state Fermentation.

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Introduction: In recent years, it has been reported that beans (*Phaseolus vulgaris* L.) besides being an economic food source has nutrients, which are important for human health. The objective of the present work was to evaluate the fermentation conditions in solid state from black beans (*Phaseolus vulgaris* L.). **Methodology:** Solid state fermentation was carried out using *A. niger* GH1, and black bean as substrate, where a Plackett Burman experimental matrix of 8 treatments was applied in triplicate. Finally, condensed tannins and hydrolyzable tannins were evaluated for each of the treatments. **Results:** The best treatment for condensed tannins was the 5, obtaining 20.35 mgCE/g and for hydrolyzable tannins the 6, obtaining 0.76 mgGAE/g. in the results It is obtained that the factors that directly influence the release of tannins are: particle size, and temperature. **Conclusions:** The results obtained are directly related to the different factors used in solid state fermentation, since in condensed tannins it is observed that the lower the temperature the higher the tannin concentration, likewise in hydrolyzable tannins it is observed that the smaller the particle size the higher the concentration of hydrolyzable tannins.

Keywords: *Phaseolus vulgaris* L., Fermentation, Tannins.



Agricultural & Food Biotechnology

Isolation and Identification of IAA-Producing Microorganisms from Coahuila for Sustainable Agriculture

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Introduction: The increasing concern regarding the ecological impact of synthetic agrochemicals has prompted a quest for sustainable alternatives in agriculture. In this context, beneficial microorganisms, such as *Bacillus* sp. and *Trichoderma* sp., have emerged as a solution due to their capability as plant growth promoters through the production of Indole-3-acetic acid (IAA), a key phytohormone in plant development. This study focused on isolating and evaluating IAA-producing strains of these microorganisms from soils in Coahuila, Mexico, with the intent to identify potential agricultural biostimulants. **Methodology:** Strains of *Bacillus* sp. and *Trichoderma* sp. were isolated and identified from rhizospheric soil samples collected in Valle de Cuatrociéngas and El Tunal, Coahuila, respectively. Strains with the highest IAA production potential were selected through a preliminary analysis in liquid fermentation. Subsequently, IAA production of these selected strains was quantified using Salkowsky's reagent in different liquid culture media. **Results:** Twelve strains of *Bacillus* sp. and five *Trichoderma* sp. strains were isolated. The bacterial strain B10 stood out for its high IAA production, reaching a maximum of 170 ppm. Among the *Trichoderma* sp. strains, strain T2 showed considerable IAA production, reaching 88 ppm. **Conclusion:** The B10 strain of *Bacillus* sp. isolated in this study presents high potential as a plant biostimulant due to its remarkable ability to produce IAA. These findings underscore the potential of *Bacillus* sp. and *Trichoderma* sp. strains as sustainable alternatives for agriculture and highlight the importance of exploring the microbial diversity of Coahuila as a source of such alternatives. The outstanding IAA production of strain T2 (88 ppm) also suggests its potential as a biostimulant, opening new avenues of research using beneficial fungi to improve agricultural production.

Keywords: *Trichoderma* sp., *Bacillus* sp., Indole-3-acetic acid, Coahuila, Biostimulants

Agricultural & Food Biotechnology

DNA-Free Genome Editing in Soybean using CRISPR/Cas9 Ribonucleoprotein Delivery

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Introduction: Soybean (*Glycine max* [(L.) Merrill]) is one of the most economically relevant crops in the world, serving as a major source of vegetable protein and feedstock for biodiesel production. CRISPR/Cas9 technology has been used to improve soybean productivity and quality by enabling rapid and precise genetic modifications. In many countries, plants developed by DNA-free gene editing methods, which utilize ribonucleoproteins (RNPs) or mRNAs, are not regulated as genetically modified organisms, hence reducing the cost and time required to release new cultivars to the market. However, DNA-free methods are still relatively underexplored for soybean molecular breeding. In this study, a DNA-free genome editing protocol was developed by bombarding soybean embryonic axes with RNPs carried by gold particles. **Methodology:** The expression optimization of the Cas9 nuclease from *Staphylococcus aureus* (SaCas9) was carried out in *E. coli* BL21(DE3) pLysS. sgRNAs were designed to target the phytoene desaturase gene (*GmPDS*). RNP activity was validated through *in vitro* cleavage assays prior to complexation with gold particles (60 µm) and embryonic axis bombardment of *G. max* var. Williams 82. Plants were regenerated *in vitro* and pre-selected based on a chlorotic phenotype, potentially caused by *GmPDS* knockout. Three sets of experiments, named A.1, A.2, and A.3 were conducted with variations in shot pressure, number of shots, and embryo sonication time. **Results:** The model used to optimize the heterologous expression of SaCas9 resulted in a maximum yield of 25 mg/L of expression. *In vitro* cleavage tests to validate the RNPs indicated an editing efficiency of nearly 90%, varying according to the ratios of SaCas9, sgRNA, and DNA template. In total, 365 embryos were bombarded. Sanger sequencing and alignment analyses identified seven individuals with indels in the sgRNA binding site, of which six were from A.3 experiment, resulting in an *in vivo* editing efficiency of 8%. **Conclusion:** This protocol presents a viable alternative for accelerating the generation of edited soybean cultivars with new agronomic traits.

Key words: SaCas9, Transgene-free, *Glycine max*, RNP



Agricultural & Food Biotechnology

Industrial Biotechnology

Exploration of the potential of *Rhizopus oryzae* and *Saccharomyces cerevisiae* in aroma generation from blackberry and strawberry residues using solid fermentation.

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Introduction: Apocarotenoids are a type of carotenoids with a skeleton of 40 carbon atoms, some apocarotenoids such as β -ionone, β -damascenone and β -damascone are of great interest in the food and cosmetic industry so an increasing demand has been generated in the non-chemical production of these compounds. The main objective of this research is to evaluate the ability of *Rhizopus oryzae* and *Saccharomyces cerevisiae* in the synthesis of β -ionone, β -damascenone and β -damascone by solid fermentation processes using blackberry and strawberry wastes as substrate. **Methodology:** Two concentrations of the precursor β -carotene (0.1 and 0.25%), malt or yeast extract (0.50%) and two types of fruit residues (blackberry and strawberry) were used. Quantification of the results was carried out by gas chromatography coupled to mass spectrometry (GC/MS) and fermentation kinetics was performed by measuring total sugars, reducing sugars, pH, temperature and humidity. **Results:** The highest concentrations of β -damascenone and β -ionone were found in the assays performed with *Rhizopus oryzae*, with a final concentration of 1 mg/kg and 1.68 mg/kg, respectively; however, no β -damascone production was found in any of the assays performed. On the other hand, a remarkable production of the molecule β -ionone-5,6-epoxide was observed, with a final concentration of 8.72 mg/kg in the assay performed with *Saccharomyces cerevisiae* on strawberry. **Conclusions:** This study highlights the feasibility of using fruit residues to obtain aromatic molecules and the possibility of using non-genetically modified microorganisms, and suggests future research to optimize fermentation conditions and explore new strains and substrates, supporting environmentally friendly solutions in the food industry.

Key words: solid fermentation, fruit waste, microorganisms, apocarotenoids.



Food Biotechnology

Preparation of a bio-compostable packing based on pectin extracted from orange peel waste and its application on a prebiotic functional food

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Introduction: The vast majority of current food packaging is made of petrochemical plastic, a material characterized for being resistant, lightweight, and economical yet highly polluting. Its overproduction, the growing consumption of industrialized food packaged in plastic, and a lack of environmental culture have created severe ecological and health problems. To help counteract this pollution, pectin from orange waste should be used to make a fully bio-compostable container capable of fulfilling the functions of conventional plastic packaging and testing its use on a functional food product. **Methodology:** This research was split into three stages: the first is the pectin extraction from orange peel, which involves acid hydrolysis. The second stage was making the compostable packaging using the previously extracted pectin. The ASTM-E96 method for vapor-water transmission was performed in each formulation. The third stage is making a functional food bar. This bar was made with jicama (*Pachirizus erosus*), a tuberous root that can be eaten. It has a high-water content and is rich in minerals, vitamin C, and oligosaccharides such as inulin, an important compound with prebiotic activity. **Results:** The pectin yield was about 21% of the orange peel, which is enough, considering only 1 gram of pectin is needed. The jicama bar presented an excellent consistency of texture and flavor. Pectin film presented characteristics worthy of being used as a bio-compostable packing. **Conclusion:** The use of orange peel as a potential raw material for food packaging presents a good alternative for conventional plastic replacement, considering the climate crisis and the health concerns surrounding micro and nano plastics in human health.

Keywords: orange waste, pectin, packaging, jicama, bio-compostable packing

Agricultural & Food Biotechnology

Antimicrobial properties and microbial dynamics of kombucha

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Introduction: Kombucha is a probiotic beverage derived from fermenting sugared *Camellia sinensis* infusion with a symbiotic culture of bacteria and yeast (SCOBY) and a starter, containing a consortium of acetic acid bacteria (AAB), yeasts, and lactic acid bacteria (LAB). Kombucha has surged in popularity due to its myriad benefits. Among these are its recognized antioxidant, anti-inflammatory, and antimicrobial properties, alongside its capacity to modulate blood pressure and cholesterol levels, thus earning it the status of a functional beverage. These effects are linked to both the inherent qualities of the tea and the metabolites synthesized by the microorganisms during fermentation. **Methodology:** Protein content, pH, saccharose content and titratable acidity were determined at 0, 7, 14 and 21 days of fermentation. Isolates were obtained through dilution and plating of kombucha and SCOBY samples and were later divided into three groups based on their Gram, oxidase and catalase activity, microscopic morphology, ability to solubilize calcium carbonate and ability to grow in a selective medium. The inhibitory effect of kombucha was evaluated at different fermentation days against *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* sbsp. *enterica* serovar *typhi*, *S. enterica* sbsp. *enterica* serovar *abony*, *Cronobacter sakazakii*, *Staphylococcus aureus* and *Bacillus cereus* using agar well diffusion method. **Results:** Titratable acidity, pH, saccharose content, and protein concentration exhibited a decreasing trend over time, whereas titratable acidity demonstrated an increasing trend. On day 0 of fermentation, a greater abundance of different morphotypes was observed; however, on day 7, the highest UFC/mL of microorganisms were recorded. The population of (AAB) increased over the days, while yeast decreased, and (LAB) remained stable. Antimicrobial activity also increased within the process, showing none to partial inhibition on days 0 and 7, but mean activity against most of the pathogens on day 14. **Conclusion:** The increase in antimicrobial activity of kombucha over the days coincides with the peak of microbial diversity and abundance of AAB, indicating a correlation between microbial metabolite production and the inhibitory capacity of the beverage. Kombucha is mainly consumed between 7 and 14 days of fermentation, meaning that its inhibitory effect could promote gastrointestinal health in consumers.

Key words: Kombucha, isolation, antagonism, probiotic.

Corn glucurono-arabinoxylan co-induces exo-glycosidases production by submerged fermentation of *Aspergillus niger* GH1

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Introduction: In recent years, the use of lignocellulosic biomass in different industries has been of great interest since the use of its components is very diverse. For this, different pretreatments must be applied, including enzymatic hydrolysis. For the polysaccharide components to be degraded to monomeric sugars, it is necessary to work with different glycosyl hydrolase enzymes, which can be used sequentially or together and thus act synergistically during the process. This work aimed to biosynthesize the exo-carbohydases from *Aspergillus niger* GH1. **Methodology:** The use of glucurono-arabinoxylan (GAX) from corn pericarp, as a carbon source by liquid fermentation. The medium was glucose-tryptone with the carbon source modified. Fermentation was carried out under the following conditions: 48 h/ 50 mL/ 30 °C/ 150 rpm. The enzymatic activity was evaluated by release of *p*-nitrophenol and reported as U mL⁻¹. **Results:** As a result, GAX had a significant effect on the production of α -glucuronidase, α -glucosidase, β -glucosidase, β -galactosidase and α -L-arabinofuranosidase enzymes. In the case of α -glucosidase at a higher GAX concentration, an inhibitory effect is shown on its production, lowering from 0.86 U mL⁻¹ (mean level of glucose / GAX) to 0.01 U mL⁻¹ (maximum GAX level). β -glucosidase (2.06 U mL⁻¹), α -L-arabinofuranosidase (6.04 U mL⁻¹), β -galactosidase (3.96 U mL⁻¹), and α -glucuronidase (0.36 U mL⁻¹); they showed their highest titers at the level with the highest concentration of GAX. **Conclusion:** The results suggest that the type of substrate can induce these enzymes during fermentation due to the different types of bonds between the monomers that make up the polysaccharide. These exo-carbohydases are of great importance in the biotechnological areas since their synergistic use helps in the complete depolymerization of the structural polysaccharides of the plant cell walls in various agro-industrial processes.

Keywords: Liquid fermentation, inductor, carbohydases.



Agricultural & Food Biotechnology

Study on enrichment of pasta with jackfruit seed flour and chickpea flour

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Pasta is among the most popular foods in many parts of the world. It is usually produced from a mixture of durum semolina (an easily digestible carbohydrate, low in fiber and other bioactive compounds.) egg, and water. However, the gluten in pasta is harmful to gluten-sensitive people such as those with celiac disease (CD). In recent years, the demand for functional foods such as high-fiber and low-calorie products have grown. Therefore, pasta is a product worthy of investigation to improve its nutrient content and make it gluten-free. In-order to replace wheat flour, two other alternatives are added, Jackfruit seed flour and chickpea flour in 3 combinations. Jackfruit seeds are a good source of nutrients and are gluten-free. Replacing wheat flour with jackfruit seed flour in pasta represents a potential alternative food for those with gluten sensitivities and helps in introducing a gluten-free fresh pasta (fettuccine). Addition of chickpea flour is a great source of protein, dietary fiber and has no presence of Gluten. Cooked chickpea flour added pasta contains 1.5 more protein, 3.2 times more fiber and 8 times more essential fatty acids than cooked durum wheat pasta per kcal energy content. Hand technique is used for the production of fresh pasta. Methods of analysis include, analyzing %moisture content, %crude fiber, %protein, % lipid, %ash, water absorption capacity, bulk density, swelling power and %carbohydrate and also sensory evaluation by five-point hedonic scale. Jackfruit seeds are generated as waste product in jackfruit processing industries, so it is essential to find application for these seeds. Therefore gluten-free pasta was developed using above mentioned ingredients, with all properties including shelf-life similar to that of commercially available pasta.

Keywords: *Pasta, jackfruit seed, gluten-free, chickpea, dietary fiber, protein, celiac disease.*



Agricultural & Food Biotechnology

Metabolism of prebiotic bacterial on different sugar

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Introduction: Prebiotic bacteria can produce a variety of metabolites such as organic acids, bacteriocins, amino acids, exopolysaccharides and vitamins; providing health benefits to those who consume. This work evaluated the prebiotic effect of 14 different sugars (glucose, fructose, sucrose, fructooligosaccharides (FOS) from agave, high molecular weight fructans from agave, lactose, glycerol, rhamnose, cellobiose, raffinose, arabinose, mannose, xylose, and galactose) on the growth of *Lactobacillus plantarum* ATCC® 8014, *Lactobacillus rhamnosus* ATCC® 53103, *Lactobacillus casei* ATCC® 334, *Lactobacillus acidophilus* ATCC® 4356 and *Bifidobacterium animalis*. **Methodology:** The prebiotic effect of the bacteria was evaluated by optical density at 595 nm for 24 hours in MRS broth, replacing glucose with 1% (10g/L) of the respective carbohydrate. With the data obtained, the specific growth rate (μ) in the exponential phase was calculated. **Results:** The different tested strains exhibited varying growth rates, the highest growth was obtained *B. animalis* with sucrose (D.O=1.58 \pm 0.05 and 0.5648 \pm 0.01) and cellobiose (D.O= 1.55 \pm 0.05 y μ = 0.3594 \pm 0.00) followed by *L. casei* with glucose (D.O=1.49 \pm 0.12 and μ =0.6883 \pm 0.00). **Conclusion:** The strains evaluated with the different substrates can be proposed to be used to develop functional foods.

Keywords: biotechnology, prebiotic bacterial, sugar sources, functional foods, biomass

Innovation and Tradition in Fermented Foods: Microorganisms in Latin American Artesanal Products

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Introduction: Fermentation is one of the oldest food transformation processes, originally developed as a preservation method. Currently, it is recognized that fermentation improves the nutritional value of foods, due to the metabolic changes made by microorganisms in food matrices. Although fermented foods are produced in various regions of the world, in Latin America the tradition has decreased over time, being limited mainly to the production of fermented beverages. To innovate in the production of fermented foods and enhance their consumption, it is necessary to recognize artisanal products and the microorganisms that participate in their fermentation processes. Therefore, the main objective of the research was to carry out a literature review to explore the importance of microorganisms in the production of artisanal fermented foods in Latin America. **Methodology:** For the development of the research, a systematic search was carried out in electronic databases such as Scopus and ScienceDirect, developing search equations that included the terms: "food fermentation", "Indigenous Fermented Foods", "Native Fermented Foods", among others. Documents such as research articles and review articles in English and Spanish are included. **Results:** Indigenous fermented foods are part of the cultural heritage of humanity and are recognized for their nutritional value. In Latin America, traditional fermented foods are elaborated with raw materials of greater consumption such as corn and cassava. The main microorganisms involved in fermentation processes are lactic acid bacteria, most of which are recognized as probiotic. **Conclusions:** Artisanal fermented foods in Latin America not only preserve cultural traditions, but also provide significant nutritional benefits thanks to the microorganisms involved in the process, so promoting and valuing these foods can encourage innovation in their production and increase their consumption in the region.

Key words: Food Biotechnology, Nutrition, Probiotics



Agricultural & Food Biotechnology

Ultrasound-assisted extraction of bioactive compounds from wine industry residues.

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Keywords: Grape pomace, Proximal analysis, Ultrasound.

Introduction: Mexico has an annual grape production of 351.31 million tons [1], of which 80% is destined for the wine industry. This industry generates between 50-60% of waste during processing, leading to challenges regarding the final disposal of these residues. These residues could be used to obtain bioactive compounds through various methodologies. The objective of the work was to valorize the waste from the wine industry, characterize it, and perform an ultrasound-assisted extraction. **Methodology:** The extraction was performed for 30 minutes using 60% acetone. The compounds of interest, including total flavonoids and condensed tannins, and their antioxidant capacity (quantification of the DPPH radical inhibition test and the ferric ion reduction test, FRAP) were quantified [2]. **Results:** The highest values obtained in the quantification of condensed tannins, 20.33 g/L catechin equivalents were obtained, and for the antioxidant activity of ferric ion reduction, 14.26 g/L trolox equivalents were achieved. **Conclusion:** The residue from the wine industry can be a source of valuable compounds for use in various industries.

[1] Planeación Agrícola Nacional 2022. México: SAGARPA.

[2] Bautista-Hernández, I., *et al.*, 2022. *Food and Bioproducts Processing*, 136, 24-35.



Agricultural & Food Biotechnology

Characterization of genes involved in development and response to abiotic factors in *Capsicum annuum*

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Introduction: *Capsicum* is a genus, that groups monoecious and autogamous flowering plants, belongs to the Solanaceae family, whose crop is agriculturally relevant. However, their yield is affected by diverse conditions such as drought. Gibberellic acid, and other bioactive gibberellins function as growth inductors and regulate several development processes in plants. Their application may help alleviate some adverse effects of drought stress. However, the signaling and physiological effects of gibberellins have not been extensively investigated in *C. annuum*. This work aimed to generate insights into the *ENO* and *WUSCHEL* gene involved in development, including fruit formation, and response to gibberellic acid application and drought stress in *C. annuum* plants. **Methodology:** We screened the *Capsicum* and *Arabidopsis thaliana* genome with sequences for the *ENO* and *WUSCHEL* gene and assayed gibberellic acid in *C. annuum* plants, treating plants with drought stress. The sequences were compared, expression of the two genes was estimated, and the yield was evaluated. **Results:** The identified sequences contained the AP2/ERF domain characteristic of proven *ENO* proteins. The expression of the *WUSCHEL* gene highly repressed in flower compared to bud, increasing with the application of gibberellic acid and decreasing with water stress compared to the control plants. The *ENO* gene showed a higher expression in flower than in bud, showing a response to gibberellic acid compared to control. A little difference, but significant, about the yield (length and weight of fruits) among the plants treated with drought stress and the others treatments. This work represents at first report on the *ENO* and *WUSCHEL* gene in *C. annuum* var. jalapeño. Progress in the characterization of these gene and the corresponding proteins will be presented. **Conclusion:** Our results corroborate that gibberellic acid and abiotic factors may modify the expression of genes involved in development in *C. annuum*.

Key words: *Capsicum annuum*, *ENO* gen, *WUSCHEL* gene, gibberellic acid, drought stress.



Agricultural & Food Biotechnology

Base para DIP elaborada de Malanga (*Colocasia esculenta*)

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Introducción: Una base para dip es un producto al cual se le puede agregar un ingrediente extra para dar sabor, ya sea una especia, un vegetal, una proteína, o una leguminosa. Desde hace algún tiempo se ha venido estudiando la incorporación en productos alimenticios, de materias primas no convencionales provenientes de raíces y tubérculos de origen local, que sean de importancia comercial y nutricional (Torres, 2014). La malanga (*Colocasia esculenta* (L.) Schott) es un tubérculo comestible perteneciente a la familia de las *Araceae* es buena fuente de fibra (0.6-0.8 %), contiene vitamina A, C, calcio, fósforo, potasio y magnesio. Estudios demuestran que el valor de la malanga radica en su alto contenido de almidón (30-85 % b.s) y proteínas (1.4-7%) (Rodríguez y cols, 2011). Este almidón presente en la malanga le ofrece propiedades necesarias para funcionar como un estabilizante, ya que ayuda a retener aceite y agua. **Metodología:** se propusieron formulaciones en la que se variaron la concentración de queso y aceite añadido a la base (malanga cocida y malanga cocida deshidratada); las formulaciones que cumplieron con las características similares a los productos comerciales, se analizaron microbiológicamente las bases antes de envasar, en esta primera etapa se descartaron tres de las seis formulaciones. Posteriormente se evaluaron sensorialmente las tres formulaciones restantes para determinar su grado de aceptación, a estas bases se añadieron sabores como cúrcuma, chile y garbanzo, además de se evaluó su composición química y vida de anaquel. **Resultados:** El producto resultante fue estable, dando una mezcla homogénea, bases aptas para ser consumidas, las cuales duran 60 días a temperatura ambiente, y durante este tiempo no se detectó contaminación microbiana y preservaron las características sensoriales aceptables. **Conclusión:** El dip elaborado es un producto inocuo y con propiedades nutricionales aceptables debido a la presencia de micro y macronutrientes presentes en la malanga.

Keywords: Dip, Malanga, No convencional, almidón, tubérculo.

Aplicación de técnicas de conservación a alimentos no convencionales, una alternativa para la alimentación

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Introducción. La situación de riqueza y vulnerabilidad en Chiapas es contradictoria y data de muchos años atrás, sin embargo, es posible que la alimentación de esta población sea sana y equilibrada ya que cuenta con recursos alimentarios suficientes, tales como los alimentos no convencionales (flores, frutos, semillas, tallos, hojas, rizomas, tubérculos o brotes tiernos), de los cuales existen aproximadamente 200 especies; estas son conocidas y se pueden adquirir en los mercados locales. El propósito de la presente investigación es dar a conocer las propiedades nutricionales y usos en la alimentación del frijol patashete (*Phaseolus Lunatus L*), malanga (*Colocasia esculenta*), pacaya (*Chamaedorea Tepejilote*), chapaya (*Astrocaryum Mexicanum Liebm*) y hongo seta (*Pleorotus ostreatus*), los cuales podrían ser retomados como parte del patrimonio alimentario local. **Metodología.** Se determinó la composición química de las materias primas, se elaboraron productos a través de propuestas metodológicas estandarizadas para asegurar calidad e inocuidad y se evaluaron sensorialmente para determinar su grado de aceptación, los resultados se analizaron estadísticamente. **Resultados:** *Hongo seta:* Presenta en estado fresco 93% de humedad lo que lo hace susceptible a descomposición temprana, alta concentración de proteína, fibra y minerales (potasio, cinc, calcio y hierro). El producto a base de este, fue aceptado y su vida útil puede extenderse hasta un año. *Frijol patashete:* La tortilla a base de este; resalta la proteína y fibra componentes en concentración superior (más del doble) al que se encuentra en una tortilla de maíz; características físicas como enrollamiento, color, olor y textura fueron aceptables, fue evaluado con alto nivel de agrado y mantiene una vida útil comparable a una tortilla tradicional. *Malanga:* El pan es sencillo y económico de elaborar, está dirigido a productores locales, presenta un alto contenido de humedad debido a la presencia de almidón y al bajo contenido de proteína, esto influyo en la textura, su vida útil es inferior a un pan de caja tradicional. Sensorialmente (productoras, vendedoras y consumidoras) mostraron un alto nivel de agrado. **Conclusión.** Es posible usar técnicas sencillas de conservación para poder disponer de ellos todo el año. La gran mayoría presenta un alto contenido de macro y micro nutrimentos, cualidad importante cuando se necesita solucionar problemas de mal nutrición en el país.

Keywords: Pacaya, malanga, frijol patashete, *Pleorotus ostreatus*



Agricultural & Food Biotechnology

Production of invertase with *Neurospora crassa* FGS#9717 by solid state culture

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Introduction: Solid state culture involves the growth of microorganisms in the absence of free water between the particles. The main difference between solid and submerged culture is the amount of water present in the system. Bacteria and fungi can grow on solid supports, with filamentous fungi being the best adapted to solid culture. *Neurospora crassa* is a fast-growing filamentous fungus, the mycelium can grow at a speed of 4 mm/h at temperatures of 30 to 32 °C with the addition of a carbon source, mineral salts, and biotin. The production of hydrolytic enzymes such as amylases, invertases, cellulases, proteases and lipases by *Neurospora crassa* has been reported. Invertase (β -D-fructofuranosidase) hydrolyzes sucrose producing a mixture of glucose and fructose, this mixture is used in the production of chocolate, bonbons, syrup, synthetic honey, jams, and jellies. **Methodology:** A strain of *Neurospora crassa* FGS#9717 auxotrophic to histidine was used. The culture in solid medium was carried out with pine sawdust as a support impregnated with Vogel medium added with 1.5% sucrose. The humidity of the culture in solid state was set at 60%. Samples were taken every 24 hours for analysis. Invertase activity was quantified by the Miller (1960) method. **Results:** Invertase synthesis kinetics were carried out from 0 to 96 hours. It was observed that after 96 hours of culture, the highest activity was obtained, 20.74 U/gmsi for invertase. **Conclusion:** The use of an organic waste for the solid-state culture of *Neurospora crassa* FGS#9717 allowed the growth of the microorganism and the production of enzymes of commercial importance.

Keywords: *Neurospora crassa*, solid state culture, inducer, enzymes



Agricultural & Food Biotechnology

Production of invertase with *Neurospora crassa* FGS#9717 by solid state culture without inducers.

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Introduction: *Neurospora crassa* is a filamentous fungus with a heterothallic life cycle that has been widely used in genetic and biochemical research over the last century. Filamentous fungi such as *Neurospora crassa* are microorganisms with characteristics that make them efficient for their application in solid state culture for the production, for example, of hydrolytic enzymes such as amylases, invertases, cellulases, proteases and lipases. Cultivation in solid medium has been used for centuries, initially for food production. In the last three decades, interest in solid culture processes has been focused on the synthesis of secondary metabolites, among other products, particularly invertase and proteases, enzymes widely used in the food industry. **Methodology:** A strain of *Neurospora crassa* FGS#9717 auxotrophic to histidine was used, the solid-state culture was carried out with pine sawdust as a support impregnated with Vogel medium with enough to reach 60% humidity. Invertase activity was quantified by the method of Miller (1960) **Results:** A kinetics of invertase synthesis was carried out to establish the time of maximum enzyme production. It was observed that at the maximum time analyzed in this study (96 hours) the activity of continues to increase, with 32.74 U/gmsi for invertase activity. **Conclusion:** An important characteristic of cultivation in solid medium is that agroindustrial waste can be used to produce metabolites. In this study, sawdust, a waste from the wood industry, was used for the growth of *Neurospora crassa* FGS#9717 and the production of invertase, enzyme of great industrial importance.

Keywords: *Neurospora crassa*, solid state culture, sawdust, enzymes



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Characterization and Evaluation of Bacteria from Volcanic Soil as Potential Plant Growth Promoters and Biocontrol Agents.

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Key words: PGPB, El Chichonal, Extremophiles, Biocontrol.

Introduction: Bacteria known for their role in promoting plant growth and naturally found adjacent to plant roots are referred to as Plant Growth Promoting Bacteria (PGPB). These bacteria operate through various mechanisms, both direct and indirect. Direct mechanisms primarily involve optimizing nutrient acquisition through the production of indole acetic acid, phosphate solubilization, and nitrogen fixation, while indirect mechanisms involve the use of bacteria for biological control of commercially important phytopathogens. The bacterial isolates were collected from the “El Chichonal” volcano located in Chiapas, Mexico, which is currently considered active with its last recorded eruption in 1982. The selection of samples was based on their resistance to high temperatures, followed by morphological analysis. **Methodology:** The capabilities of these bacteria were subsequently evaluated in relation to the different mechanisms of action contributing to plant growth. Indole acetic acid quantification was measured using a previously prepared standard calibration curve, utilizing Indole-3-acetic acid (IAA) and Salkowski's reagent for the detection of the phytohormone in the bacterial culture. Nitrogen fixation was detected through streaking and bacterial growth in Ashby medium, and phosphate solubilization capacity was evaluated through bacterial growth on Petri dishes with NBRIP medium (National Botanical Research Institute's Phosphate Growth Medium). Additionally, the potential of these bacteria as biocontrol agents against various phytopathogens was assessed through direct confrontation.

Results: The results demonstrated a possible advantage of bacteria obtained from extremophilic environments in competing under adverse conditions, showing a high capacity for phytopathogen inhibition and nutrient production. **Conclusion:** The findings present a new perspective on the use of potential PGPB in the agro-industry, as well as the application of these microorganisms to counteract pests of economic importance.



Agricultural & Food Biotechnology

Isolation of microorganisms with probiotic potential and evaluation of the antimicrobial activity of Mother of Vinegar against pathogens causing ATE.

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Introduction. Mother of vinegar, a vinegar static production method, facilitates the formation of a bacterial cellulose biofilm, providing an optimal microenvironment for yeast and lactic acid bacteria fermentation, and ethanol oxidation by acetic acid bacteria. This matrix serves as a microbial consortium and a starter culture for artisanal vinegar production in Mexico. This study aims to assess pH, titratable acidity, antimicrobial activity against foodborne pathogens, and isolate potential probiotic microorganisms in mother of vinegar and artisanal vinegar. **Methodology.** pH, titratable acidity, and isolation were conducted on fermentation days 0 and 7. Isolation used specific culture media: Man, Rogosa, and Sharpe agar (MRS), yeast extract glucose agar with calcium carbonate (GYC), mannitol egg yolk agar (MY), and acidified potato dextrose agar (PDA). Potential probiotic isolates were pools and tested for antimicrobial activity against *Salmonella* Typhi, *Salmonella* Abony, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, y *Cronobacter sakazakii*, using the modified Kirby Bauer method. Antimicrobial activity of mother of vinegar and artisanal vinegar was evaluated at 0, 3, and 7 fermentation days. **Results.** At day 0, vinegar pH was 3 with a titratable acidity of 4 meq; at day 7, pH was 4, acidity remained constant. 100 isolates were obtained (65 cellulose matrix, 35 vinegar) with 60% Gram negative bacteria, 20% Gram positive bacteria, 10% yeasts, and 10% yeast-Gram negative bacteria consortium. 27.7% (5/18) of the pools showed antimicrobial activity against all pathogens. Mother of vinegar and artisanal vinegar exhibited inhibition against all pathogens at 0 and 3 days except for *B.cereus* on day 7. **Conclusion.** During mother of vinegar fermentation at different periods of time a variation of pH was observed, while the titratable acidity remained constant. The microbial population with the highest predominance in the sample was Gram negative bacteria. The pool isolates showed antimicrobial activity against all the pathogens used in this study, as well as the mother of Vinegar and the artisanal vinegar.

Key words: Mother of Vinegar, antimicrobial activity, probiotic potential, pathogens



Agricultural & Food Biotechnology

Herbicide potential of compounds produced by *P. putida* in a liquid culture system.

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Introduction: The excessive use of synthetic herbicides has generated adverse effects such as damage to human health, soil residues and herbicide-resistant weeds. Because of this, it is important to look for new environmentally friendly products. Microbial herbicides are an alternative as they tend to be less toxic. In this sense, bacteria such as *Pseudomonas* play an important role, as they can produce different metabolites with agricultural application. The objective of this project was to evaluate the herbicide capacity of compounds produced by *P. putida* in a liquid culture system. Methodology: Liquid cultures were carried out in flasks with Luria Bertani medium and samples were taken every hour for 32 h. Auxin content and bacterial growth were quantified in each sample. Subsequently, pre-emergence and post-emergence herbicide evaluation was performed with *H. annuus* and *A. sativa* as model plants. The pre-emergent evaluation was carried out in germination trays with 12 cavities containing 75% peatmoss and 25% perlite as substrate, the seeds were placed and then the auxin treatment was applied. For the post-emergent evaluation, pots with substrate in the same proportions mentioned were used, the plants were sown and after 10 days, the auxin treatment was applied. **Results:** The compounds produced by *P. putida* reduced the germination of *A. sativa* and *H. annuus* seeds by 20 and 30 % respectively. In addition, they affected the development of the seedlings since smaller sizes were observed in the hypocotyls and roots. **Conclusions:** The bacterium *P. putida* can produce auxins such as indole-3-acetic acid that can affect plant development and germination.

Key words: Bioherbicide, *Pseudomonas*, auxins, weeds



Agricultural & Food Biotechnology

Influence of ultrasound on the physicochemical properties of coacervates complexes isolated pea protein-citrus pectin

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Introduction: Protein preconditioning methods play an important role in improving their functional properties and their use for the formulation of colloidal systems such as coacervate complexes. This allows the generation of structures that are stable to various environmental factors, taking advantage of the chemical nature and surface characteristics of individual elements to direct and control interactions between biomolecules of opposite charge. **Methodology:** Solutions of pea protein isolate (PPI, 1 wt%) preconditioned with ultrasound (80% amplitude), were prepared at different exposure times (15, 20, 25 min), in addition to a solution of citrus pectin (CP, 1 wt%). The zeta potential was determined as a function of pH (2.0-5.0) to identify the maximum interaction between PPI and CP. PPI-CP coacervates (CC) were formed at different ratios (3.0:1.0 to 5.0:1.0) with the previously identified optimal pH. CC were centrifuged, decanted and dehydrated (55°C) to calculate the coacervation yield. FTIR spectra of PPI and CC were obtained to identify changes in the protein structure and interactions formed during the coacervation process, respectively. In addition, the viscoelastic properties (G' and G'') of CC were determined, at 1% deformation and frequency sweep of 0.01-100 Hz. **Results:** The maximum stoichiometric difference between the PPI and CP dispersions was located at pH 3.0, this value was used as the optimal pH for CC formation. Coacervation yields were obtained in a range of 35.87-68.32%. When performing a deconvolution process on the FTIR spectra in the Amide I region of the treatments, changes were observed in the secondary structure of PPI caused by the ultrasound process, which also caused a greater interaction for the formation of CC. For their part, the CC showed a predominantly solid viscoelastic behavior ($G' > G''$). The above is attributed to the increase in the proportions between the biopolymers used, as well as a longer exposure time to the PPI ultrasonication process. **Conclusion:** Ultrasound treatment influenced the interactions between PPI and CP, promoting coacervation. The viscoelastic properties of PPI-CP were affected by the pH, stoichiometry and concentration of the biopolymers used. These CC can be used as a possible delivery system for bioactives with application in foods.

Keywords: Coacervates complexes, Coacervation yield, Ultrasound, Viscoelastic behavior.



Agricultural & Food Biotechnology

Comparative Study of the Antioxidant Activity of Melanins Obtained from Edible Mushrooms.

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Introduction: Melanin is a polymer synthesized by a wide variety of organisms. It can be used as a pigment in beverages and foods and has biological properties that may be beneficial to human health, including antioxidant activity. This study compares the antioxidant capacity of melanin extracted from the fruiting bodies of huitlacoche (MH), champignon (MC), and *Pleurotus ostreatus* (MP). **Methodology:** Melanin was extracted from the fresh fruiting bodies of the mushrooms using an 80°C NaOH solution for 3 hours.

The solution was then cooled, and the pH was lowered to less than 2 with HCl. The mixture was allowed to precipitate for one day and then washed with water, chloroform, methanol, and ethyl acetate to partially purify the melanin. The dried melanin was made into a 5% (w/v) solution in DMSO to measure antioxidant activity using the DPPH, ABTS, CUPRAC, FRAP, and iron chelation with ferrozine as an indicator methods. **Results:** With the DPPH radical, the percentage of inhibition for MH was 54.78%±0.2%, for MC 82.6%±0.9%, and for MP 70.52±0.5%. The percentage of inhibition of the ABTS radical for MH was 11.82%±0.07%, for MC 7.77%±0.03%, and MP 5.71%±0.0012%. Iron chelation with MH was 88.46%±0.9%, for MC 64.1%±0.7%, and for MP 36.54%±0.09%. In the case of CUPRAC, the TROLOX concentration for MH was 5.5±0.002µg/mL, for MC 24.58±0.07µg/mL, and for MP 47.55±0.09µg/mL. In the case of FRAP, the TROLOX concentration for MH was 9.97±0.01µg/mL, for MC 18.43±0.07µg/mL, and for MP 23.18±0.09µg/mL. **Conclusion:** All melanin samples showed antioxidant activity in all methodologies, indicating that they could be used as ingredients in food and provide antioxidant activity to the product, turning a simple food into a functional food.

Keywords: Melanin, antioxidant capacity, CUPRAC, ABTS, DPPH.



Agricultural & Food Biotechnology

Comparative Study of Melanins Obtained from Banana Peel and Pulp.

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Introduction: Melanin is a natural pigment used to color beverages and foods. In fruits, it is produced by enzymatic browning, which is generally undesirable. However, studies have shown that melanin has antioxidant and chelating activities that may be beneficial to human health. This study analyzed the melanin produced by ripe bananas (45 minutes after cutting) in both the peel (MC) and pulp (MP) to determine which has higher antioxidant and chelating activity. **Methodology:** Melanin was extracted from the peel and pulp separately using an 85°C NaOH solution for 3 hours. The solution was then cooled, and the pH was lowered to 2 with an HCl solution. The mixture was allowed to precipitate for one day and then washed with water, chloroform, methanol, and ethyl acetate to partially purify the melanin. The dried and pulverized melanin was made into a 5% (w/v) solution in DMSO. Total polyphenols were quantified using the Folin-Ciocalteu method, and antioxidant activity was measured using the DPPH, ABTS, CUPRAC, FRAP, and iron chelation with ferrozine methods. **Results:** The total polyphenol concentration of MC was 17.9 ± 0.04 meq gallic acid/g of peel, and for MP, it was 11.8 ± 0.07 meq gallic acid/g of banana pulp. With the DPPH radical, the percentage of inhibition for MC was $6.31\% \pm 0.002\%$ and for MP $7.2 \pm 0.001\%$. The percentage of inhibition of the ABTS radical for MC was $59.9\% \pm 0.41\%$ and MP $60.1\% \pm 0.2\%$. Iron chelation with ferrozine as an indicator for MC was $20.7\% \pm 0.01\%$ and for MP $19.81\% \pm 0.03\%$. In the case of CUPRAC, the TROLOX concentration for MC was 36.3 ± 0.12 $\mu\text{g/mL}$ and for MP 35.4 ± 0.09 $\mu\text{g/mL}$. In the case of FRAP, the TROLOX concentration for MC was 21.3 ± 0.05 $\mu\text{g/mL}$ and for MP 19.7 ± 0.09 $\mu\text{g/mL}$. **Conclusion:** Melanin obtained from banana peel has higher polyphenol content and antioxidant activity compared to melanin obtained from banana pulp.

Keywords: Melanin, antioxidant capacity, Banana, ABTS, DPPH.



Agricultural & Food Biotechnology

Role of TiO₂ nanoparticles on the biocontrol activity of *Trichoderma* species

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Introduction: An alternative to the chemical management of pathogens in agriculture is biological control, highlighting *Trichoderma* fungi. Moreover, it has been shown that some metallic and metal oxide nanoparticles (NPs) can also be used to inhibit the growth of phytopathogens. In addition, the combined use of NPs and beneficial plant organisms with application in agriculture has begun to be studied. The objective of this work is to determine if TiO₂ NPs influence *Trichoderma* species, and improve their biocontrol activity on apple pathogenic fungi, as well as to evaluate the expression of genes associated with this activity. **Methodology:** TiO₂ NPs were synthesized by the oil-in-water microemulsion method and characterized. Subsequently, *Trichoderma* tolerance assays to the NPs were performed at different concentrations of the NPs. Once the highest tolerance concentration was obtained, confrontations were carried out between *Trichoderma* and three apple pathogenic fungi. Expression assays of genes associated with *Trichoderma* biocontrol were performed. **Results:** TiO₂ NPs have been obtained, in addition to which characterization has been carried out. Tests were performed with the NPs at 0, 25, 50, 50, 100, 200 and 500 ppm to determine the tolerance of *Trichoderma*. The concentration at which *Trichoderma* continues to grow without showing apparent stress was selected and confrontations with apple pathogenic fungi were carried out. In addition to obtaining differential expression in genes associated with biocontrol, including chitinases, hydrophobins and LysM genes. **Conclusions:** TiO₂ NPs influence the biocontrol activity of *Trichoderma* species and their genes associated with this activity.

Key words: *Trichoderma*, Biocontrol, nanoparticles of TiO₂, apple phytopathogens

Agricultural and food biotechnology

Solid-state fermentation with *Rhizopus oryzae* and the evaluation of its effect on functional characteristics of pigmented corn

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Introduction: Corn is one of the most widely cultivated/harvested cereals in the world, and contains a large number of antioxidant compounds, such as phenolic compounds. However, some of them are bound to components of the cell wall, and pretreatments are required to release them. Solid-state fermentation (SSF) with *Rhizopus oryzae* has been used to increase the antioxidant capacity of grains and legumes. However, very little evidence has been found for its use in pigmented corn. The objective of the present study was to evaluate the growth of *R. oryzae* during SSF on pigmented corn as a support and its effect on the release of phenolic compounds with antioxidant capacity. **Methodology:** The grain was submitted to soaking to adjust moisture to 35%. Then, 300 g of corn was mixed with 5 mL of salt medium (1 g/L KH₂PO₄, 1 g/L MgSO₄·7H₂O, 2 g/L (NH₄)₂SO₄) previously inoculated with 1x10⁶ spores/gdm of *R. oryzae*. This mixture (12 g wet mass) was packed in tray reactors (54 cm³) and incubated at 30 °C for 72 h, sampling every 12 h to determine the best time for phenolic compounds (condensed and hydrolyzed) release and antioxidant capacity (ABTS, DPPH and FRAP). The analysis of free and bound phenols by high-performance liquid chromatography-mass spectrometry (HPLC-MS) was performed to identify phenolic compounds present in fermented corn grain. **Results:** The results obtained showed that *R. oryzae* was able to use in pigmented corn grain for growth to a support, and release phenolic compounds, reaching the highest value at 60 h of culture (6.27 mg/gdm). The use of SSF increased the total phenolic content and antioxidant capacity of corn up to 161 and 94 %, respectively, with respect to the unfermented control. The phenolic compounds identified were Caffeic acid 4-O-glucoside, Caffeoyl tartaric acid, 2-S-Glutathionyl caftaric acid, 5-Heptadecylresorcinol, p-Coumaroyl tartaric acid glucosidic ester and Rosmanol, are reported for their anti-inflammatory properties and are used in the prevention of various diseases. **Conclusion:** SSF represents a sustainable alternative to increase the content of phenolic compounds in pigmented corn grain.

Key words: *Zea mays*, antioxidant capacity, phenolic content, HPLC-MS.



Agricultural & Food Biotechnology

Ultrasound-Assisted Extraction and Flash Fractionation of Polyphenolic Compounds from *Punica granatum*

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Introduction: Ellagitannins are bioactive compounds classified as hydrolyzable tannins. They are characterized by their low molecular weight and the various structural linkages they can form. Ellagitannins are present in a wide variety of plants, fruits, and some everyday consumer products. They are not essential for metabolism, meaning they do not provide nutritional benefits. However, the ingestion of ellagitannins is of utmost importance due to their antioxidant, antifungal, antibacterial, antitumor, and anti-inflammatory properties. The objective of this study was to extract, purify, and characterize ellagitannins from pomegranate peel. **Methodology:** The raw material (pomegranate peel) was obtained. Subsequently an experimental matrix was employed using the Taguchi L9 (3³) design for the ultrasound-assisted extraction of phenolic compounds. The optimal treatment was selected based on the total polyphenol content as measured by the Folin-Ciocalteu method. Once the optimal treatment was determined, fractionation was performed using a Pure C-850 Flash liquid chromatograph, followed by characterization of the fractions obtained via HPLC/ESI/MS. **Results:** The optimal ellagitannin extraction treatment was as follows: 20 min extraction time, 40 % ethanol (v/v), solid-to-liquid ratio of 1:12, yielding a total polyphenol content of $8,801.89 \pm 621.41$ GAmEq/L, surpassing the signal-to-noise ratio expected by statistical analysis (77.43) with a value of 88.43 ± 0.66 . Four fractions were obtained containing ellagitannins, primarily including punicalin α , pedunculagin I, punicalin α , punicalin β , granatin B, and ellagic acid. **Conclusion:** An optimal ultrasound-assisted extraction treatment for ellagitannins was successfully developed. Additionally, the extract was fractionated, and the ellagitannins present in each fraction were characterized.

Key words: *Punica granatum L.*, Ultrasound-assisted extraction, Ellagitanins.



Agricultural & Food Biotechnology

Utilization of high-impact agro-industrial residues nationwide for cultivating lion's mane mushroom (*Hericium erinaceus*) with nutraceutical properties.

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Introduction: Agave in Mexico generates large amounts of waste, reporting around 1,776,720.56 tons in 2021, with half of the plant being agro-industrial waste. This waste can be used as a substrate to cultivate the edible mushroom *Hericium erinaceus*, reducing pollution and generating income for farmers. This mushroom, known for its antioxidant and nutraceutical properties, promotes sustainable production and scientific research on its health benefits. **Methodology:** In the ejido Las Mangas, Coahuila, two species of agave (*A. salmiana* and *A. duranguensis*) were collected. The corncob was obtained from the city's central supply market. The agave samples were divided into treated and untreated groups. A proximal analysis of the five samples (*A. salmiana*, treated *A. salmiana*, *A. duranguensis*, treated *A. duranguensis*, and corncob) was conducted, evaluating moisture, ash, protein, fiber, fats, and carbohydrates using AOAC methods. **Results:** It was demonstrated that the treatment removed secondary metabolites in the agaves, as the sugar concentration decreased by 9% compared to the untreated samples. The protein content was higher in the corncob (17.93%), while the ash content was higher in the agaves due to their nature. **Conclusion:** *Agave salmiana*, *duranguensis* residues, and corncob are suitable for producing edible mushrooms. It is suggested to combine agave and corncob for greater biological efficiency and to reduce colonization time by supplementing the nitrogen and carbon requirements.

Keywords: cultivation, lion's mane, agro-industrial residues, chemical composition.



Agricultural & Food Biotechnology

Application of pigment extracted from *Hibiscus sabdariffa* (hibiscus flower) in chocolate cookies.

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Introduction: The natural pigments used for foods have taken great importance in recent years, mainly due to its promoting effects of health and the fewer allergies they cause in consumers (García 2021). The hibiscus flower comes from the plant *Hibiscus sabdariffa* and the coloration of its petals vary from yellowish green to intense red. They contain a large number of anthocyanins which give them their reddish colour. Anthocyanins (glycosylated anthocyanidins) are flavonoid compounds found naturally in fruits and flowers, these compounds participate in vital biological functions with their antioxidant activity, their most important property. This project was carried out to extract pigments from hibiscus flowers that were previously used to infuse a flavoured beverage and thus give a second use to this waste. In this way, a raw material (pigment) that can be applied in food is obtained. In this study it was applied in the production of chocolate cookies, resulting in an improvement in the colour of the cookie according to the sensory evaluation tests.

Methodology: The anthocyanins are water-soluble, so in this work we obtained the pigments from Soxhlet extraction using 96% ethanol as diluent and citric acid was added as a colour stabilizer. The alcohol was evaporated in a rotavapor equipment, obtaining the solid pigment, which was used as an ingredient to produce chocolate cookies. A control was made using all the ingredients except the pigment in the preparation of the cookies and another control using the allura red pigment.

Results: 4.3% of the dry pigment was obtained with respect to the weight of the hibiscus flower used for extraction. In the sensory analysis carried out, the acceptance of the product made with hibiscus extract compared to a control product made without the extract was a 60% preference.

Conclusion: It is possible to give a second use to hibiscus flower waste by obtaining pigments considered safe to use in food preparation, improving their appearance and acceptance by consumers

Keywords: pigments, safe, waste, anthocyanins

Ultrasound extraction of bioactive compounds from mandarin peel

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Introduction: Although widely consumed, the processing of citrus fruits generates large amounts of waste, such as peels and seeds. Citrus peels are rich in bioactive compounds with beneficial properties, which can be extracted using different methods. The main objective was to evaluate ultrasound assisted extraction UAE for the recovery of phenolic compounds from mandarin peel, assessing the antioxidant capacity of the extracts. **Methodology:** The citrus fruit was obtained from a local producer in Huasteca Potosina, Ciudad Valles, SLP. The peel was removed, cut into small pieces, and dehydrated at 50°C for 2 days in a drying oven. Subsequently, it was pulverized and sieved for storage in hermetically sealed bags. UAE was performed using a BRANSON model 2800 ultrasonic bath operating at 40 kHz. Extractions were conducted using 1 g of material with a mass/solvent ratio of 1:10 (m/v) with 70% ethanol. Extraction times of 5 to 50 minutes were used. The extracts were filtered and stored for phenolic content and antioxidant capacity analysis. The phenolic content was measured using the Folin-Ciocalteu method, and antioxidant capacity was determined by DPPH and ABTS radical inhibition methods. **Results:** The Folin-Ciocalteu method showed that the concentration of polyphenols varied significantly with extraction time. In 40 min a yield of polyphenols of 315.35 mg PT eq. gallic acid/L was achieved, a similar yield was obtained at 15 and 30 min of extraction, with values of polyphenols from 296.96 and 272.81 mg PT eq. gallic acid/L respectively. Similar results were obtained in the antioxidant capacity of the extracts, with the DPPH radical inhibition method, where the times of 40, 30 and 15 minutes showed the highest inhibition with a range of 62 to 68% without significant differences between them. However, ABTS radical inhibition did not show significant differences in any of the samples according to the ordinary one-way ANOVA and Tukey's multiple comparisons test. **Conclusion:** Although no significant differences were observed in ABTS inhibition, the consistency in the DPPH results and polyphenol concentration suggests that 40 minutes is the optimal time to maximize the extraction of antioxidant compounds under the specific conditions of the experiment.

Keywords: Polyphenols, Citrus peel, Ultrasound assisted extraction (UAE).



Agricultural & Food Biotechnology

Identificación y cuantificación de compuestos orgánicos volátiles (COVs) producidos por *Trichoderma harzianum* A15

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Introduction: *Trichoderma* is an Ascomycete fungus (Hypocreales) and one of the most widely used soil-dwelling microorganisms in agriculture, which are beneficial to producers worldwide. These fungi are characterized by having several species capable of benefiting plants through their mechanisms of action, which are competition for space and food, antibiosis and mycoparasitism. Antibiosis is one of the biological control mechanisms that *Trichoderma* exerts on other phytopathogenic fungi through the production of secondary metabolites such as volatile organic compounds (VOCs). **Methodology:** The analysis was performed by headspace (HS) technique using solid phase microextraction on gas chromatography coupled to mass (SPME-GC-MS). The capillary composition was divinylbenzene/carboxene/polydimethylsiloxane. VOCs produced by *T. harzianum* A15 on day 1 were analyzed. **Results:** 4 compounds were identified. Most of the VOCs identified belonged to the groups of alcohols, sesquiterpenes and phenolic acid compounds. The most abundant compounds were gentisic acid, 1-hexadecanol, trichoacorenol and phenylethyl alcohol which have been reported as antifungal, antibacterial. The VOC profile produced by *T. harzianum* A15 contains components of importance for agriculture.

Keywords: *Trichoderma*, VOCs, Biological control, Agriculture, Interaction, Food



Agricultural & Food Biotechnology

Isolation and identification of microorganisms present in agricultural soils of the Valles de Jalisco region, Mexico

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Introduction: The analysis of microbial biodiversity in agricultural soils is essential for understanding terrestrial ecosystems and their influence on agriculture. Microbial consortia, or plant growth-promoting microorganisms, play a crucial role in the decomposition of organic matter and nutrient cycling, with potential applications in biotechnology. The Valles de Jalisco region stands out for its agricultural diversity and economic importance, making it an ideal area to study microorganisms and their contribution to soil biogeochemical cycles. Identifying and characterizing these microbial consortia in this region can have significant implications for promoting sustainable agriculture and industrial development. The objective of this study is to isolate and molecularly identify species of microbial consortia present in agricultural soils in the Valles de Jalisco region, Mexico.

Methodology: Soil Sample Collection: Representative soil samples will be collected from various agricultural areas in the Valles de Jalisco region. Isolation of Microbial Consortia: Plant growth-promoting microorganisms will be isolated from the soil samples using appropriate culture and selection techniques. Evaluation of Metabolic Activity: The metabolic activity of the isolated microorganisms will be evaluated to understand their function in the soil. DNA Extraction and ITS rDNA Region Amplification: DNA will be extracted from the microorganisms and the ITS region of the rDNA will be amplified for molecular analysis. PCR-RFLP Technique: The PCR-RFLP technique will be applied to identify the species of isolated microorganisms. Chemical Characterization of Strains: Chemical analyses will be carried out to characterize the strains of microorganisms and their potential use in biotechnological applications. Evaluation of Potential for Biofertilizers: The potential of the isolated strains of microorganisms for use as biofertilizers in agriculture will be evaluated.

Expected Results: A detailed characterization of the species of microbial consortia or plant growth-promoting microorganisms present in the agricultural soils of the Valles de Jalisco region is expected. Additionally, their biotechnological potential and possible application in sustainable agriculture will be identified. This study will contribute to the understanding of microbial biodiversity in agricultural soils and its relevance in the agricultural ecosystems of this specific region.

Keywords: Microorganisms, agricultural soils, Jalisco, Valles region.

Agricultural & Food Biotechnology

Fortification of blue maize tortilla with ayocote and quelite: Exploring underutilized vegetables potential for enhanced nutritional value and health benefits.

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Introduction: Many underutilized vegetables hold great potential in enhancing nutrition and health, as well as diversifying diet and promoting agricultural sustainability. Blue maize, ayocote (*Phaseolus coccineus*), and quintonil (*Amaranthus hybridus*) are underutilised plant species native to Mexico that are rich sources of macro- and micronutrients, antioxidants, and fibre. Maize tortillas are a staple food in Mexico and other American countries, thus blue maize tortillas were used as food matrix to incorporate ayocote and quelite flours. This combination aims to enhance the nutritional and nutraceutical properties of tortilla, encouraging the consumption and recognition of native plant species as viable alternatives for improving the overall well-being of the population.

Methodology: Two blue maize flour-based formulations were developed to assess the effect of incorporating ayocote and quintonil at different concentrations (6% and 9%). With respect to tortillas functionality, the content of total phenolic compounds (TPC) was quantified, and the antioxidant capacity was evaluated by means of DPPH and ABTS assays. **Results:** The addition of ayocote and quelite flour significantly increased the nutrient composition of blue maize tortillas. Formulations enriched with 9% exhibited the best results, increasing protein and ash content by 31% and 49%, respectively, and decreasing fat content by a 21% with respect to control (100% blue maize). Moreover, TPC and scavenging activity against DPPH and ABTS radicals were not affected by the partial substitution of blue maize with ayocote and quelite flours. **Conclusion:** The incorporation of ayocote and quelite flours successfully improved the nutritional value of blue maize tortillas while preserving their functional characteristics. These results support the revalorization of blue maize, ayocote, and quelite, not only in their use for the development of foods with enhanced nutritional profiles and health benefits, but also in preserving traditional foods of great culinary relevance for Mexico.

Keywords: Blue corn, bioactivity, functional food, underutilised plant species



Agricultural & Food Biotechnology

The effects of bioactive compounds extracted from *Cucumis melo* var. *cantalupensis* seeds and pulp on metabolic disorders.

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Introduction: The *cantalupensis* melon contains several compounds, such as vitamin C, zinc, β -carotene, and polyphenols, that contribute to its high nutritional value, antioxidant properties, and biological activity. Microwave-assisted extraction is an alternative method that offers advantages such as reduced extraction time, precise control of conditions, and less solvent usage. Millions of people die every year from metabolic illnesses, which are on the rise globally; but it is important to find alternatives that help the complication pathologies. The aim is to identify the potential health advantages of exploiting these extracts of value-added substances. **Methodology:** With a microwave (Mars 6) using a central composite design incorporating three variables, temperature, retention duration, and percentage of solvent. The total phenolic content was assessed using the Folin Ciocalteu method, while antioxidant activity was determined through measurements of 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) inhibition capacity, Ferric Ion Reducing Antioxidant Power (FRAP), and inhibition of linoleic acid oxidation (LPO). Total sugar was quantified by the Anthrone Method, reduced sugar with Dinitro Salicylic Acid (DNS), and the antidiabetic inhibition enzyme using α -glucosidase. The cytotoxicity was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol bromide (MTT) and with trypan blue. The STATISTICA 7® program performed a response surface design and analysis of variance. **Results:** Total Polyphenol content in seed extracts was higher than pulp, with Seed Extract at 4.53 ± 0.59 mg Gallic Acid Equivalent (GAE/g) and Pulp Extract at 3.75 ± 0.59 mg GAE/g. Both extracts showed antioxidant activity and sugar content using different methods. Some extracts have more capacity to inhibit α -glucosidase. **Conclusion:** *Cantalupensis* biological features have not been extensively studied. Considering the prevalence of other well-studied varieties such as *agrestis*, it is imperative to research this fruit to identify and analyze bioactive compounds to enhance human health. Simultaneously, this extraction method enables the rapid and controlled production of substantial amounts of bioactive compounds, surpassing the capabilities of conventional extraction methods. This highlights the importance of exploring alternative technologies to investigate the potential benefits of functional foods and their applications in human health and nutrition.

Keywords: Bioactive compounds, Antioxidant Capacity, Antidiabetic activity, Hepatocarcinoma.



Agricultural and Food Biotechnology

Revalorization of byproduct of nixtamalized maize flour industry: Optimization of laccase bleaching conditions by Taguchi DOE Methodology

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Maize is the second most cultivated cereal worldwide and a key ingredient in products like tortillas. During nixtamalization, a process where maize is cooked with lime, nixtamalized maize pericarp (NMP) is produced as a byproduct, rich in phenolic compounds. However, NMP's yellowish color makes it difficult to integrate into white maize flours. Various bleaching methods have been investigated, but many have adverse health effects. Enzymes, such as laccases, offer a natural alternative for bleaching. This study evaluates the effect of enzymatic bleaching with laccases on NMP and its impact on the release of reducing sugars and total phenols. The Taguchi design determined that optimal conditions for enzymatic bleaching include the use of 1000 U/Kg of NMP, pH 5, solid-liquid ratio 10, and 35°C. Although laccases had a positive effect on the release of phenolic compounds and reducing sugars, the effect on color was not statistically significant. NMP treated with laccases has high potential to be integrated into the tortilla industry for value-added products.

Key words: Enzymatic bleaching, Laccase, nixtamalized maize pericarp



Agricultural & Food Biotechnology

Valorization of *Stenocereus thurberi* and *Rubus idaeus* to obtain wine and its quality analyses.

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Abstract

The natural resources of less favored areas of Mexico have important bioactive components that can improve health through the development of new food products, in addition to adding value to products and generating economic income. The pitaya (*Stenocereus thurberi*) is a species of the cactus family, and raspberry (*Rubus idaeus*) is a polydrupe with a strong and sweet flavor, it grows most often in forest glades or meadows. These fruits are beneficial for humans because they have antioxidant properties and contain nutrients in greater quantities, compared to other fruits, however they are little exploited. Wine, an alcoholic beverage is produced from fermentation of fruit juice especially grape which has a chemical balance that allows them to ferment without addition of sugar, acids, enzymes or other nutrients. Other than grape, other fruits have been used in wine production by researchers and qualified the wine with the name of the fruit. The aim of the present study was to produce fruit wines from a mixture of pitaya and raspberry, and evaluate the physicochemical characteristics, antioxidant content, and sensory parameters. The two wine samples were analysed for titratable acidity, total soluble solids, vitamin C, color parameters, total flavonoids, total phenolic compounds, and antioxidant activity by ABTS and DPPH. All analyses were carried out by triplicate and the results were analysed using a one-way ANOVA test and then a Fisher test ($p < 0.05$) was applied for the significant differences. A sensorial evaluation was conducted with 12 trained judges to evaluate the changes in the sensory properties of the two wines. The sensory test applied was a hedonic test using a five points scale. The results for titratable acidity, vitamin C, and polyphenol content, were significant different ($p < 0.05$) due to the microorganism strain used for the fermentation of the pitaya-raspberry wine samples. The results of the sensory evaluation were also affected. There were significant differences ($p < 0.05$) between samples regarding the attributes of odor and flavor. However, there were no significant differences ($p < 0.05$) for attributes of color, appearance, clarity and global acceptance.

Keywords: pitaya-raspberry wine, fruit wine, antioxidant activity, bioactive compounds, sensory evaluation.

Agricultural & Food Biotechnology

Carbon source increases *Bacillus amyloliquefaciens* antifungal activity against postharvest phytopathogenic fungi

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Introduction: The food production industry has different challenges. One of the main challenges is the control of phytopathogenic fungi on crops since these fungi can infect plants from the beginning of the crop and reach the postharvest stages. With this goal, the *Bacillus* genus has been considered an alternative to produce antifungal metabolites, and the search for the composition of the culture medium or the fermentation parameters to increase production. The present study aims to evaluate different carbon sources in media to increase the antifungal activity of cell-free extracts produced by *Bacillus amyloliquefaciens*. **Methodology:** Different extracts were produced by changing the composition of TSA (Tryptic-casein soy) medium. Glucose, sucrose, and starch replaced the carbon source at 25 g/L concentration. The extract production was performed for 48 h, sampling every 6 h. The obtained extracts were centrifugated at 10,000 rpm for 15 min and filtered with a 0.22 µm nylon filter. The antifungal activity of the extracts was evaluated with the poison medium technique in PDA (Potato dextrose agar) plates against three phytopathogenic fungi (*Botrytis cinerea*, *Penicillium expansum*, and *Pestalotiosis* sp.) at dilution of 1:300 v/v. **Results:** The results showed increased antifungal activity in the extracts obtained with glucose and starch as carbon sources. The highest inhibition percentage was 47%, with the extract produced in the starch-added medium at 42 h fermentation. **Conclusion:** Modifying the media culture to produce cell-free extracts with high antifungal activity can be a biological alternative to controlling different fungi diseases related to the evaluated strains.

Keywords: Starch, *Bacillus* fermentation, fungal growth inhibition, biological control.



Agricultural & Food Biotechnology

Proximal analysis, phytochemical and antioxidant characterization of jacube (*Acanthocereus tetragonus* (L) Hummelinck).

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Abstract

Introduction: *Acanthocereus tetragonus* known as jacube is a cactus plant. Cultivated in states such as Veracruz and San Luis Potosí in the Huasteca Potosina, it is used as an ornamental hedge and in Mexican gastronomy it is consumed in stewed pieces, in its medicinal use it has been observed that it has hypoglycemic properties. Therefore, the objective of this work was to evaluate the physicochemical, phytochemical and antioxidant properties of jacube. **Methodology:** proximal chemical analysis was carried out on immature jacube stems (moisture, ash, fats, proteins, total sugars and crude fiber). To assess the phytochemical components, a qualitative phytochemical analysis was performed, and quantitatively the flavonoid content (with AlCl₃), polyphenols (Folin-Ciocalteu technique) and antioxidant potential were determined by reduction of the DPPH-radical). **Results:** the crude fiber content of jacube is 65.51 ± 2.52 % on a dry weight per 100 g, comparable to the fiber content of nopal (50.00 ± 10.00 %), besides being rich in nutrients such as minerals. Among the phytochemicals, flavonoids, phenolics and coumarins predominate, which can be attributed with the antioxidant capacity of more than 80% present. **Conclusions:** jacube is a healthy food option due to its nutritional and functional properties that can have a beneficial impact on health.

Key words: Antioxidant, cactus, fiber and phytochemicals.



Agricultural & Food biotechnology

Proximal analysis and conservation of the jobo fruit (*Spondias mombin*) from the Huasteca Potosina

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Introduction: The jobo fruit (*Spondias mombin*) is a seasonal fruit native to the Amazon, which has spread to the tropical areas of Mexico, including the Huasteca Potosina region. Despite its great potential, this fruit lacks comprehensive studies revealing its nutritional and chemical content. Its delicate nature results in significant losses during handling and the ripening process. Dehydration is one of the most effective preservation methods, as it reduces volume and extends the shelf life of foods. **Methodology:** The components of the fruit were separated, recovering the pulp and peel of the jobo. Both parts were dehydrated using heat at 37°C. A proximal analysis was performed, evaluating dry matter, moisture, fat, fiber, protein, and minerals in both the pulp and the peel. Additionally, carbohydrate content (total and reducing sugars), colorimetry, and other parameters (pH, titratable acidity, °Brix) were determined according to AOAC-regulated methods. To determine the existence of significant differences between the components, a statistical analysis was performed, with a 95% confidence level. **Results:** The content of each of the fruit's components was determined, highlighting the high sugar content of $67.25 \pm 2.89\%$ in the pulp and $22.07 \pm 0.41\%$ in the peel. The proximal analysis data revealed significant differences between the pulp and the peel, except for pH. **Conclusion:** It is concluded that the pulp and peel of the jobo fruit show significant differences between them. Additionally, valuable information about the fruit was generated, providing a solid basis for future research seeking to utilize this resource.

Key words: *Spondias mombin*, proximal analysis, conservation.

Chitosan-double-stranded RNA nanocomplexes for enhanced *Piezodorus guildinii* pest control

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Introduction: *Piezodorus guildinii* (Hemiptera: Pentatomidae) causes economic losses in soybean crops by reducing productivity, affecting grain size, oil content, and germination power. RNA interference (RNAi) is a conserved gene regulation process triggered by double-stranded RNA with sequence homology to messenger RNA and could potentially be used as a safe technique to combine with integrated pest management strategies. RNAi efficiency can be enhanced by conjugating dsRNA with cationic polymers such as chitosan, which protects dsRNA from degradation and promotes cell delivery. In this study we aimed to evaluate the effect of dsRNA administration by injection and ingestion on the viability of *P. guildinii*, and the effect of dsRNA-chitosan nanocomplexes as a novel pest control strategy. **Methodology:** Eleven target genes were selected and dsRNAs were designed and synthesized for each one. Mortality was monitored for 14 days, and gene level expression levels was determined by qRT-PCR after injection and ingestion of 1 µg of dsRNA. dsRNA-chitosan nanocomplexes were produced, characterized and mortality was evaluated after feeding in *P. guildinii* adults. **Results:** Eleven target genes were selected, and dsRNAs were designed and synthesized for each. In injection assays, significant differences with mortality exceeding 76% were observed, while mortality after ingestion had a lower effect with significant differences, reaching 49%. Interestingly, chitosan:dsRNA nanoparticles demonstrated higher stability under RNase treatments in vitro and a cumulative mortality of 66%. Consistently, RT-qPCR analysis revealed that gene expression levels in treated insects were significantly lower compared to control at 24, 48, and 72 hours post treatment for targeted genes in treated insects. **Conclusion:** In this work we designed and evaluated the administration of dsRNA molecules by injection and ingestion on the viability of *P. guildinii* and determined its molecular silencing effects. Furthermore, we synthesized and characterized chitosan:dsRNA nanocomplexes, that showed an enhanced effect on mortality and stability, showing that this technology could represent a new strategy for the integrated pest management of this insect.

Key words: RNAi, nanocomplexes, pest control



Agricultural & Food Biotechnology

Evaluation of the *in vivo* antagonistic activity of secondary metabolites of *Trichoderma spp.* against *Fusarium oxysporum* f. sp. *lycopersici* in tomato (*Solanum lycopersicum*) plants.

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Introduction: The presence of *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) in tomato crops can reduce yields by up to 70 % worldwide. Currently, synthetic fungicides are the primary control method, but they can harm the environment. Recently, beneficial microorganisms, such as fungi from the *Trichoderma* genus, have been promoted as sustainable alternatives to reduce reliance on synthetic fungicides. Secondary metabolites produced by *Trichoderma spp.* are crucial for biocontrol of *FOL* due to their antagonistic activity. This study aimed to evaluate the antagonistic activity of secondary metabolites produced by *Trichoderma spp.* **Methodology:** The *in vivo* antagonistic activity against *FOL* in tomato plants under greenhouse conditions was evaluated using secondary metabolites produced from two *Trichoderma* species, both individually and in synergy, to provide a biocontrol effect. *Trichoderma spp.* strains were obtained from tomato crop soil at the Universidad Autónoma Agraria Antonio Narro (UAAAN). A completely randomized design was used with six treatments and three replicates each one used: 1) Secondary metabolites of *T. harzianum*, 2) Secondary metabolites of *T. yunnanense*, 3) Combined secondary metabolites of *T. harzianum* and *T. yunnanense*, 4) Commercial control (Trichobiol 1.5 L/ha), 5) Inoculated control (1x10⁵ *FOL* spores/mL), and 6) Absolute control. Plant morphometric variables (height, stem diameter, number of leaflets, chlorophyll, root length, and wet and dry weight of part area and root) and *FOL* incidence and severity were evaluated. **Results:** Significant differences were observed in the treatment combining secondary metabolites of both *Trichoderma* species compared to the other treatments, showing lower *FOL* incidence and severity. Additionally, this same treatment showed statistical differences compared to the other treatments in terms of growth stimulating activity, increasing the number of leaflets, diameter and dry weight of the plants. It also showed similar behavior to the commercial control in terms of chlorophyll content and plant height. **Conclusion:** The treatment with secondary metabolites of *Trichoderma spp.* in synergy proved to be more effective by presenting greater antagonistic activity against *FOL* in tomato plants under greenhouse conditions.

Key words: Biocontrol, antagonistic activity, secondary metabolites (SM), *Trichoderma spp.*, phytopathogen.



Agricultural & Food Biotechnology

Impact of in vitro gastrointestinal digestion on fermented grape pomace extract: antioxidant activity behavior

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Abstract

Introduction: Grape pomace (*Cabernet sauvignon*) is a by-product of the wine industry, rich in polyphenols with significant antioxidant properties. These polyphenols offer various health benefits, including improving cardiovascular health, reducing the risk of diabetes and inflammation, and promoting gut microbiota health. However, for these polyphenols to exert their beneficial effects, they must be bioaccessible, meaning they need to be released from the food matrix during gastrointestinal digestion and be available for absorption in the intestine. **Methodology:** The extract, previously obtained through a solid-state fermentation process, was subjected to in vitro digestion following the standardized INFOGEST 2.0 protocol [1]. The digestion process included three phases: oral, gastric, and intestinal. Samples were collected at each phase to assess the antioxidant potential of the compounds as they moved through the gastrointestinal tract. Antioxidant activity was measured using ABTS and DPPH radical inhibition assays, as well as the ferric ion reduction (FRAP) method. **Results:** The in vitro digestibility results indicated variations in antioxidant activity across the different digestive phases. Notably, during the intestinal phase, there was a reduction in antioxidant potential by 18.23% (FRAP), 16.88% (ABTS), and 67.88% (DPPH). This decrease in activity could be attributed to the harsh conditions that simulate the digestive tract, including the reagents used and pH fluctuations. These conditions can lead to the degradation of polyphenols and chemical modifications, resulting in a partial loss of bioactivity. **Conclusions:** The study's findings demonstrate that despite some loss of antioxidant potential during digestion, grape pomace from the wine industry retains promising bioactivity. This suggests its potential as a valuable ingredient or food additive, contributing to the valorization of wine industry waste.

Key words: Grape marc, INFOGEST, Flavonoids.

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Agricultural & Food Biotechnology

Nutritional and functional characteristics of beer bagasse as a potential food ingredient

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Introduction: Brewery bagasse is the largest waste from the beer brewing process; it is mainly made up of spent grains of barley, which originally has different compounds that could generate a health benefit, such as dietary fiber, including arabinoxylans, and β -glucans, among others. The main objective of this work was to characterize beer bagasse from different recipes to establish its potential as a source of dietary fiber and as a food ingredient. **Methodology:** The beer bagasse was obtained from a local craft brewery (Valparaiso City, Chile). Freeze dried beer bagasse samples - Stout, Ale and IPA categories- were characterized using the Weende scheme. Also, the presence of dietary fiber (TDF), insoluble fiber (IDF) and soluble fiber (SDF) was determined by enzymatic-gravimetric method (Megazyme TDF kit); the content of β -glucans (BG) was determined by enzymatic method (Megazyme K-BGLU kit), and the arabinoxylans (AX) was determined by Orcinol method. **Results and Discussions:** The TDF levels of the Stout, Ale and IPA samples are 42, 64 and 59%, respectively. The results are higher than those obtained in unexhausted barley (15.6%). In all cases, the IDF content is greater than the SDF, observing IDF/SDF ratios of 19.96, 41.86 and 23.66, respectively. For BG determination, the samples evaluated present contents between 0.52 and 1.07%, similar values to those observed in unexhausted barley. Regarding AX content, values between 3.5 and 14% were observed, with the Stout type being the sample with the best results. Regarding other compounds, the presence of protein of over 12% in all samples stands out, and the lipid content (etheral extract) of up to 6.43%. **Conclusion:** The different types of brewing bagasse are an interesting source of fiber dietary, suggesting different food applications on the base of the IDF/SDF ratio. **Acknowledgments:** Project FONDEF ID22i10292, Project Regional R23F0004.

Key words: Revaluation of agri-food waste, beer bagasse, dietary fiber, arabinoxylans, food ingredient.



Agricultural & Food Technology

Study on Nutrient-Enriched Pasta using Jackfruit seed Flour and Chickpea Flour

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Introduction: Pasta is one of the most widely consumed foods across the world. It is usually produced from a mixture of durum semolina, egg, and water. However, the gluten content in pasta is harmful to the people who are sensitive towards its intake like those suffering from celiac disease (CD). In the recent years, the demand for functional foods, such as high-fibre and low-calorie products, has grown steeply. Therefore, pasta is a product worthy of investigation to improve its nutrient content and make it gluten-free. **Methodology:** In order to replace wheat flour, two other alternatives, jackfruit seed flour and chickpea flour were added. The mixture was standardized using three different proportions of jackfruit seed flour (10%, 15%, and 20%), combined with chickpea flour, olive oil, egg, and salt. Additionally, the product with 71% wheat was prepared and was taken as control. All the combinations were prepared into pasta. The products obtained were analysed by their bulk density, moisture content, crude protein, fat content, ash content, water absorption capacity, swelling power, crude fibre, carbohydrate, and sensory evaluation (5-point hedonic scale). **Results:** Based on the analysis made, it was found that sample 3 (20% Jackfruit seed flour) was optimum for consumption. This sample showed a water absorption capacity of 0.9ml/gm, moisture content 30.12%, Crude Protein 21.51gm, Carbohydrate 35.32gm, fat 8.32%, ash content 2.23%, bulk density of 6.5gm/cm³, Crude fiber content of 2.5% and swelling power of 6.3 gm/gm. The product developed was nutritionally rich, texturally superior and safe for long-term storage. **Conclusion:** Jackfruit seeds are generated as a waste product in jackfruit processing industries, so it is essential to find application for these seeds. Therefore, gluten-free pasta was developed using the above-mentioned ingredients, with all properties including shelf-life similar to that of commercially available pasta.

Keywords: *pasta, jackfruit seed, gluten-free, chickpea, dietary fibre, protein, celiac disease*

Agricultural & Food Technology

Biopolymeric micro-nano systems loaded with phytohormones of microbial origin to control water stress.

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Introduction: *Lasiodiplodia theobromae* (LT) is a cosmopolitan fungus producer of phytohormones. These bioproducts are of great commercial interest in the agricultural area, and their applications allow stimulating resistance mechanisms against biotic and abiotic factors, where one of the most relevant is drought, one of the most important problems due to its relationship with the production and yield of crops. In this work, a strain of *Lasiodiplodia theobromae* was isolated and identified from mango (*Manguifera caesia*) (LDTM) and compared with a strain previously isolated from cocoa (*Theobroma Cacao*) (LDTC) to evaluate the production of indole acetic acid (IAA), then the appropriate conditions were established to optimize the production of IAA, through a liquid fermentation process. **Methodology:** From mango (*Manguifera caesia*) samples with symptoms of necrosis and downward death, five mm² tissue sections were washed, disinfected, dried, and sown in Petri dishes in PDA medium at 28°C for seven days. Microscopic and macroscopic identification of LT based on their morphological characteristics. Preliminary evaluation and optimization used a Miersch medium with modifications, and phytohormone quantification was performed by rapid auxin detection by microplate. A Box-Behnken experimental design was used for the optimization stage, using time, working volume, and yeast factors. **Results:** A strain of LT was isolated from mango (*Manguifera caesia*), and a solitary black and globose pycnidium and mature conidia were observed (Dhandhukia & Thakkar, 2007; Laredo-Alcalá *et al.*, 2016). In the preliminary stage, 1.04 ppm and 0.85 ppm were obtained for LDTC and LDTM, respectively. Proceeded to the next stage with the LDTC strain. Subsequently, 127 ppm of indole-3-acetic acid was obtained in the optimization stage. **Conclusion:** It was possible to obtain an LT strain from mango (*Manguifera caesia*), determine its AIA production capacity, and establish the best conditions for AIA production.

Keywords: *Lasiodiplodia Theobromae*, Phytohormones Liquid Fermentation, AIA



Agricultural & Food Biotechnology

Invertase production by *Aspergillus brasiliensis* ATCC 9642 in solid-state culture with stout beer residues

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Introduction: *Aspergillus brasiliensis* ATCC 9642 is a reference microorganism in the standards of the American Society for Testing and Materials (ASTM) and has been studied to produce metabolites of industrial interest such as organic acids and enzymes such as xylanase, thermostable β -xylosidase and polygalacturonase. Recent studies have shown that *A. brasiliensis* also produces a series of extracellular enzymes, such as invertase, both in solid culture and in liquid culture, being produced industrially through the latter. However, solid culture has potential for the industrial production of enzymes due to the high productivity of enzymes and the low production costs for this purpose, considering that agroindustrial waste can be used as natural supports. This study aimed to evaluate the production of extracellular invertase by *A. brasiliensis* in solid state culture without the addition of sucrose. **Methodology:** *Aspergillus brasiliensis* ATCC 9642 was used for the invertase production in solid culture, using waste from stout beer production as a support, impregnated with Pontecorvo medium, without additional sucrose, with a humidity of 60% and culture temperature of 30 °C. It was sampled every 12 h to obtain kinetics of enzymatic activity, quantifying the products of said reaction by the Miller (1960) method. **Results:** Invertase kinetics showed a proportional increase in activity over time, obtaining 91.08 ± 2.42 U/gmsi after 48 h of culture. **Conclusion:** Stout beer residues, without the addition of sucrose, showed to be a good natural support for the compounds production of industrial interest such as the invertase enzyme, from *Aspergillus brasiliensis* ATCC 9642 in solid culture.

Keywords: *A. brasiliensis*, stout beer residue, solid state, enzymes



Agricultural & Food Biotechnology

Invertase production by *Aspergillus brasiliensis* with lager beer residues in solid state fermentation

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Introduction: Various species of the genus *Aspergillus* are used for a large-scale production of enzymes such as amylases, xylanases, galactosidases, pectinases and organic acids. *Aspergillus brasiliensis* ATCC 9642 has been used for enzymes production and hydrocarbon biodegradation at lab level. Most of these processes are performed by submerged fermentation (SmF); however, solid-state fermentation (SSF) processes offer several advantages over SmF, such as the reduction in catabolite repression and substrate inhibition greater enzyme yields and volumetric productivities, extended product stability and low production costs. This study aimed to evaluate the production of extracellular invertase by *A. brasiliensis* in solid state culture without sucrose addition. **Methodology:** Waste from lager beer production was used as a natural support impregnated with Pontecorvo medium, without additional sucrose, and *Aspergillus brasiliensis* ATCC 9642 as an invertase-producing microorganism in solid state fermentation, with a humidity of 60%, at 30 °C and 48 h of cultivation. Once the fermentation started, it was sampled every 12 h to carry out kinetics of enzymatic activity and the products obtained from said reaction were quantified by the Miller (1960) method. **Results:** Invertase enzyme activity increased as the culture time increased, obtaining an activity of 60.88 ± 2.74 U/gmsi after 48 h of culture. Although it was not possible to find a maximum of activity in the times analyzed, these results are greater than those reported by other authors using sucrose as a substrate. **Conclusion:** *Aspergillus brasiliensis* ATCC 9642 was able to produce invertase from lager beer waste, without the addition of sucrose, in solid culture, which allows us to appreciate the potential of solid-state fermentation and the use of agroindustrial waste for the production of metabolites of industrial interest.

Keywords: *A. brasiliensis*, lager beer residue, solid state fermentation, invertase



Agricultural & Food Biotechnology

Use of High Moisture Extrusion Cooking (HMEC) technology to obtain plant-based protein meat analogues

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Introduction: Every day more people choose a vegan or vegetarian diet, which is attributable to various causes, among which consumer concern for the environment and animal welfare stands out; and to a lesser extent, the need for good nutrition and health. That is why an alternative route is proposed in order to reduce the production numbers of meat of animal origin and also satisfy the growing demand; which consists of meat analogues based on vegetable proteins, which, in parallel, has gained high popularity in the market. This research project aims to develop these analogues. **Methodology:** The procedure is based on the texturing of preselected vegetable protein using High Moisture Extrusion Cooking (HMEC) technology, which allows obtaining fibrous protein foods with sensory characteristics comparable to that of meat muscle. A mixture of soy protein isolate, pectin, and avocado oil was used to obtain the meat analogues. Proximal chemical analysis was carried out on the extruded samples using various methodologies to determine the percentage amount of moisture, proteins, fibers, ashes and fats present in the products. The texture profile analysis (TPA) was carried out on the extrudates obtained and their physicochemical characteristics were studied. **Results:** The studies carried out showed that the products obtained with pectin presented anisotropic structures comparable to the meat muscle, while those extruded without pectin did not show the formation of fibrous structures. The proximal chemical analysis showed that the macronutrient composition in the products obtained is in the range of commercial brands, as was the case with the TPA results. **Conclusions:** This project showed that it is possible to obtain plant-based meat that simulates the muscle of animal-based meat. The incorporation of pectin favorably contributed to the formation of fibrous structures and avocado oil to improve the fatty acid profile.

Key words: Extrusion, plant-based meat, fibrous structures



4th **BIO IBEROAMERICA**

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BIOCATALYSIS & BIOTRANSFORMATION

Magnetic immobilization of *Rhodotorula toruloides* and reuse in consecutive fermentation cycles

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RESUMEN

Introduction: *Rhodotorula toruloides* is a red oleaginous yeast that synthesizing valuable metabolites in various industries, including lipids, carotenoids, and enzymes. Using magnetic nanoparticles (MNP) in surface-adhesion fermentation offers several advantages over submerged fermentation technology and conventional cell immobilization methods due to their remarkable magnetic properties, low toxicity, good biocompatibility, and simple synthesis process. Magnetic nanoparticles can adhere to cell surfaces through electrostatic or hydrophobic interactions, thereby facilitating the transport of metabolites across membranes. The present study aims to develop magnetic immobilization technology to separate and reuse yeast cells during fermentation for extracellular invertase production. **Methodology:** The magnetic immobilization of *R. toruloides* and reuse in fermentation cycles were evaluated using chitosan-coated manganese ferrite magnetic nanoparticles (MnFe₂O₄-Ch), synthesized through a one-step chemical coprecipitation method. Characterization studies were performed using X-ray diffractograms, magnetometry, Fourier-transform infrared spectroscopy (FT-IR), DLS, and scanning electron microscopy (SEM) analysis. The immobilized biomass was assessed with and without adding magnetic nanoparticles in various fermentation cycles. Invertase activity was assayed in cell-free culture media by DNS method. **Results:** MnFe₂O₄-Ch with a spinel structure and a crystallite size of 20.73 nm was obtained, with a magnetic saturation of 39.6 emu/g at 20 kOe and 300 K and an average hydrodynamic particle size of 181.7 nm. FT-IR confirmed the chitosan presence. Through surface-adhesion fermentation, the growth of *R. toruloides* was enhanced with MnFe₂O₄-Ch. SEM showed yeast cell and MNP interaction. The microorganism was immobilized on MNP and reused in various fermentation cycles. In the third cycle of reusing immobilized yeast with the addition of MnFe₂O₄-Ch, enzymatic activity increased compared to the first two reuse cycles, reaching 1.88 IU/mL without significant difference compared to the initial surface-adhesion fermentation enzymatic activity. **Conclusions:** Surface adhesion fermentation with MNP could be a promising method for extracellular yeast enzyme production, allowing the reuse of biomass separated with a magnetic field in several fermentation cycles.

KEYWORDS: *Rhodotorula toruloides*, manganese ferrite magnetic nanoparticles, magnetic immobilization.

Topic: Biocatalysis

Green extraction of polyphenolic bioactive compounds from *Lippia graveolens* using fermentative techniques and their antifungal activities

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Introduction: Phytopathogenic fungal attacks cause fruit waste, spoilage, reduced quality, and economic loss in the supply chain [1]. Approximately 30% of harvested fruits do not reach consumers because of fungal diseases, highlighting the urgent need for effective control measures [2,3]. Innovative strategies, such as plant extracts, essential oils, and biocontrol agents, are being explored to combat decay [4]. **Methodology:** The leaves, flowers, and stems of *Lippia graveolens* were subjected to fermentative extraction processes involving the use of the filamentous fungus *Trichoderma asperellum* for 120 h in both the solid and liquid states. The extracts were then analyzed for their polyphenolic constituents using Folin-Ciocalteu reagent and HCl butanol and were further assayed in vitro against *Fusarium oxysporum* and *Alternaria alternata* using HPLC-MS. The antioxidant potential of the extracts was also tested using the FRAP, ABTS, and DPPH assays. **Results:** The results showed that the solid-state fermentation *L. graveolens* leaf extract at 36 h contained hydrolysable tannins (7.46 mg/g), while the solid-state *L. graveolens* stems extract showed the highest condensed tannins (6.25 mg/g) at 72 h. At 72 h, the solid-state *L. graveolens* stem extract inhibited the growth of *A. alternata* and *F. oxysporum* by 65.4% and 76.3 %, respectively. All extracts demonstrated significant antioxidant potential in ABTS, DPPH, and FRAP assays. Twelve major polyphenolic compounds were identified, namely kaempferol, quercetin, luteolin, 3,4-DHPEA, elenolic acid, NDGA, 5-Heptadecylresorcinol, 3,7-Dimethylquercetin, dihydroflavonolsand, luteolin 7-O-(2-apiosyl-glucoside), dihydroquercetin, and kaempferide. **Conclusion:** Stem extracts had strong fungistatic effects against phytopathogenic fungi, demonstrating their potential as bioactive ingredients in biopesticide formulations.

Keywords: Solid State fermentation, liquid state fermentation, *Fusarium oxysporum*, *Alternaria alternata*, Mexican oregano

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Biocatalysis and Biotransformation

Quercetin modification using a laccase from *Trametes sanguineus*

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Introduction: Quercetin, a widely studied flavanol, is present in various natural sources like red cherries, apples, grapes, blueberries, blackberries, citrus fruits, broccoli, tea, cocoa, onions, and green leafy vegetables. It offers numerous health benefits such as senolytic and angioprotective properties, antioxidant effects, inhibition of allergic reactions, and potential anti-cancer properties. Despite its advantageous attributes, the effectiveness of quercetin as a drug is challenged by its poor absorption, limited aqueous solubility, instability in physiological environments, low permeability, and lack of bioavailability. To address these limitations, enzymatic modifications have been explored to improve the stability and solubility of flavonoids. **Methodology:** In our study, a crude enzymatic laccase extract from *Trametes sanguineus* culture was utilized to modify quercetin. The experimental conditions included phosphate buffer (100 mM, pH 3.0 and 5.6) and acetate buffer (100 mM, pH 4.5), with a crude extract containing laccase activity at 0.8 U/mL, operated at temperatures of 45°C and 50°C. Quercetin was maintained at a concentration of 10 mM, with a reaction period of 24 hours under agitation at 200 rpm. The modifications in the quercetin structure were identified through UV-Vis spectroscopy (200-400 nm). **Results:** Different UV-Vis spectra patterns were observed between quercetin and the quercetin after a 24-hour reaction catalyzed with laccase. Remarkable changes were noted in the reaction employing phosphate buffer at pH 5.6 and 45°C. Most samples exhibited enhanced QR solubility, with total or partial solubilization of the flavonoid. **Conclusion:** Under the specified conditions, the crude enzymatic extract from *T. sanguineus* partially oxidized quercetin. The modified QR from the reaction using pH 5.6 and 45°C in phosphate buffer will undergo further investigation regarding its impact on cancerous cellular lines. Structural elucidation of the modified QR will be conducted through FTIR spectra and ¹H and ¹³C NMR. This research contributes to the progress of biocatalytic methods for altering natural compounds with potential therapeutic applications using an enzyme produced by a robust fungus.

Keywords: enzyme, cancer, solubility, flavonoids



Biocatalysis & Biotransformation

Lipase/esterase activity of bacteria isolated from soils contaminated with polypropylene

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Introduction: Lipases and esterases are crucial enzymes for hydrolyzing carboxylic esters. Due to their stability and extracellular nature, they have significant applications in various industries, such as pharmaceuticals, food, and cosmetics. This study explores bacteria from polypropylene-contaminated soils as potential enzyme sources, given their potential ability to metabolize complex carbon sources like synthetic plastics. **Methodology:** Polypropylene samples exhibiting signs of deterioration or soil traces were collected from a landfill in Mazamitla, Jalisco. Microorganisms were recovered in YPD and nutrient agar. Plates were incubated at 30°C for 24 hours. Distinct colonies were isolated and identified using MALDI-TOF MS. Screening on tributyrin emulsion media exhibited lipase/esterase activity. Positive strains were fermented in minimal media, and enzyme activity was measured using p-nitrophenyl butyrate. **Results:** From 55 isolates, 50 were identified, mainly belonging to *Pseudomonas* and *Bacillus* genera. Screening revealed that 23 strains exhibited lipase/esterase activity, with 10 classified as high producers, 8 as medium producers, and 3 as low producers. Liquid culture assays revealed that enzyme activity peaked at 48 hours, with the highest activities observed in an unidentified strain, *Pseudomonas jessenii*, and *Pseudomonas kilonensis*. These strains demonstrated significant potential for enzyme production, with enzyme activity increasing over time. **Conclusion:** The study has identified bacteria from polypropylene-contaminated soils with significant lipase and esterase activities. The strains, particularly an unidentified strain, *Pseudomonas jessenii*, and *Pseudomonas kilonensis*, have shown promising capabilities for enzyme production. These findings strongly suggest that such strains could be further optimized and characterized for industrial biocatalyst applications, offering a sustainable approach to enzyme production.

Key words: Lipase activity; Esterase activity; Polypropylene degradation; soil bacteria.

Cutinase activity of microorganisms isolated from PET contaminated soils in Mazamitla

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Introduction: The ubiquitous presence of plastics in the environment is a global concern, due to their persistence and the challenges associated with their degradation. Among various strategies to mitigate plastic pollution, microorganisms capable of producing hydrolytic enzymes, particularly hydrolases, are of interest. Esterases, lipases, and cutinases, target the ester bonds in plastic polymers, initiating their depolymerization. Soils contaminated with plastics, such as dumpsites, represents a habitat for microorganisms with potential biodegradation capabilities. Since cutinases are structurally similar to PETases, the aim of this work was to identify cutinase producing bacteria, from PET contaminated soils. **Methodology:** PET with signs of deterioration and soil samples were collected from an open dump site in Mazamitla, Jalisco. Sediments and plastics were resuspended in water and plated in YPD and nutrient agar plates. Isolated colonies were identified by MALDI-TOF. Cutinase activity was evaluated using an induction medium containing (g/L): tryptone 10, yeast extract 5, agar 20, and a linseed oil emulsion at 10 g/L. The plates were inoculated by single streak and incubated at 30°C for 48 hours. The presence of a white halo indicated cutinase activity. Positive strains were evaluated for extracellular activity and activity measured by the p-NPB method. **Results:** 117 strains were isolated, identifying 84.8% of them using Maldi-TOF. From the identified strains 95.1% were bacteria and 4.8% yeasts. After screening in selective media, 49 strains showed hydrolysis halo. The 24 strains classified as high activity in the agar screening method were selected to evaluate the extracellular production of cutinase using flaxseed oil. *Comamonas testosterini*, *Pseudomonas koreensis*, and *Acinetobacter beijerinckii* showed the highest levels of extracellular activity. **Conclusion:** Contaminated soils are a good source for the isolation of microbial strains with potential enzymes for plastic biodegradation. All the strains classified as good producer in agar screening showed extracellular activity. These strains could be furtherly studied to improve the production of cutinases.

Key words: Cutinase activity, PET degradation, soil contamination, extracellular enzymes.

Spatial variations of β -etherase and phloroglucinol pathway enzymes in a lagoon type anaerobic biodigester fed with cattle manure.

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Introduction: Valorizing lignocellulosic biomass offers a compelling path toward renewable energy production. Anaerobic digestion of organic fractions from agricultural residues has emerged as an effective strategy for waste management and the generation of renewable energy. However, reaching the maximum biogas potential is often difficult due to the presence of recalcitrant lignin barrier. Anaerobic degradation of lignin requires specialized microorganisms for efficient biocatalysis. In this work characterization and purification of two key enzymes from the few anaerobic lignin-degrading pathways, *viz.*, C α -dehydrogenase from the β -etherase pathway and phloroglucinol reductase from the phloroglucinol pathway were determined from different longitudinal sampling points of a full-scale lagoon type anaerobe biodigester (40 x 40 x 8 m) fed with cattle manure. **Methodology:** The samples were collected from different sites, *viz.*, the influent, beginning, middle, and effluent of biodigester. The protein fractions were analyzed by Tricine SDS-PAGE and characterized for iso-electric point by 2-D gel electrophoresis. The kinetic parameters (K_m and V_{max}) of C α -dehydrogenase were determined using guaiacylglycerol- β -guaiacyl as substrate, and phloroglucinol for phloroglucinol reductase. **Results:** The major activity for both enzymes was found on inlet fraction with $k_m=1.46$ mM and $V_{max} = 512$ μ mol/s for phloroglucinol, $k_m=0.36$ mM, and $V_{max}= 100$ μ mol/s for guaiacylglycerol- β -guaiacyl ether, for phloroglucinol reductase and C α -dehydrogenase respectively and the corresponding molecular weight was 31 and 33 kDa. **Conclusion:** Our findings reveal significant disparities in protein fractions across spatial variations within a full-scale anaerobic biodigester. It can be observed that lignin degradation primarily occurs in the inlet fraction; the residual activity of phloroglucinol reductase was found to decrease in the outlet fraction. This indicates that lignin degradation products were assimilated by microbial consortia throughout the flow in the biodigester. These insights could help to enhance lignin degradation within biodigesters to maximize methane yield.

Key words: Anaerobic lignin degradation, C α -dehydrogenase, phloroglucinol reductase.

Biocatalysis & biotransformation

Fungal bioprocess in solid state on tarbush (*Flourensia cernua*) to obtain extracts with bioherbicidal effect on model seeds and weeds

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Introduction: Due to the increase in cancer cases associated with glyphosate, a synthetic herbicide, natural alternatives have been investigated to gradually eliminate its use. Consequently, it has been found that tarbush (*Flourensia cernua*), a semi-desert plant, produces secondary metabolites considered as allelochemicals to which bioherbicidal activity is attributed. The bioavailability of these compounds can be increased by exposing the plant material to filamentous fungi through solid-state fermentations to obtain extracts that can be used as a biodegradable alternative to glyphosate. **Methodology:** A solid fermentation was carried out with *Aspergillus niger* using *Flourensia cernua* plant material as a substrate to obtain an extract with which allelopathic tests were carried out on model seeds of corn (*Zea mays*), beans (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), and weed (*Lolium perenne*). **Results:** A greater allelopathic effect was observed in the seeds when the extract was applied at the higher concentration. Complete inhibition was not obtained in the model seeds, but the quality and size of the stem and roots were significantly reduced. In *Lolium perenne* seed, the fermented extract applied undiluted inhibited 100% of the seeds. **Conclusion:** Solid state bioprocessing managed to promote the release of allelochemicals with the capacity to affect vital processes for plant development, demonstrating to have a limiting effect on the growth of the three model seeds and inhibition on the germination of *Lolium perenne*.

Key words: Bioprocess, glyphosate, bioherbicide, *Flourensia cernua*, *Aspergillus niger*

Biocatalysis and biotransformation

Biotransformation of spent coffee grounds waste through optimization of solid-state fermentation to obtain polyphenols.

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Introduction: Spent Coffee grounds are an agro-industrial waste that contributes damage to the environment. It has been shown that it contains compounds of interest, which is why using a bioprocess such as solid-state fermentation with this substrate can obtain bioactive compounds with biological activities. **Methodology:** An exploratory design was carried out for solid-state fermentation of coffee grounds (*Trichoderma harzianum* and *Rhizopus oryzae*), using the Box Hunter & Hunter exploratory design. The objective was to identify key factors, such as temperature, humidity, and inoculation, evaluating minimum and maximum levels with a fixed time of 48 hours. Tests for hydrolysable and condensed tannins were carried out, reporting the sum as the total polyphenols. The results were analyzed with the STATISTICA 7 program, comparing means. The factors were evaluated at levels -1, 0, 1 using the Box-Benken optimization design. Finally, the best treatment was selected and antioxidant tests (DPPH, ABTS, FRAP). **Results:** The exploratory design favored the T3 treatment (0.279 ± 0.002 mg/g coffee grounds) of *Trichoderma harzianum* to obtain the total polyphenol content, and for *Rhizopus oryzae* the T3 treatment (0.250 ± 0.011 mg/g). The factor that favored the process corresponds to the minimum value (1×10^7 spores g^{-1}) inoculum size. The extracts of both mushrooms had affinity for the DPPH radical. In the Box design Behnken favored the T12 treatment (0.511 ± 0.017 mg/g) of *Trichoderma harzianum* and for *Rhizopus oryzae* the T9 treatment (0.636 ± 0.003 mg/g). **Conclusion:** Optimizing the fermentation of coffee grounds in solid state maximizes the production of total polyphenol compounds, with antioxidant activity.

Keywords: Bioprocess, Polyphenols, Spent coffee grounds



Biocatalysis & Biotransformation

Hyperthermia in *Rhodotorula toruloides* using manganese ferrite nanoparticles (MnFe₂O₄)

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Introduction: *Rhodotorula toruloides* is a pigmented oleaginous yeast capable of producing carotenenes with applications in the pharmaceutical and food industries. Carotenoids have antioxidant, anti-inflammatory, and anti-cancer properties. These metabolites accumulate intracellularly, which is why different cell disruption techniques are used. A novel method that could be used is magnetic fluid hyperthermia (MFH) treatment, a technique that facilitates selective heating of cells. **Methodology:** Manganese ferrite (MnFe₂O₄) magnetic nanoparticles (MNP) were synthesized using the coprecipitation method. The sample was characterized by X-ray diffraction (XRD), magnetometry, and dynamic light scattering (DLS). **Results:** XRD analysis confirmed the presence of single-phase MnFe₂O₄ with cubic spinel structure, and the crystallite size was 16 nm. Magnetic saturation was 42.7±0.3 emu/g at 20 kOe and 300 K. The average hydrodynamic diameter of scattering was 344 nm. The heating capacity of MNP was studied with an induction heating system under different magnetic field intensities of 10 kA/m varying concentrations of nanoparticles at a fixed frequency of 231.1 kHz. Cell viability was evaluated, finding a decrease of up to 70 % at a temperature of 53 °C at a concentration of 10 mg/mL of MNP. **Conclusion:** It is the first work on applying hyperthermia in biotechnological processes. Optimization of carotenoid extraction conditions is required to achieve greater efficiency.

Keywords: Nanoparticles, Magnetic hyperthermia, *Rhodotorula toruloides*



Biocatalysis and Biotransformation

Enzymatic modification of quercetin and caffeic acid by laccases

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Introduction: Natural compounds such as flavonoids and phenolic compounds exhibit diverse biological properties, including antimicrobial, antioxidant, antiviral, anticancer, or anti-inflammatory activities. Among these, quercetin demonstrates such activities and also finds application in treating obesity, cardiovascular diseases and diabetes. However, polyphenols present significant drawbacks such as low water solubility and stability, limiting their application since these compounds cannot be efficiently transported to the target sites within the organism. Considering this, the main aim of this study is to develop derivatives of quercetin+caffeic acid (QC+Caf) with improved solubility while retaining their antioxidant potential. **Methodology:** Quercetin and caffeic acid were co-modified using acetate buffer at pH4.5 and 40% methanol to dissolve the compounds. Laccase was added to a final concentration of 0.5 U/mL and the reaction mixture was incubated at 30 °C, 200 rpm for 24 h. Subsequently, modifications in the compounds were assessed using UV-Vis spectrophotometry and HPLC. Additionally, the UV stability, solubility and the antioxidant activity were evaluated. **Results:** UV-Vis spectra exhibited distinct patterns following laccase addition. HPLC confirm these observations, revealing alterations in the quercetin and caffeic acid after laccase addition. Furthermore, these derivatives retained antioxidant activity, albeit showing approximately 20-30% reduction compared to quercetin and caffeic acid (control). **Conclusion:** QC+Caf derivatives were obtained by using laccases, which exhibit antioxidant activity and enhanced solubility. These derivatives are currently undergoing evaluation on cancer cells, while efforts are also directed towards elucidating their structure.

Keywords: Flavonoids, phenolic compounds, laccase, derivatives

Biocatalysis and biotransformation.

Bioactive properties of *Flourensia cernua* extracts through solid state fermentation by *Aspergillus niger*, a biotechnological approach.

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Introduction: *Flourensia cernua* is a semi-desertic plant used in traditional medicine to treat gastrointestinal conditions, it contains high amounts of phenolic compounds, which give it potential for various applications. In this study, the in vitro bioactive properties of *Flourensia cernua* extract obtained by solid state fermentation (SSF) with *Aspergillus niger* were investigated. **Methodology:** Phytochemicals macerated from the foliage of the plant material were characterized using hydrolyzable phenols, total flavonoids, DPPH antioxidant activity and ABTS techniques. In addition, the culture conditions of *Aspergillus niger* on *Flourensia cernua* were established with a Box-Benhken experimental design with three factors and three levels, where the results were maximized according to the total phenolic content (TPC), and finally the cytotoxic effects of the extracts and their antioxidant properties were evaluated. **Results:** For the characterization, values of 9.52 mg GAE/g for hydrolyzable phenols and 52.5 mg QE/g for total flavonoids were obtained in the macerated extract, values of 5.16 mg GAE/g for hydrolyzable phenols, and 11.31 mg QE/g for total flavonoids in the fermented extract; however, the fermented extract presented greater antioxidant activity. Implementing the experimental design, it was possible to maximize the obtained results during the fermentation, resulting 43.440 mg GAE/g for hydrolyzable phenols, and 15.255 mg QE/g for total flavonoids. In addition, cell viability studies with the extracts showed non-cytotoxic effects on human monocytes and enhanced metabolic activity in fibroblasts of the 3T3 line and porcine bone cells after 24 hours with the fermented extracts. **Conclusion:** It was determined that SSF increases the phenolic content and in turn exhibits an increase in antioxidant activity, promoting greater cell viability in vitro, conferring to the fermented *Flourensia cernua* extract a potential for biomedical applications.

Key words: *Flourensia cernua*, Solid state fermentation, *Aspergillus niger*, bioactive properties.



4th **BIO IBEROAMERICA**

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3-6 SEPTEMBER 2024

BIOECONOMY & SUSTAINABILITY

Topic: Biocatalysis

Ultrasound Assisted Extraction and Characterization of Polyphenolic compounds from Coffee Residues

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Introduction: The pursuit of eco-friendly pigment sources has garnered significant attention in modern research, as industries strive to identify alternatives to minimize environmental harm. Among the potential alternatives under investigation are coffee residues rich in bioactive compounds, including pigments [1], [2]. **Methodology:** Extraction of coffee pulp was accomplished using non-conventional ultrasound-assisted extraction techniques at a ratio of 1:20 (w/v) coffee pulp to solvent. Extraction was carried out using ethanol at various concentrations, including 100:0, 25:75, 50:50, 60:40, 75:25, and 0:100. The extraction process was conducted at different temperatures ranging from 30 °C to 50 °C and durations of 30, 40, 50, 60, and 70 min. The polyphenolic compound content in the extracted pigments was evaluated using the Folin and HCl butanol tests. **Results:** The highest quantity of hydrolyzable tannins, amounting to 20.2 mg GAE, was obtained from 100 % ethanol of UAE, which was subjected to 40 °C for 40 min. Conversely, the aqueous extract of UAE kept at 30 °C for 30 min yielded the highest amount of condensed tannins, totaling 32.5 mg CE/g. The polyphenolic content is contingent on time and temperature. The antioxidant activity of the extracts was directly proportional to the amount of polyphenolic compounds present. **Conclusion:** UAE offers significant advantages in terms of efficiency, cost-effectiveness, and extraction quality, making it an ideal method for extracting bioactive polyphenols from coffee pulp.

Keywords: Coffee pulp, ultrasound-assisted extraction, Polyphenolic compounds

Reference:[1] S. Ruhil and K. Nagpal, "Microbial Pigments as Vegan Colors for Food and Pharmaceuticals: A Sustainable Approach," 2024, pp. 419–438. doi: 10.1007/978-981-97-1152-9_16. [2]A. S. Khandeparkar, R. Paul, A. Sridhar, V. V. Lakshmaiah, and P. Nagella, "Eco-friendly innovations in food packaging: A sustainable revolution," Sustain. Chem. Pharm., vol. 39, p. 101579, Jun. 2024, doi: 10.1016/j.scp.2024.101579.

Bioeconomy and Sustainability

Enhancing Procyanidin Extraction Efficiency from Coffee Pulp: A Comparative Investigation of Microwave, Ultrasound, and Hybrid Extraction Techniques

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Procyanidins, versatile secondary metabolites present in diverse plant sources, including coffee pulp, an agro-industrial residue, offer a myriad of health benefits, spanning from antioxidant to neuroprotective effects [1]. Despite their promising attributes, the optimal method for procyanidin extraction from coffee pulp remains an active area of investigation. This study evaluates the efficacy of microwave, ultrasound, and hybrid extraction techniques in recovering the compound from coffee pulp.

To optimize extraction efficiency, various factors and variables such as solvent concentration and solid-to-liquid ratios were systematically evaluated. A factorial experimental design incorporated ethanol and acetone at concentrations of 30%, 70%, and 100%, along with solid-to-liquid ratios of 1:10, 1:30, and 1:60 w/v. Procyanidins in the extracts were quantified using the HCl-Butanol test.

The microwave extraction method yielded 137.27 ± 33.3 mg/100g with 70% ethanol at 1:30 w/v, and 176.94 ± 18.1 mg/100g with 70% acetone at the same ratio. Ultrasound extraction resulted in a yield of 143.54 ± 15.1 mg/100g using 30% ethanol at 1:60 w/v, and 211.14 ± 61.7 mg/100g with 70% acetone at the same ratio. The hybrid extraction approach produced a yield of 201.14 ± 39.7 mg/100g with 70% ethanol at 1:60 w/v, and 306.57 ± 18.1 mg/100g with 70% acetone at 1:30 w/v. These results represent a significant improvement over previous reports, with ultrasound extraction previously yielding 200 mg/g and microwave extractions achieving 30.7 mg/g.

The study uncovered that employing a hybrid extraction method with 70% acetone at a 1:30 w/v ratio yielded the highest procyanidin output. This research not only enhances procyanidin extraction from coffee pulp, offering a sustainable solution for agro-industrial waste but also paves the path for innovating new extraction techniques for plant-based secondary metabolites, fostering innovation across multiple domains.

Keywords: *Neuroprotective effect, Coffee pulp, Procyanidin, Health benefits, Ultrasound, Microwave*

Evaluation of bioactive compounds from prickly pear peel for the development of functional packaging in food.

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Introduction: The byproducts generated by the food agroindustry represent a valuable source of phytonutrients, such as phenolic compounds. These compounds, known for their bioactive properties, including antioxidant and antimicrobial capacity, can be explored in the natural products industry. In this context, a research study has been conducted on obtaining phenolic compounds from the peels of *Opuntia ficus-indica* (prickly pear) using two methodologies: ultrasound-assisted extraction and solid-state fermentation. The objective is to add value to this agroindustrial waste and contribute to environmental impact mitigation. **Methodology:** Solid state fermentation (SSF) is a method used in various industries to produce metabolites from microorganisms using a solid support instead of a liquid medium. In this study, dried and ground green prickly pear shells were used as substrate. The fungus *A. niger* GH1 was used under controlled conditions of temperature, humidity and inoculum. After 52 hours of fermentation, the process was stopped and the compounds were extracted with absolute ethanol. In addition, an ultrasound extraction with absolute ethanol was performed on dried and ground prickly pear peels, controlling three factors: solid-liquid ratio, temperature and sonication time. The different extracts obtained were analyzed for condensed tannins by the terbutanol-HCl method and hydrolyzable tannins by the Folin-ciocalteu method. The objective was to compare both processes in obtaining phenolic compounds. **Results:** In the study, solid state fermentation (SSF) using dried and ground green prickly pear peels produced 50.18 mg/g of condensed tannins. In contrast, ultrasound-assisted extraction reached a maximum of 4.58 mg/g of condensed tannins. In addition, hydrolyzable tannins obtained by solid-state fermentation of the same substrate was a maximum of 7.1 mg/g, while ultrasound-assisted extraction showed a maximum of 0.935 mg/g. **Conclusion:** In summary, the results indicate that solid-state fermentation achieved a higher yield of condensed tannins compared to ultrasound-assisted extraction. In addition, solid-state fermentation also resulted in a higher yield of hydrolyzable tannins. These findings suggest that SSF could be an effective strategy to obtain phenolic compounds from dried and ground green prickly pear peels.

Keywords: Tannins, Extraction, Agroindustrial waste.

Bioeconomy & Sustainability

Cassava starch-based films and rosemary essential oil: Antibacterial activity and physicochemical properties

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Introduction: Currently, single-use food packaging made of non-biodegradable polymers is causing high levels of environmental pollution. Therefore, there is a great interest in the production of environmentally friendly materials. In addition to containment, food packaging requires functionalities such as oxidation control, and antimicrobial properties, among others. Thus, bioplastics made from renewable sources and bio-active components are desirable in food packaging. **Methodology:** Bioplastic films were prepared with cassava starch and rosemary essential oil using a casting methodology. The antibacterial activity, water vapor transmission, mechanical resistance, and microstructure were measured in the films. The films were exposed to pathogenic bacteria such as *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*. **Results:** Antibacterial activity was evidenced for the pathogens evaluated except for *B. cereus*. The films showed average values of water vapor transmission 3.6988 (10^{-14} g/Pa s m), tensile strength 8.90 MPa, young modulus 1679.72 MPa, and elongation at break 4.33%. The microphotographs taken by scanning electron microscopy showed good adhesion between the components of the bioplastic matrix. **Conclusion:** These results show the potential of the bioplastics of cassava starch and rosemary oil for food packaging, mainly in the packaging of fruits or products made with eggs or chicken.

Keywords: antibacterial, essential oil, bioplastic, cassava starch.



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BIOENERGY & BIOREFINERY



Bioenergy & Biorefinery

Hydrothermal pretreatment of *panicum virgatum* to enhance an enzymatic hydrolysis

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Introduction: Given the need to replace fossil fuels with a renewable alternative, the concept of biorefinery arises. Biorefineries use biomass as raw material, which is a renewable resource and multiple high value-added products such as biofuels are produced from it. Switchgrass or *Panicum virgatum* is a native grass from Mexico with great potential as a raw material in the production of bioethanol. The bioethanol production process from a material such as switchgrass consists of four main stages: pretreatment, hydrolysis, fermentation, and distillation. **Methodology:** This study started with a dry harvested switchgrass which was prepared by cutting and grinding it to be able to use it at different pretreatment conditions. Three pretreatments were carried out at various temperature and residence times, these being: 160 °C for 10 min, 160 °C for 40 min, and 170 °C for 25 min. Selecting the conditions that offered the highest percentage of cellulose, an enzymatic hydrolysis was performed by means of cellulase enzymes to obtain glucose for 72 hours. **Results:** The third pretreatment offered the highest percentage of cellulose, being 45.28% with a severity factor of 3.92. The product of the hydrolysis was a concentration of 9.96 g/L of glucose in the sample and a productivity of 0.66 g/L/h after 10 hours of reaction. **Conclusion:** A hydrothermal pretreatment is beneficial in the processing of this material enabling an enzymatic hydrolysis of it to produce fermentable sugars. With the potential to further process this biomass, Switchgrass is positioned as a promising material for the production of biofuels.

Key words: Hydrothermal Pretreatment, Enzymatic Hydrolysis, *Panicum virgatum*



Bioenergy & Biorefinery

Evaluation of *S. platensis* growth in a pilot-scale open photobioreactor to promote biocompounds of interest

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Introduction: *S. platensis*, a nutrient-rich microalgae recognized as a superfood and it is cultivated using the Open Raceway Ponds (ORP) system which accounts for 95% of the worldwide microalgae biomass production for its cost-effective cultivation mode and an easily scale-up photobioreactor. The objective was to scale-up a microalgae cultivation to 140 L and to characterize its biomass to quantify biocompounds of interest **Methodology:** A 140 L cultivation of *S. platensis* is performed in a 300 L glass fiber open raceway pond using a modified version of Zarrouk media of half of its concentration. The photobioreactor was run indoors in the months of April and May 2024 and used submersible and non-submersible LED light to provide for proper illumination [200-500 $\mu\text{mol}/(\text{s}\cdot\text{m}^2)$]. Samples to measure biomass growth are taken every three days and it is determined using a standard curve of optical density and dry weight; conductivity and dissolved oxygen are also measured. Biomass characterization was performed using a sequential methodology of disruption of wall cell and quantification of proteins by Bradford technique, total sugars by Anthrone method, and lipids by phospho-vainillin reagent. **Results:** At the end of the cultivation, the system showed a suitable biomass productivity of 0.01 g/(L*day) and biomass concentration of 0.45 g/L, with a constant pH of 9.6 ± 0.2 which is ideal for *S. platensis*. Regarding biomass characterization the results gave a quantification of 0.39 ± 0.2 , 0.36 ± 0.01 , and 0.09 ± 0.02 (g/g) of proteins, total sugars, and lipids, respectively. **Conclusion:** ORP photobioreactors are a more operational friendly scale-up system due to their simple operation and ability to produce biomass effectively. This biomass has the potential to enhance the production of desired biomolecules, including a high-quality protein content.

Keywords: Biorefinery, aquatic biomass, protein content, open cultivation systems



Bioenergy & Biorefinery

Xylooligosaccharides derived from hemicellulose by fractionation of sugarcane bagasse using hydrothermal processing.

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Introduction: Xylooligosaccharides (XOS) derived from hemicellulose are high-added value oligomers considered an emerging class of prebiotics, with benefits such as reducing blood cholesterol and enhancing nutrient absorption. XOS are extracted from the lignocellulosic materials (LCM), such as sugarcane bagasse (SB), through hydrothermal pretreatment, resulting in the depolymerization of hemicellulose in the SB and the subsequent production of XOS. The objective of this project is to study the kinetic models of degradation of the liquid fraction rich in hemicellulose to obtain XOS from SB and its application on a food matrix. **Methodology:** The methodology focused on: 1) Chemical characterization of SB, 2) The kinetics of depolymerization of SB was carried out by means of a hydrothermal process. The operating conditions of time and temperature are 0-60 min at 150°C and pressure of 4.4 kg/cm². Chemical characterization was performed by quantitative acid hydrolysis. For the liquid fraction, rich in hemicellulose, two samples were taken to measure the number of oligomers recovered. In this case, posthydrolysis was carried out. The severity factor [$\log_{10} (R_0)$] was calculated by integrating the heating ramps, isotherms (residence time dependent), and cooling ramps of each hydrothermal pretreatment. **Results:** Phase first reported a concentration by weight of cellulose 25.51%(w/w), hemicellulose 11.10%(w/w) and lignin 25.40% (w/w). For the second phase, 1) The severity factor ($[\log_{10} (R_0)]$), reporting a $[\log_{10} (R_0)] = 3.07$ at 0 min of processing up to $[\log_{10} (R_0)] = 4.43$ at 60 min, the increase in $[\log_{10} (R_0)]$ increase with processing time. 2) Heating rate, the HP had the same behavior, with an average of 15.2°C/min. 3) pH, a pH=4.36 was reported at 0 min of processing and a pH=3.82 at 60 min, the acidification of the medium is a function of the processing time. 4)% solids yield, maximum solids yield of 82.1%. 5) XOS concentration, at 150°C and 60 min of processing, an XOS concentration of 9.2 g/L was determined. **Conclusion:** with the strategic HP conditions, increasing the processing time, a greater depolymerization of the hemicellulose present in the SB is obtained, being a sustainable alternative for the extraction of XOS.

Keywords: Biorefinery, Pretreatment, Lignocellulosic material, Autohydrolysis, Severity factor.

Bioenergy and Biorefinery

Impact of autohydrolysis processing on sugarcane bagasse

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Introduction: The objective of this study was to characterize sugarcane bagasse (SCB) biomass and evaluate the impact of hydrothermal pretreatment on parameters such as severity factor, solid yield, pH, and heating rate under various hydrothermal conditions. Hydrothermal pretreatment (HP) also called subcritical water treatment or autohydrolysis is an eco-friendly method that utilizes elevated temperatures ranging from 150-230°C for 10-50 minutes and pressures (4.9-20 bars), to maintain water in liquid state. **Methodology:** The biomass characterization involved treating 0.5 g of 0.3 mm SCB with 5 mL of 72% (w/w) H₂SO₄, stirring for 1 hour at 30°C. The mixture was then transferred to a Schott bottle, and the volume was adjusted to 148.67 mL. The flask was autoclaved for 1 hour at 121°C to achieve complete hydrolysis of oligomers. The mixture was filtered, with the residue quantified as Klason lignin and the filtrate analyzed via HPLC to determine cellulose and hemicellulose content. Using a central composite design, Hydrothermal processing was conducted using 160 mL stainless steel reactors with PID temperature control under various conditions (170°C, 180°C, 190°C for 30 min, 40 min, 50 min) with a solid-to-liquid ratio of 1:10 (g : mL). After the hydrothermal pretreatment, the samples were subjected to acid hydrolysis for the characterization of the lignocellulosic contents. **Results:** The chemical analysis of untreated biomass was 33.13 ± 0.30 % cellulose, 15.13 ± 0.08 % hemicellulose and 28.60 ± 2.23 % lignin content. After the hydrothermal pretreatment, the hemicellulose content reduced drastically as the intensity of the treatment increased: 11.605 ± 0.02 % of 170°C/30 min to 0.626 ± 0.025 % of 190°C/50 min. The condition of 190°C/40 min resulted in the highest severity factor of 4.48, indicating prolonged retention time and higher temperature. The 170°C/30 min condition exhibited the highest heating rate (12.88°C/min), solid yield (69.7%), and a pH of 3.97, attributed to the lower temperature and shorter reaction time. **Conclusion:** Overall, the chemical characterization of sugarcane bagasse confirms its significant cellulose content, which is expected to increase with further treatments.

Keywords: Agro-industrial waste, biorefinery, hydrothermal pretreatment, subcritical water.



Bioenergy & Biorefinery

Endemic microalgae from the regions of Coahuila for use within the biorefinery concept

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Introduction: Microalgae species from Mexico are proposed as a new and natural source of high value-added compounds important for industries such as food, feed and energetic, as well as in applications in colour technology. The objective of this work was to find Mexico's semi-desert region microalgae for its application under a biorefinery concept using alternatives cultures for their cultivation. **Methodology:** The isolated microalgae were identified and grown in the laboratory. For the above were used synthetic mediums for microalgae specie *Oscillatoria Syn. Limnothrix Redekrei* as cyanobacteria; and for genus *Pseudochlorella* and *Chlorella* as chlorophytes. After that, the *Oscillatoria Redekrei* was chosen due its major yields to evaluate its growth in a 40% wet weight of Extract Rice Husk (ERH40) as alternative organic culture. Also, the microalgal biomass was characterized in protein content and was extracted the phycocyanin by ohmic heating. **Results:** Biomass obtained; 1.45 ± 0.10 g/L for *Pseudochlorella*, 1.28 ± 0.07 g/L for *Chlorella sp.*, and, 2.55 ± 0.74 g/L for *Oscillatoria*. The production obtained for high value-added compounds from biomass for *Chlorella sp.*, *Pseudochlorella*, and *Oscillatoria* in grams per grams of biomass was: 0.39 ± 0.02 , 0.40 ± 0.02 , and 0.17 ± 0.02 respectively for lipids. 0.41 ± 0.03 , 0.33 ± 0.03 , and 0.16 ± 0.01 respectively for carbohydrates. 0.14 ± 0.03 , 0.14 ± 0.01 , and 0.23 ± 0.01 respectively for protein. For total carotenoids were 0.97 ± 0.02 , 0.60 ± 0.16 , and 0.51 ± 0.02 mg/g respectively. Finally, for phycocyanin were obtained 0.09 ± 0.01 for *Oscillatoria* and for chlorophytes effectively it was not obtained as say in the literature. High value compounds obtained were comparable with commercial species such as *Spirulina Platensis*. The microalgae had growth in ERH40 obtaining 0.66 ± 0.08 grams of protein and 0.03 ± 0.05 grams of phycocyanin for each gram of biomass. The Rice Husk has 32.48 ± 1.63 % of lignin, 38.26 ± 1.30 % of cellulose, 8.12 ± 0.58 % of hemicellulose and 4.02 ± 0.31 % of extractives, this last includes lipids, proteins, resins, tannins and essential oils and 17.12 ± 0.38 % of minerals, this last containing 15.63 ± 0.06 % of Si, 1.24 ± 0.02 % of K and 0.95 ± 0.04 % of Mg between other elements in less proportion (% in dry wight). **Conclusions:** Microalgae from Coahuila contains high value-added compounds of great industrial interest and in a combination of second and third biorefinery generation, rice husk contains the nutrients necessary for microalgae production, therefore, it can be proposed as an alternative culture to replace synthetic media. **Keywords:** Sustainability, Algae, Biomass, rice husk, ohmic heating.

Evaluation of alternative medium for microalgae growth

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Introduction: Because of the high-value compounds found in their cellular structure, *Chlorella* sp. is being considered as a model microalgae for human consumption. These microorganisms can grow in a variety of media, and some studies on rice husk extract have been used to support their growth to approach the residues generated in the agroindustry. **Methodology:** The raw material was subjected to moisture, ashes, solvent extractives (water and ethanol) and elemental composition using an X-ray fluorescence analysis. Additionally, the content of the main polysaccharides and Klason lignin was determined according to the standard analytical procedures. For the kinetic growth of *Chlorella* sp., the essays were carried out in 250 mL with 10% w/v of inoculum in mixotrophic mode at 27°C – 29°C under white light with an intensity of 82.89 $\mu\text{mol m}^{-2}\text{s}^{-1}$, evaluating rice husk extract and synthetic medium of MiEB12, measuring the optical density at 415nm with a microplate reader and pH. **Results:** The chemical composition of rice husk obtained (% total dry weight) was, moisture content 10.90%. The most present components were cellulose with 38.12 ± 1.72 , hemicellulose with 5.80 ± 1.06 , and lignin Klason, 47.44 ± 3.6 , ashes 19.33 ± 0.58 and extractives 4.55 ± 0.4 . The trace metals related to microalgae growth, were found in rice husk as Si^+ $85.95 \pm 0.36\%$, Mg^+ $5.03 \pm 0.24\%$, K^+ $4.80 \pm 0.38\%$, Ca^{2+} $1.58 \pm 0.19\%$. The kinetic growth rate obtained in MiEB12 medium after 16 days of cultivation was $0.12 \pm 0.012 \text{ day}^{-1}$, meanwhile in rice husk extract after 6 days of cultivation was $0.16 \pm 0.01 \text{ day}^{-1}$. The kinetic growth of *Chlorella* sp. showed a good adaptation in organic medium, in which it can use as an alternative to growth in replaced synthetic medium such as MiEB12, and the integration of an agroindustrial waste to reach a circular economy can be implemented.

Key words: Agroindustrial waste, circular bioeconomy, quality, rice husk extract

Bioenergy and Biorefinery

Blasting extrusion pretreatment of sweet sorghum bagasse for saccharification yield enhancement and fermentation using *Pichia kudriavzevii* for ethanol production

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Blasting extrusion pretreatment (BEP) was used for obtaining sugar enriched saccharified extracts from sweet sorghum bagasse (SSB) for ethanol fermentation using a x yeast. A factorial design was used for studying the effect of last barrel zone temperature (TLBZ) and the screw configuration (SC) on sugar release and sugar extraction yield, the experiment with the higher glucose release was selected for a latter fermentation using *Pichia kudriavzevii* for ethanol production. The best results were obtained in experiment 1 (E1) when using a BEP with a TLBZ of 190°C and a screw configuration composed of one polygon element and a shear zone composed of seven reverse elements. E1 showed the highest total sugar, glucose, xylose, and mannose/arabinose release after enzymatic hydrolysis using the enzymes Cellic® CTec2 and Cellic® HTec2, dosed at 5.4% and 0.6% of the total amount of cellulose. The total sugars and glucose released were increased by 3.5 and 3.1 folds when compared to the unextruded SSB control. These results also represent a 11.46% and 10.27% increase in total sugar and glucose release when comparted to traditional twin extrusion technology. Enzymatic hydrolysis product was adequate for fermentation with *Pichia kudriavzevii*. BEP with the correct screw configuration enhanced the bioconversion efficiency SSB into bioethanol.

Key words: lignocellulosic matrix, bioethanol, sugar extraction, thermos resistant yeast, bioenergy

Carotenoids and bioenergy co-products extraction from mixed microalgae culture

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Introduction: Microalgae have shown great promise for biofuels generation, but their utilization for energy production is still unprofitable due to high initial investments. However, the biorefinery model offers a viable strategy that maximizes biomass value by yielding several products, including biofuels and high-value products. This work aimed to produce microalgae biomass for the extraction of carotenoids and analyze the remaining biomass for bioenergetic purposes.

Methodology: Microalgae biomass corresponds to a mixed culture of *Chlorella* sp. and *Scenedesmus* sp. The mixed culture was grown for 14 days using 1 mL L⁻¹ Bayfolan® Forte fertilizer as the culture medium. The microalgae biomass was then subjected to carotenoid extraction using supercritical fluids under 20 and 30 MPa and three temperature conditions (40, 50, and 60 °C). β -carotene identification was carried out by high-performance liquid chromatography (HPLC). The biomass before extraction (BBE) and after extraction (BAE) of carotenoids underwent lipid extraction using the Bligh and Dyer method and hydrothermal liquefaction (HTL) for biocrude production. **Results:** The increase in temperature up to 60 °C causes an increase in β -carotene extraction yields. These results suggest that β -carotene is extracted in greater proportion at 60 °C and 30 MPa. BBE exhibited a higher lipid and biocrude proportion, at 12.9% and 19.2%, respectively, while BAE at 30 MPa showed a diminished lipid content of 6.7% and 13.9% biocrude yield. **Conclusion:** The use of supercritical CO₂ and ethanol as a cosolvent allowed the extraction of carotenoids. The overall performance of successive extractions at 30 MPa with different temperatures was better compared to the process at 20 MPa. When carotenoids are extracted, a portion of lipids is also extracted, which impacts the production of biocrude. Given the importance of carotenoids as high-value products, experiments are ongoing to optimize processes for carotenoids, lipids, and biocrude extraction.

Keywords: Microalgae, Biorefinery, carotenoids, lipids, biocrude.

Bioenergy & Biorefinery

Enzymatic Hydrolysis using *Populous Grandidentata* as a Biomass to Produce Fermentable Sugars.

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Introduction: Biorefineries emerge in order to find an alternative to fossil fuels due to is a polluting and finite source. A biorefinery integrates processes that helps with the conversion of lignocellulosic biomass in multiple high value-added products such as bioethanol. Biofuels are divided in 3 groups: first generation use crops and food as raw material, second generation use agro-industrial waste and third generation biomass is macro and micro algae. This study evaluates the production of fermentable sugars from enzymatic hydrolysis process at different pretreatment conditions, using *Populous grandidentata* as biomass which is a native tree from north-central and northeastern United States and southeastern of Canada. **Methodology:** Initially a characterization of *Populous grandidentata* following the quantitative acid hydrolysis method was made to find out if the biomass has a viable content of cellulose for bioethanol production. After that five pretreatments were carried out of a different operating conditions using a high-pressure reactor, these being: 150°C for 10 min, 150°C for 25 min, 150°C for 40 min, 180°C for 10 min and 180°C for 25 min in order to evaluate their feasibility in the production of fermentable sugars. A characterization of the pretreatments was made in order to obtain the highest operation conditions results of cellulose percentage, more pretreated biomass was obtained from these pretreatment to finally perform the hydrolysis adding cellulase enzymes in 3 flasks in constant agitation for 72 h, taking samples at certain times defined by the methodology and analyzed by HPLC chromatography. **Results:** The characterization of the biomass throw a percentage of 48.71 of cellulose that allowed to realize five pretreatments resulting in a higher percentage of cellulose under the conditions of 180°C for 10 min, being 60.91±4. The result in the enzymatic hydrolysis was 6.141 g/L of glucose in the sample and a productivity of 0.14 g/Lh after 24 hours. **Conclusion:** *Populous grandidentata* is considered as a promising second generation lignocellulosic material to produce fermentable sugars. Through enzymatic hydrolysis of pretreated material, good sugar yields can be obtained and the chemical reaction rate can determined the most viable times in wich said reaction occurs.

Keywords: Bioethanol, Enzymatic Hydrolysis, *Populous grandidentata*.



4th **BIO IBEROAMERICA**

IBERO-AMERICAN CONGRESS
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BIOENGINEERING & BIOPROCESSES



Bioengineering & Bioprocesses

New methodologies for the characterization of materials from bioengineering and/or bioprocesses.

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This section contains information related to the advances in microscopic techniques for characterizing materials that have been generated by bioprocesses and/or bioengineering. Current microscopic techniques contribute to the complete understanding of chemical reactions, phase transformations and reaction mechanisms. The best way to use them for the study of different materials is in a synchronized and orderly manner. Digital microscopy is presented as an excellent option for the exploration of surfaces, as well as the different textures in samples (it also includes innovations such as image analyzers and also a roughness tester, both in 3D). In ultra-high-resolution electron microscopy (Scanning and Transmission) the analyses are explored with detectors such as the gentle beam (scans at 1 mm from the surface and with voltages as low as 0.1KeV) and STEM modes. Finally, the best options in the use of dual beam microscopy and its contribution in these areas will be reviewed.

Key words: Digital Microscopy, Stereography, Characterization in Bioprocesses, Characterization in Bioengineering, Ultra High Resolution Microscopy.



Bioengineering & Bioprocesses

Effect of the absence of PhbP2 and PhbP3 phasins in the bacterial growth, production and molecular weight of P3HB in *A. vinelandii*

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Introduction: *Azotobacter vinelandii* is a bacteria with a high capacity to synthesize intracellularly the bioplastic poly-3-hydroxybutyrate (P3HB). P3HB granules are surrounded by different proteins, some of them, the phasins, which carry out activities related to the metabolism of P3HB. The aim of this study was to evaluate the effect of the absence of phasins PhbP2 (OP-phbP2⁻ strain) and PhbP3 (OP-phbP3⁻ strain), and OP strain, as control, on the bacterial growth, production and molecular mass (Mw) of P3HB, in cultures under low and high oxygen transfer rates (OTR). **Methodology:** Batch cultures were carried out in a 3 L bioreactor, using PYS medium (peptone, yeast extract and sucrose), T= 29°C, 1 vvm and pH=7.2 under two OTR conditions (8 and 3 mmol L⁻¹ h⁻¹) during 72 h. **Results:** Under high OTR_{max} conditions (8.6 ± 0.6 mmol L⁻¹ h⁻¹), the absence of phasins PhbP2 and PhbP3 resulted in a strong negative effect on the specific growth rate. Interestingly, this behavior was not observed under conditions at low oxygen transfer rate (3.9 ± 0.71 mmol L⁻¹ h⁻¹); where both growth and oxygen consumption were the same in the different strains evaluated. This behavior could be associated with the role of PhbP2 and PhbP3 phasins, as chaperone proteins that protect the bacteria against oxidative stress under conditions of high OTR. It was observed that the absence of phasin PhbP3 harms the P3HB synthesis, finding a decrease of 19% in the polymer production at the end of cultivation; however, the molecular weight of the polymer remains constant. This behavior is related with the absence of depolymerases activity in the culture with OP-phbP3⁻ strain. **Conclusion:** It can be concluded that both growth and polymer synthesis were affected when phasin PhbP2 and PhbP3 were absent from the P3HB granule,

Key words: Phasins, *A. vinelandii*, bacterial growth, P3HB.



Bioengineering & Bioprocesses

Study of medium composition for laccase production by yeast surface display

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Introduction: Laccase is a multicopper oxidase enzyme with diverse biotechnological applications. Yeast surface display is a powerful tool for protein engineering, allowing the expression and immobilization of heterologous proteins on the cell surface (1). A drawback of this system is the low expression of the enzyme on the display system, which prompted the improvement of the culture medium. **Materials and Methods:** Yeast strain EBY100 *S. cerevisiae* expressing laccase TK14-OB1 was grown in a selective medium and a laccase expression medium in 1000 mL flasks containing 200 mL medium at 30°C and 150 rpm. The effect of TRPE expression medium composition (galactose, casein peptone, yeast extract, and copper sulfate) on the production of laccase TK14-OB1 was evaluated applying a rational approach involving a central composite design and statistical analysis. Laccase activity was measured using a spectrophotometer with ABTS as substrate and gravimetrically measured biomass concentration. **Results and Discussion:** A matrix of 30 different treatments was obtained to evaluate the concentrations of the expression medium components. The results indicate that laccase production is influenced by the concentration of the medium components. The optimized culture medium contains a lower concentration of galactose and a higher concentration of casein peptone and yeast extract. With the optimized medium, laccase expression was increased by 5000 U/g compared to the control medium, resulting in a 30% reduction in production cost. **Conclusions:** In this work, we have shown that for laccase expression in the yeast surface display system, the composition of the expression medium must be considered to achieve higher enzyme expression and reduce production costs by using a lower galactose concentration.

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Key words: Laccase, heterologous expression, optimization.

Physicochemical Characterization of Creosote Bush (*Larrea tridentata*)

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Introduction: Creosote bush (*Larrea tridentata*) is a shrub distributed over nearly 19 Mha in the arid zones of central and northern Mexico and the southern United States. This plant can grow in unfavourable climatic conditions. Traditionally, this plant has been used to make infusions to treat various conditions because it contains several bioactive molecules such as NDGA, quercetin, and kaempferol, among others. These molecules have antioxidant, anti-inflammatory, antiproliferative, and antimicrobial activities. **Methodology:** The physicochemical analysis of the plant material was carried out according to the methodology reported by Cerda-Cejudo *et al.*, 2022. The proximal analysis of plant material was performed following the methodologies defined by the AOAC for the quantification of fat, fiber, protein, and ash. Total sugars were determined using the Anthrone method. **Results:** According to the physical analysis, the creosote bush presented a water absorption capacity of 3.62 ± 0.02 %, a moisture of 5.00 ± 0.01 % and supports a maximum moisture value of 73.76 ± 0.01 %. The proximal analysis revealed that the creosote bush has a fat content of 5.11 ± 0.08 %, a fiber content of 16.82 ± 3.47 %, a protein content of 1.28 ± 0.00 %, a total sugar content of 37.06 ± 0.09 %, and finally an ash content of 9.41 ± 0.087 %. **Conclusions:** The physicochemical characterization is a preliminary determination of highest importance when proposing the use of a plant as a support for a bioprocess such as solid-state fermentation, since with this information it is possible to plan and verify the conditions under which the bioprocess can be carried out.

Keywords: Bioactive molecules, NDGA, quercetin, sugars, protein, fiber.



Agricultural & Food Biotechnology

BioIberoamerica is a biannual event that strengthens the bonds between Latin America and the Iberian Peninsula and the international forum most important in this region.

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Introduction: BioIberoamérica 2024 will take place from 3-6 September 2024 in Monterrey, one of the most dynamic cities in the world, located in the northeast of Mexico, a region bordering the USA, characterized by a dynamic strategy for economics, social development, innovative, with educative institutions recognized globally and a great biotechnological sector including the main biotech cluster in Mexico. The main objective is to get together all the scientific community interested in biotechnological developments, including outstanding scientific leaders. **Methodology:** The event proposes a broad program of activities with oral presentations, poster sessions, round tables, workshops, and parallel technical sessions, to bring together experts to promote the exchange of experiences among the different actors of the biotechnology sector, to identify the demands of research, innovation and technology transfe. **Results:** Bioiberoamerica has become one of the leading biotech events in Iberoamerica, being the perfect setting for forging new partnerships, scientific and academic collaboration, and strengthening existing business relationships in the biotechnological global sector. **Conclusion:** BioIberoamerica 2024 will bring together more than 500 participants working in health, sustainable agri-food, and climate change solutions and biotechnological innovations. The event is attended by professionals, students, exhibitors, and investors.

Keywords: BioIberoamerica, Mexico, biotechnology.



Bioengineering & Bioprocesses

Isolation and molecular identification of fatty acids-producing microorganisms

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Introduction: Fatty acids are essential for brain development and the prevention of various diseases in humans; they are primarily obtained from the diet, especially from animal-derived foods like fish. However, many people avoid these foods due to being vegetarian, vegan, or personal preference. Additionally, overfishing poses a risk to biodiversity. A promising alternative for obtaining fatty acids is oleaginous microorganisms, such as *Mortierella* spp, *Rhodotorula glutinis*, and *Penicillium* sp, among others. This study focused on *Mortierella alpina* due to its high production of long-chain fatty acids, including arachidonic acid. **Methodology:** To isolate this fungus, soil samples were taken from apple plantations in Arteaga, Coahuila, Mexico and isolated in PDA media through serial dilution. Strains were discarded until one with morphological characteristics similar to *Mortierella alpina* was found. Micro and macroscopic identification was performed, using lactophenol blue staining for microscopic analysis. Fermentation was then conducted at 28 °C and 120 rpm for seven days to recover biomass, followed by lipid extraction using the Bligh & Dyer (1959) method. Molecular identification was performed by extracting DNA using the enzymatic method (thin-prep) and utilizing the ITS4 and ITS5 regions for PCR. Sequencing was done using the Sanger method and compared in the NCBI database using the Megablast algorithm. **Results:** Comparison in the database confirmed that the extracted DNA corresponded to *Mortierella alpina* with 100% similarity, which present a yield of lipids 87%, and fatty acids 43% of the dry biomass. **Conclusion:** The new isolated strain of *Mortierella alpina* proved to be an efficient producer of fatty acids, with a production greater than 20%, hence necessary to identify the fatty acids produced for future application.

Key words: *Mortierella alpina*, fatty acids, lipid extraction

Production and purification of a protein fragment A from recombinant *E. coli* as a potential ligand for the purification of monoclonal antibodies

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Introduction: Both production and purification of monoclonal antibodies (mAb) have crucially become important in medical treatments, so demand is high. Protein A affinity chromatography has been highlighted as a key technology for this type of purification. However, the molar mass of the most used ligand, protein A, limits the adsorption capacity of the stationary phase. Thus, giving the opportunity to pursue synthesis of smaller fragments with the help of genetic engineering, while retaining the affinity for mAbs. **Methodology:** a previously genetic engineered *E. coli* system capable of excreting a 15 kDa fragment was used to optimize the production of this fragment. Different compositions of culture medium, as well as different conditions of induction by arabinose, were explored. Kinetic characterization of the system was also performed. After selecting the culture medium, where the microorganism exhibited a better growth and the expression of the protein A, evidence in SDS-Page, was more notorious, the next step was to produce the fragment in a batch bioreactor, in a 3 L Biostat® B jacketed fermenter (Sartorius Stedim Biotech), used at 50% of its capacity and a double Rushton impeller. **Results:** The optimization and characterization of the fragmentation process for the recombinant strain of *E. coli* producing the 15 kDa protein A fragment was achieved, and the growth kinetics consumption agree with literature reports, indicating that the mineral medium, culture conditions and induction conditions favor a correct expression of the protein A fragment and do not compromise the integrity of the biological system. From the SDS-PAGE gels, it can be assured that induction is taking place and that separation is possible either by direct injection or by pre-processing. On the other hand, it was possible to print polypropylene devices and synthesize GMA-EDMA monoliths inside them, further binding of protein A fragment is needed to evaluate the capacity of its affinity chromatographic monolith in the separation of mAbs, as well as its comparison with commercial membranes, like Sartobind Rapid A membrane characterized previously. **Conclusion:** The study demonstrates the successful optimization of a genetically engineered *E. coli* system to produce a 15 kDa protein A fragment. By refining culture media and induction conditions, researchers achieved effective protein expression, as confirmed by SDS-PAGE. Additionally, the synthesis of GMA-EDMA monoliths for affinity chromatography indicates a promising direction for enhancing monoclonal antibody purification, highlighting the potential of genetic engineering in meeting the growing demand for therapeutic mAbs.

Key words: Protein A, purification, induction, production, batch bioreactor, monoclonal antibody



Bioengineering & Bioprocesses

Production of a recombinant protein A fragment as a potential ligand for affinity chromatography

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Introduction: Production of monoclonal antibodies (mAb) is crucial in protein therapeutics development. Protein A affinity chromatography is a key technology for this process. However, the molar mass of the most used ligand, protein A, limits the adsorption capacity of the stationary phase. Synthesis of small fragments maintaining affinity for mAbs with the aid of genetic engineering, as well its production is of prime importance. **Methodology:** a genetic engineered *E. coli* system capable of excreting a 15 kDa fragment was generated. The strain was evaluated for production of this fragment in a batch bioreactor (3 L Biostat® B, Sartorius) under different culture conditions by using arabinose as inductor. **Results:** The engineered *E. coli* was able to produce and excrete a 15 kDa small fragment with bioactivity as demonstrated by western blot. Maximal production of the fragment was achieved after 6 h of cultivation when using arabinose as inducer and glucose (6 g/L) as carbon source. SDS-PAGE revealed a 15 kDa band indicating production of the fragment and a straightforward separation of the fragment. **Conclusion:** Genetically engineered *E. coli* designed produced and excreted a 15 kDa protein A fragment. By refining culture media and induction conditions, increased protein expression was attained. This approach might open a window to produce novel biomolecules with promising application in production of mAbs.

Key words: Protein A, purification, induction, production, batch bioreactor, monoclonal antibody



Bioengineering & Bioprocesses

Intracellular metabolomic profile of microalgae *Scenedesmus* sp. under biogas atmosphere

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The environmental crisis caused by the use of fossil fuels has led humans to look for alternatives like natural fuels, such as biogas. Biogas is a product of anaerobic digestion of organic waste, which can be generated naturally or as a derived from some agro-industrial processes. Biogas due to its composition (75% CH₄ and 25% CO₂) requires a purification process to remove the CO₂ content and thereby increase the heat capacity conferred by CH₄. We propose the use of photosynthetic microorganisms such as the green microalgae *Scenedesmus* sp. endemic of Chapala Lake, Jalisco, México for this bioremediation process. However, the high concentrations of CO₂ might generate a stressful environment for the microalgae, thus by a metabolomics analysis of nuclear magnetic resonance proton experiments (¹H NMR) we propose to evaluate the intracellular metabolomic profile of *Scenedesmus* sp. under biogas (treatment) and air (control) conditions, and so analyze the effect of biogas on the microalgae metabolism, and thus propose an optimal biologic method for CO₂ removal from biogas. It was shown that biogas conditions stimulate the growth of the microalgae positively, such as the production of metabolites like carbohydrates, lipids, proteins and pigments. Likewise, metabolites like glycine, xylose, adenine and thymine were identified as possible markers for adaptation of the culture conditions of *Scenedesmus* sp. under a biogas atmosphere. This suggests to us that the biological method of removal of CO₂ from biogas, by the use of green microalgae from the genera *Scenedesmus* is efficient, also obtaining biomass rich in nutrients for its potential biotechnology applications on different fields.

Key words: Metabolomics, Microalgae, Biogas



Bioengineering & Bioprocess

Valorization of Mexican rambutan peel through the recovery of ellagic acid by solid-state fermentation using a yeast

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Introduction: Rambutan (*Nephelium lappaceum* L.) is a tropical fruit which is original from South East Asia and it was introduced to Mexico in the 60's, the fruit's peel is known to possess ellagitannins such as ellagic acid which confer the peel of great biological activity, solid state fermentation has been used to obtain said compounds and rambutan peel can be used as a fermentation support/substrate, this work aims to obtain, identify and quantify ellagic acid obtained by SSF with a strain of yeast.

Methodology: Water absorption index and the support's maximum moisture were determined. To determine the ideal conditions for ellagic acid accumulation a Box-Behnken 3^k experimental design was applied using variables such as temperature, moisture, and inoculum.

Results: The maximum accumulation time of ellagic acid by solid-state fermentation was determined to be at 48 h with ideal conditions of 30°C, 60% moisture and 1.5x10⁷ cells/g using *S.cerevisiae* and high performance liquid chromatography identified ellagic acid, geraniin, and corilagin as the most abundant compounds. The maximum recovery of ellagic acid was 458 ± 44.6 mg/g. HPLC/ESI/MS analysis at 48 h fermentation showed the biodegradation of geraniin and corilagin to ellagic acid.

Conclusion: Mexican rambutan peel has demonstrated to be a suitable substrate for SSF.

Key words: bioprocess, ellagic acid, yeast, ellagitannins.

Bioengineering & Bioprocesses

Bioprocessing of *Caesalpinia coriaria* pods to obtain bioactive phenolic compounds.

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Introduction: The cascalote tree (*Caesalpinia coriaria*) produces pods that used only for tanning and as fodder. Cascalote pods are rich in bioactive phenolic compounds, however, most of them are bound to cell wall components, which makes their extraction and recovery difficult. *Aspergillus niger* GH1 is a fungus capable of bioprocess several rich in phenolic compounds and increase their recovery. This work was aimed the bioprocessing of cascalote pods by solid-state fermentation (SSF) using *A. niger* in SSF and its effect on the release of bioactive phenolic compounds

Methodology: Cascalote pods (1>mm) were mixed with polyurethane foam (90:10 w/w ratio) and impregnated with Czapek-Dox (Moisture 50%) previously inoculated with *A. niger* GH1 (1x10⁶ spores/g of support). The wet mixture was packed in column reactors (31.5 mm x 180 mm) and incubated at 30°C for 72 h, sampling every 12 h. The extracts were recovered with 9 mL of 50 % ethanol, and the content of phenolic compounds (Folin-Ciocalteu) and antioxidant capacity (DPPH) were determined. **Results:** *A. niger* GH1 was able to grow using cascalote pods as substrate in SSF, improving the extraction and recovery of phenolic compounds, with the maximum release and antioxidant capacity at 12 h (168.63 mgGAE/gdw and 1132.63 mgTE/gdw, respectively). The use of SSF increased the total phenolic content and antioxidant capacity by 52.3 and 33.75 %, with respect to the unfermented control. **Conclusions:** SSF represents a biotechnological and sustainable alternative to increase the content of bioactive phenolic compounds in cascalote pods.

Key words: Solid-state fermentation, *A. niger*, bioprocesses, phenolic content, antioxidant capacity.

Establishing sustainable bioproduction of lactones from metabolic engineering of industrial cell factory systems: *Ashbya gossypii*

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Introduction: Lactones are volatile organic compounds derived from lipid metabolism. They have a wide range of applications, notably as fragrances and flavours, with γ -decalactone being particularly valuable. Lactones significantly contribute to the smell and taste of fruits like strawberries and peaches, but traditional extraction methods have many disadvantages. Microbial production has emerged as a more sustainable alternative but depends on the biotransformation of valuable limited hydroxylated fatty acids, each producing specific lactones¹. *Ashbya gossypii*, a filamentous fungus, naturally synthesizes γ -lactones *de novo* from carbon sources² and is a notable industrial cell factory for riboflavin (vitamin B2) production³. It can also produce phenylethyl alcohol, another valuable fragrance compound. Additionally, metabolically enhanced *A. gossypii* strains have been explored for lipid production, valorising biomass into biofuels^{4,5}. **Methodology:** In this work, *A. gossypii* strains were engineered for improved *de novo* lactone biosynthesis, focusing on fine-tuning different steps of the lactone biosynthetic pathway, namely fatty acid desaturation. A fatty acid desaturase gene from *Fragaria ananassa* (strawberry) was selected to improve this step. A versatile Golden Gate-based toolbox of integrative cassettes was developed to facilitate the generation of new strains. Process optimization involved testing various carbon sources and agro-industrial residues as substrate for lactone production. **Results:** A strain with increased expression of a native desaturase produced significantly higher γ -decalactone titres, reaching 114 ± 16 mg/L using molasses as the carbon source and corn-steep liquor as supplementation. Phenylethyl alcohol production reached 18 ± 2.6 mg/L under these conditions. Bioreactor production allowed for further optimization of production conditions. Moreover, the development and optimization of a Golden Gate-based toolbox of integrative cassettes with improved transformation efficiency accelerated strain engineering. **Conclusion:** High titres of γ -decalactone were achieved with engineered strains and precision fermentation, contributing to biorefinery and the valorisation of agro-industrial residues. The developed Golden Gate-based toolbox with improved transformation efficiency is a valuable tool for *A. gossypii* strain engineering. Insights gained into the lactone biosynthesis pathway will be beneficial for improved production and large-scale optimization.

Keywords: Metabolic engineering, Lactone, Precision fermentation, Biorefinery, Residue valorisation

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Bioengineering & Bioprocesses

Exploration of the potential of *Rhizopus oryzae* and *Saccharomyces cerevisiae* in aroma generation from blackberry and strawberry residues using solid fermentation.

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Introduction: Apocarotenoids are a type of carotenoids with a skeleton of 40 carbon atoms, some apocarotenoids such as β -ionone, β -damascenone and β -damascone are of great interest in the food and cosmetic industry so an increasing demand has been generated in the non-chemical production of these compounds. The main objective of this research is to evaluate the ability of *Rhizopus oryzae* and *Saccharomyces cerevisiae* in the synthesis of β -ionone, β -damascenone and β -damascone by solid fermentation processes using blackberry and strawberry wastes as substrate. **Methodology:** Two concentrations of the precursor β -carotene (0.1 and 0.25%), malt or yeast extract (0.50%) and two types of fruit residues (blackberry and strawberry) were used. Quantification of the results was carried out by gas chromatography coupled to mass spectrometry (GC/MS) and fermentation kinetics was performed by measuring total sugars, reducing sugars, pH, temperature and humidity. **Results:** The highest concentrations of β -damascenone and β -ionone were found in the assays performed with *Rhizopus oryzae*, with a final concentration of 1 mg/kg and 1.68 mg/kg, respectively; however, no β -damascone production was found in any of the assays performed. On the other hand, a remarkable production of the molecule β -ionone-5,6-epoxide was observed, with a final concentration of 8.72 mg/kg in the assay performed with *Saccharomyces cerevisiae* on strawberry. **Conclusions:** This study highlights the feasibility of using fruit residues to obtain aromatic molecules and the possibility of using non-genetically modified microorganisms, and suggests future research to optimize fermentation conditions and explore new strains and substrates, supporting environmentally friendly solutions in the food industry.

Key words: solid fermentation, fruit waste, microorganisms, apocarotenoids.



Bioengineering & Bioprocesses

Impact of co-substrates on the production of poly (3-hydroxybutyrate-co-3-hydroxyvalerate hydroxy valerate) by *Burkholderia thailandensis* E264

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Introduction: Conventional plastics obtained from non-renewable resources are fundamental in everyday life. Due to their lightness, versatility, and low cost, their exploitation has been promoted worldwide. Plastic products obtained from non-renewable resources have little or no biodegradability, and approximately 50% of plastics are discarded after a single use, creating a waste management problem. PHBV (poly (3-hydroxybutyrate-co-3-hydroxyvalerate) is a thermoplastic biopolymer that belongs to the family of polyhydroxyalkanoates (PHA). It is a biodegradable and biocompatible material, making it ideal for a wide range of applications, such as biodegradable packaging medical products. **Methodology:** PHBV production by *Burkholderia thailandensis* E264 (BtE264) was evaluated using levulinic acid (LA), valeric acid (VA), and sodium propionate (SP) as precursors for biopolymer synthesis. Biomass production was determined using the dry weight method, substrate consumption, and PHBV identification and quantification by chromatography using reference standards. **Results:** BtE264 produced the co-polymer PHBV when any of the three co-substrates was added to the culture media, and no co-polymer was detected in the base media (without co-substrate). The LA showed a higher positive effect on microbial growth ($8.4 \text{ g}\cdot\text{L}^{-1}$) and PHA production ($3.91 \pm 0.4 \text{ g}\cdot\text{L}^{-1}$), representing 78 y 22 mol % of 3HB and 3HV, respectively, at 120 h culture. **Conclusions:** This study has revealed that BtE264 can synthesize the PHB homopolymer and the PHBV co-polymer under the specific culture conditions examined. Notably, using LA as a co-substrate significantly positively affected the % mol, indicating its potential to enhance PHBV production. These findings underscore the importance of co-substrates, particularly LA, in biopolymer synthesis and could pave the way for further research.

Keywords: Biopolymer, co-substrates, poly (3-hydroxybutyrate-co-3-hydroxyvalerate)

Bioengineering & Bioprocess

Enhancing *C. vulgaris* biomass production via spend coffee grounds hydrolysates

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Introduction: Coffee is one of the most consumed beverages globally, yet only about 10 % of the coffee fruit is used for the beverage, resulting in substantial waste. The Spend Coffee Grounds (SCG), rich on carbohydrates, lignin, proteins, and minerals, offers a potential substrate for microalgal growth. This study evaluates the use of alkaline hydrolysates of SCG to enhance biomass production in *Chlorella vulgaris*. **Methodology:** The hydrolysate of SCG was obtained by alkaline hydrolysis with 2% w/v of NaOH and autoclaved at 121°C at 15 minutes. The preliminary experiments were done with different concentrations of the hydrolysate using a control, 0.125%, 0.25%, 0.5%, 1%, 2% and 4% of the hydrolysate with Basal Bold Medium to determine the better concentration of hydrolysate SCG. The kinetics of the different treatments of Glucose at 1% w/v, Hydrolysate of SCG at 1% v/v and Basal Bold Medium were growth under 175 rpm on orbital agitation, the cell count and the photosynthetic compounds were evaluated daily after the inoculation until 7 days with 2 different photoperiod (Light: Dark): 12:12 and 18:6. **Results:** Preliminary experiments indicate a highly significant increment of the cell production at 2% SCG hydrolysate concentration (p -value<0.01), The kinetic data shows a low growth of *Chlorella vulgaris* in SCG Hydrolysates and exponential growth in Glucose. **Conclusion:** The results confirm the possible use of Hydrolysate SCG like nutrients source to *Chlorella vulgaris*, but it needs more experimentation to confirm the possibility to use in Pilot- Scale production.

Key words: Spend Coffee Grounds, *Chlorella vulgaris*, hydrolysate, biomass production.

Sustainable technology for protein extraction from *Tenebrio molitor* flour

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Introduction: Regarding the Earth's population growth rate, it is evident that the demand for protein will continue to increase, leading to impacts in terms of sustainability across many sectors. Due to this, it is urgent to find efficient alternative protein sources, where insects such as *Tenebrio molitor* (yellow mealworm) are prominent candidates. *T. molitor* is highly rich in proteins and other nutrients when compared to other usual sources of proteins (poultry and cattle). Another advantage is the low environmental impact and economic costs of production and maintenance, which are crucial to comply with the global protein needs. A concern when using insects as food is consumer acceptability, which in the West is still a hurdle. One way to overcome this concern is by carrying out protein extraction and formulating new food products with higher tolerance by the end user.

Methodology: Design of experiments (DOE) was applied to optimize the extraction operating conditions, namely temperature, time of extraction, and salt concentration in aqueous solution. The solvent was mixed with the yellow mealworm flour, the solution was centrifuged, and the supernatant was collected for further analysis of the extracted proteins. After this, isoelectric precipitation was applied to recover the proteins in solution, followed by centrifugation, and freezing the pellet. Then, the samples were to be freeze-dried to obtain a pure dried protein powder. Protein in solution was quantified by the BCA method, whereas the precipitated protein was determined by weight. In the extracted samples, the total protein profile was analysed by SDS-PAGE. **Results:** The best results were achieved with an extraction yield in solution ranging between 44 and 49 g of protein per 100 g of insect flour. In the final protein powder form, the best results correspond to 25 g of protein per 100 g of insect flour. **Conclusion:** From the obtained results, the possibility of integrating insects' protein into new food products is a promising strategy. Scaling-up tests are being carried out to address the technology potential at the industrial level.

Key words: Insects; Protein extraction, Sustainability, Food products.

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Phenolic content and antioxidant capacity from pistachio green hull subjected to solid-state fermentation.

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Introduction: The pistachio green hull (PGH) is a by-product of the pistachio production, that comprises the 60% of the total production. In the PGH there are different phenolic compounds with antioxidant capacity, located free or bound form to the PGH cell wall. These bound compounds can be released through solid-state fermentation (SSF), using filamentous fungi that produce extracellular enzymes, which hydrolyze the bonds to these compounds and thus release them of the cell wall components. This work was aimed to evaluate the time of the higher release of phenolic content and antioxidant capacity from fermented PGH. **Methodology:** For the SSF process, the PGH was impregnated with Czapek Dox mineral medium previously inoculated with *Aspergillus niger* GH1 strain (1×10^6 spores/gdm) and the moisture content was adjusted to 50%. Seven gwm were packed in reactors (4 x 4 x 4 cm) and were incubated at 30 °C for 96 h. Every 12 h, the content of hydrolysable phenols (HP) and condensed phenols (CP), as well as antioxidant capacity by DPPH, ABTS and FRAP methods was determined. **Results:** An increase of 15.7% in HP and 30.3% in CP was obtained after of 24 h of fermentation compared to control, with quantities of 18 mg EAG/gdm and 6.2 mg EC/gdm for HP and CP, respectively. For the antioxidant capacity, the results show increases of 16.5%, 3.7% y 31.2% by ABTS, DPPH y FRAP, respectively, corresponding to quantities of 87.6 mg ETrolox/gdm, 74.6 mg ETrolox/gdm y 58.6 mgFe⁺²/gdm, respectively. **Conclusion:** The highest antioxidant capacity and total phenolic content occurred at 24 h, it shows the effectiveness of the FES to increase recovery of phenolic compounds from PGH, it allows an alternative of valorization for this waste.

Key words: Solid-state fermentation, pistachio green hull, *Aspergillus niger*, phenolic compounds, antioxidant capacity.

Economical production of *Pichia pastoris* single cell protein from glycerine at pilot scale.

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Introduction: Glycerine synthesized from fat hydrolysis is a potentially sustainable resource for biomanufacturing. The use of glycerine as a raw material to produce single-cell protein (SCP) is an alternative to alleviate the high global demand for proteins of animal origin. The yeast *Pichia pastoris* is an ideal host for the synthesis of glycerine-based SCP. However, improving glycerine utilization, protein content of *P. pastoris* are also current challenges, which are of great significance for the economical industrial application using glycerine as raw material for SCP production. **Methodology:** The production of SCP with *P. pastoris* was carried out using glycerine, in a fed-batch system with basal culture medium, in a 23 L stirred tank Applikon bioreactor. The bioprocess was controlled and verified at; 600 rpm, 1 vvm, pH 5 for 7 days at 30°C. For determination of total protein and biomass, was used Bradford method. The centrifuged cells were dried at 105 °C until a constant weight was maintained. Total nitrogen content was measured using the Kjeldahl method. The analysis of the amino acid profile was performed through an automatic amino acid analyzer. The total protein, nitrogen and amino acid content are related to the dry yeast mass (%). **Results:** Finally, the engineered strain produced high levels of SCP from glycerine in a pilot-scale fed-batch culture at 30 °C with a biomass of 153.57 g DCW/L, glycerine conversion rate of 0.73 g DCW/g, and protein content of 0.836 g/g DCW. SCP obtained from *P. pastoris* contains a higher percentage of protein compared to conventional foods such as soy, fish, meat, whole milk and is a source of essential amino acids (%), including methionine (2.12), lysine (8.45) and amino acids branched chain, valine (4.78), isoleucine (5.64), leucine (8.12). **Conclusion:** The SCP of *P. pastoris* using glycerine is a promising alternative to obtain a protein resource with environmental, economic and nutritional benefits, which is expected to become a sustainable mode of production in the future. To our best knowledge, this is the first report demonstrating the feasibility of using *P. pastoris* as the chassis for the production of amino acids from glycerine.

Keywords: *Pichia pastoris*, Glycerine, Single cell protein, Amino acids, Economical benefit

Bioengineering and Bioprocesses

Influence of agitation rate on the 3HV composition of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer in continuous cultures of *Azotobacter vinelandii* OP

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Introduction: *Azotobacter vinelandii* is a Gram-negative bacterium that can fix nitrogen and exhibit high respiration rates. It is capable of synthesizing PHBV through the addition of valeric acid. In this work, the effect of different agitation rates on the 3HV fraction of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) in continuous cultures of *Azotobacter vinelandii* OP was evaluated. **Methodology:** Chemostats were developed under diazotrophic conditions in a 3L Applikon bioreactor using a working volume of 1.5 L, with sucrose (20 g L⁻¹) as a carbon and energy source. The chemostat was operated at a dilution rate of 0.04 h⁻¹, at 30°C, pH 7.0, 1 vvm, and different agitation rates (150, 250, 450, 650 and 850 rpm), using 10 mM of valeric acid in the feed tank. The oxygen transfer rate (OTR) and carbon dioxide transfer rate (CTR) were calculated by analyzing fermentation gases. The PHBV and monomeric composition quantification were analyzed by gas chromatography with a flame ionization detector (FID), utilizing propanolysis of previously dried biomass. Steady state was achieved considering biomass and OTR as criteria. **Results:** Highest biomass concentration was obtained at 450 rpm, reaching 3.67±0.10 g L⁻¹, whereas, at extreme agitation rates (150 and 850 rpm), it was approximately 1.0 g L⁻¹. PHBV accumulation was highest 65 % (w w⁻¹) between 150 and 450 rpm and decreased to 32 % (w w⁻¹) with increased agitation, indicating no adverse effects on PHBV accumulation between 150 and 450 rpm. 3HV fraction in PHBV was 10 mol % at 450 rpm, increasing to 36.4 mol % under extreme agitation (150 and 850 rpm). OTR and CTR increased 5 and 8.5-fold, respectively, compared to low agitation. Respiratory quotient increased from 0.4 at 150 rpm to 0.7 at 450 and 850 rpm, indicating a high oxygen consumption due to valeric acid utilization in PHBV synthesis. **Conclusion:** The present study shows that agitation rates significantly affect PHBV biosynthesis and biomass production in *A. vinelandii* OP cultures. Intermediate agitation (450 rpm) promotes biomass growth and PHBV accumulation, whereas extreme agitation (150 and 850 rpm) increases the 3HV monomeric fraction of PHBV.

Keywords: *Azotobacter vinelandii* - Chemostat - Oxygen transfer rate - Poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

Bioengineering & Bioprocesses

Alginate production and transcription levels of alginate lyases to different agitation rates in *Azotobacter vinelandii* cultures.

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Introduction: Alginate is a polysaccharide used in a wide range of industrial applications, and its functional properties depend on its molecular weight. In this study, we aimed to evaluate alginate production and the expression of genes involved in depolymerization in batch cultures of *Azotobacter vinelandii* at different agitations rates.

Methodology: *A. vinelandii* ATCC 9046 was used. The experiments were developed in a 3-L bioreactor (Applikon, Schiedam, Netherlands) using a working volume of 1.5 L and aerated at 1.5 Lmin⁻¹ (1 vvm). The alginate MMW was determined using gel permeation chromatography (GPC) in an HPLC (Jasco, Japan). Real-time PCR was performed in an AriaMx Real-Time PCR system (Agilent Technologies, USA).

Results: The expression of depolymerization genes was evaluated at different agitation rates (100, 300, 500, 700 and 900 rpm). A higher level of *alyA1* transcription was obtained at 700 and 900 rpm (4- and 17-fold). On the contrary, at 100 and 300 rpm there was no high gene expression. Considering that in some culture conditions (700 and 900 rpm) after the cell growth phase a decrease in the MMW of alginate was observed (from 320 to 126 kDa), it is possible that transcription of the *alyA1* gene could explain this decrease. The *alyA3* it was observed that there is greater transcription at 700 y 900 rpm between 2 and 14 times compared to the control in any phase of the culture. This enzyme is correlated with *alyA1*; the activation of this gene could be correlated with the decrease in alginate MMW. Regarding the *algL* gene, it was observed that it had a 15-fold higher gene expression at 900 rpm (24h). It is believed that an alteration of this gene generates an increase in the concentration of alginate that in this case it is around (4.8 g L⁻¹).

Conclusions: An increase in agitation rate allows increased oxygen transfer in batch cultures of *A. vinelandii*. The transcription levels of *alyA1* and *alyA3* were more affected at 700 and 900 rpm in the depolymerization in alginate biosynthesis causing the decrease in molecular weight could be decisive in determining the quality of alginate.

Key words: Lyases, Mean molecular weight, Alginate, *Azotobacter vinelandii*, Batch cultures.

Bioengineering and bioprocess

Respirometric analysis of solid-state fermentation with *Rhizopus oryzae* using pigmented corn as substrate in tray bioreactor.

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Introduction: *Rhizopus oryzae* is a microorganism of interest for the industry due to its great adaptability to grow in diverse substrates, it can be used to produce metabolites of industrial interest, such as enzymes, proteins, pigments and phenolic compounds. This work was aimed to evaluate the growth of *R. oryzae* in solid-state fermentation (SSF) using pigmented corn kernels as a support, in terms of O₂ consumption and CO₂ production. **Methodology:** Therefore, the growth of *Rhizopus oryzae* on PC grains (inoculated with 1x10⁷ spores/gdm) was evaluated using tray bioreactors (TB). During the SSF (60 h), the TB were incubated at 30°C with air flow rate supplied of 1 mL/gwm min. Microbial growth was estimated indirectly through carbon dioxide production (CDP) and oxygen consumption (OC). **Results:** Kinetic parameters of the culture, such as the specific CDP rate (0.084 (1/h)), the CDP per OC yield (0.86 mgCO₂/mgO₂), and the maintenance coefficient (0.015 mgCO₂/mgO₂ h) were estimated by the logistic equation and Pirt model. At the end of the bioprocess, a total CDP of ~35 mg/gidm and OC of ~29 mg/gidm were reached. At 19 h, the respiratory quotient presented values >0.85 molCO₂/molO₂. **Conclusion:** These results indicate that *R. oryzae* presents aerobic metabolism at this stage of the culture. Respirometric analysis demonstrates that *R. oryzae* can be growth in grains of pigmented corn and can be used to obtain various metabolites of interest.

Key words: carbon dioxide production, oxygen consumption, bioprocesses, Pirt model.

Bioengineering & Bioprocess

Sensitivity analysis to evaluate transport phenomena of a solid-state fermentation packed bed bioreactor for protease production using agro-industrial wastes.

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Introduction: Solid State Fermentation under the conception of the circular economy, allows recovery of agro-industrial wastes for production of secondary metabolites such as proteases. However, one of the great disadvantages for the industrial-scale implementation of this technology is the negative impact of oxygen and temperature transfer on microorganism growth and on the production of metabolites due to the poor thermal conductivity transfer. **Methodology:** The system is a cylindrical bioreactor, packed with solid agroindustrial wasted. A model was numerically discretized by orthogonal collocation, the set of differential equations was solved by a fourth order Runge-Kutta-Fehleberg method in Fortran code and the results were assessed by experiments using *Yarrowia lipolytica* 2.2ab. A sensitivity analysis was performed, evaluating the effect of the characteristic times on the behavior of a conventional bioreactor to explain the dominant mass and heat transfer phenomena in the bed. **Results:** It was observed that the evaporation process is one of the dominant mechanisms in heat transfer and from the beginning of the process it determinate the temperature of the fluid phase. At smaller characteristic times of evaporation, the CO₂ in the solid phase diminished along with the rate of protease production because the temperature rise in both phases. **Conclusion:** The sensitivity analysis was performed evaluating the effect of the characteristic times on the behavior of a conventional bioreactor, allowing to explain the dominant mass and heat transfer phenomena in the bed.

Key words: Bioreactor modeling, Solid State Fermentation, Sensitivity analysis, protease production, agro-industrial wastes

Standardization of the fermentation process of mesquite pod flour hydrolysates for the xylitol production

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Introduction: In Mexico, the mesquite pod is a widely distributed vegetable source, which has a high fiber content. This characteristic indicates that it could be a potential substrate for bioprocesses. The objective of the study was to standardize the fermentation process of mesquite pod flour hydrolysates for the xylitol synthesis. **Methodology:** The mesquite hydrolysates were generated with 17% citric acid in a 1:10 (w/v) ratio. Subsequently, the monosaccharide profile of the hydrolysates was performed through HPLC. For fermentation, 50 mL of hydrolysate inoculated with 1, 2.5, and 4 g/L of *Candida guilliermondii* was used, which was carried out under constant stirring at 150 rpm for 50, 84, and 120 h. Sampling was carried out every 12 h. To analyze the ferments generated, a spectrophotometric method was used to quantify biomass, cell viability by means of counting in a Neubauer chamber, and the dinitrosalicylic acid method for reducing sugars. The quantification of xylitol bioconversion was carried out by the mathematical model applied by Tizazu et al. (2018). The results were analyzed using a central composite design. **Results:** The best fermentation treatment was a time of 84 h and an inoculum concentration of 4 g/L, resulting in a 14% decrease in the concentration of reducing sugars ($p < 0.05$) and a reduction ($p > 0.05$) of 6% in cell viability. Biomass increased ($p < 0.05$) by 138% compared to the initial concentration. The response variables showed correlations ($p < 0.05$) with the initial xylose concentration of mesquite flour. The synthesis of xylitol in conventional fermentation reached 3.52 g/L under these conditions. **Conclusions:** These results indicate that mesquite pod is a promising raw material to produce xylitol. The production is only 18% lower than that of other similar plant sources. Furthermore, the bioprocess stands out for its sustainability and low toxicological risk.

Keywords: *Bioprocess, Citric acid, Candida guilliermondii*

Formation of a PLA biofilm by adding pecan shell previously treated with cold plasma

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Introduction: Polylactic acid (PLA) is being explored as an alternative solution to solve the ecological problem of plastic waste accumulation, with a major focus on packaging. PLA, due to its biodegradation ability, presents the major advantage to enter in the natural cycle implying its return to the biomass. Currently, PLA-based packaging and containers including bottled water, juice and yoghurt are used in Europe, Japan and North America for supermarket products [1]. Pecan shell (PS) is an agro-industrial waste that can be used as raw material in the production of plastics [2]. The wide-ranging benefits offered by bioplastics mean that research and industry objectives are moving towards a transition from petroleum-derived plastics to biomaterials [3]. Arc plasma and frequency plasma were applied, varying time, to analyze their degradation with the objective of fabricating a biofilm from this treated waste and PLA. **Methodology:** PS was treated in triplicate with arc plasma and frequency plasma, the conditions used were: frequency 500 Hz, power 60 W and times of 1, 5 and 10 minutes respectively. The results were characterized by FTIR, TGA, DSC, contact angle and solubility. **Results:** For arc plasma, the percentage of degradability with respect to time: 1 minute 0.93 %, 5 minutes 3.9 % and 10 minutes 6.12 %. For frequency plasma with respect to time, the percentage of degradability was: 1 minute 4.03 %, 5 minutes 4.4 % and 10 minutes 5.55 %. Regarding arc plasma, between minutes 1 and 10, the increase was 6 times. On the other hand, in frequency plasma between 1 and 10 minutes, the increase was 27%, that means that, at minute 1, the degradability was 73%. Regarding the FTIR, it is verified that there is no grafting of any functional group, therefore PS is only degrading. **Conclusion:** PS was degraded by plasma, which added to the biodegradable nature of PLA, could be applied in food industry packaging.

Keywords: Polylactic acid, pecan shell, food packaging, cold plasma, biodegradability.

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4th **BIO IBEROAMERICA**

IBERO-AMERICAN CONGRESS
ON BIOTECHNOLOGY

3-6 SEPTEMBER 2024

BIOSYSTEMS



Biosystems & Synthetic Biology

Effect of bacteria isolated from *Scenedesmus* sp. cultures on its growth under a biogas atmosphere

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Introduction The use of microalgae to carry out CO₂ removal from industrial gaseous effluents such as biogas is nowadays a biotechnological strategy implemented worldwide. Biological interactions between microalgae and bacteria have been proposed to enhance the growth and metabolic capabilities of microalgae during biotechnological processes. Consequently, there has been increased interest in finding bacteria capable of promoting the growth and metabolism of microalgae under the growth conditions used in biotechnological processes. **Methodology** The study aimed to analyze the growth-promoting capacity of two bacterial strains isolated from *Scenedesmus* sp. cultures under an air atmosphere applied at two concentrations (0.5 and 1 DO) to axenic cultures of the same microalgae for 144 hours under a synthetic biogas atmosphere (75% CH₄ + 25% CO₂) and compare it with the growth of this microalgae (axenic and non-axenic). **Results** Non-axenic *Scenedesmus* sp. cultures showed better growth at the end of the experiment than the other treatments. The tested bacterial strains showed growth promotion at 96 hours of incubation; however, at 144 hours, the growth of the microalgae interacting with these bacteria was similar to the growth shown by the axenic cultures. **Conclusion** Bacteria present in non-axenic cultures of *Scenedesmus* sp. facilitate the growth of the microalgae under a biogas atmosphere.

Key words: *Scenedesmus*; Biogas; Microalga Growth-Promoting Bacteria.

Gene Ontology analysis of the proteome of a natural photoheterotrophic consortium

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Introduction: Microbial consortia are paramount in bioproduction as they are more robust than single cultures. In this work, we analyse the proteome of a natural photoheterotrophic consortium (known as C4), mainly composed of *Clostridium* spp. and *Rhodopseudomonas palustris*, which can transform organic acids into biohydrogen (bioH₂) and polyhydroxybutyrate using light as an energy source.

Methodology: C4 was cultivated under growing (NH₄ repleted), and non-growing conditions (NH₄ depleted and a mix of organic acids (acetate, lactate, butyrate, and propionate). In the latter condition biohydrogen (bioH₂) is produced. Protein was extracted from both cultures and then identified by shotgun proteomics using HPLC-MS². The relative content of each protein in C4 under non-growing conditions was compared against growing conditions and then expressed as the log₂ fold change (LFC). Statistically significant (SS) proteins with an absolute (LFC) higher than 0.5 were subject to Gene Ontology analysis. UniProt database for *C. pasteurianum* and *R. palustris* CGA009 were used as references, and Wolfram Mathematica was used for data analysis.

Results: Approximately 790 proteins were SS and had an LFC higher than 0.5 for both microorganisms. Proteins related to hydrogen regulation, stress response, and the light-harvesting system were among the top upregulated proteins in *R. palustris*. On the other hand, in *C. pasteurianum*, upregulated proteins were mainly related to translation, sporulation, and transport, they had a low LFC though. The top 20 more frequent GO terms showed that twelve GO terms were common for both bacteria, eight were unique for *R. palustris*, and eight were unique for *C. pasteurianum*. Among the unique in *R. palustris*, we found the Biological Process (BP) terms: proteolysis, nitrogen fixation, signal transduction, and cobalamin biosynthesis. For *C. pasteurianum*, the unique BP terms were translation, carbohydrate metabolic process, and phosphorylation. An overrepresentation analysis (ORA) showed that four GO terms related to translation were SS overrepresented in *C. pasteurianum*, but none was found SS in *R. palustris*.

Conclusion: Proteomic analysis revealed that the main regulated processes under bioH₂ production conditions in C4 for *R. palustris* and *C. pasteurianum* were related to nitrogen metabolism and protein synthesis.

Keywords: Proteomics, photoheterotrophy, consortium, gene ontology

Biosystems and synthetic biology

“Isolation of growth-promoting bacteria for microalgae present in cultures of *Scenedesmus* sp.”

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Introduction: *Scenedesmus* sp., a unicellular green microalga which is globally distributed and studied for applications in biofuels, food, and pharmaceuticals. In nature, microalgae like *Scenedesmus* sp. interact with bacteria, forming positive, negative, or neutral relationships. Symbiotic interactions are common and in these interactions microalgae provide nutrients and oxygen to bacteria, while bacteria assist in nitrogen fixation. Add to this, bacteria can promote microalgal growth by producing organic compounds such as amino acids, vitamins, and organic acids, although negative interactions through nutrient competition could also occur. In laboratory conditions the maintenance of microalgae cultures are always in risk of bacterial contaminants which can produce negative or positive effects on microalgae growth. This study hypothesizes that *Scenedesmus* sp. cultures maintaining in laboratory conditions contain bacterial strains that either benefit or hinder the microalga's growth and metabolism. **Methodology:** Non-axenic *Scenedesmus* sp. cultures were centrifuged at 1,000 rpm, and the supernatant was spread on nutrient agar plates, followed by incubation at 27°C for 48 hours. Isolated colonies were characterized macroscopically and microscopically. Axenic *Scenedesmus* sp. cultures were inoculated with each bacterial strain at two concentrations (0.5 and 1 OD), and the growth of both microorganisms was monitored every 48 hours for six days. **Results:** Five bacterial strains were identified (strains 1-5). Strains 2 and 5 were the most abundant and for this reason were selected for further study. Strain 2 promoted microalgal growth at both concentrations being the effect proportional to the bacterial concentration. Strain 5 promoted growth only at the higher concentration, while the lower concentration negatively affected microalgal growth. **Conclusion:** This work demonstrates that *Scenedesmus* sp. cultures harbor bacterial strains that can promote microalgal growth, and the kind of effect produced on microalgae growth will depend of the bacterial concentration. Higher concentrations enhance growth promotion, whereas lower concentrations can have adverse effects. Identifying such growth-promoting bacteria is crucial for advancing microalgal biotechnology.

Key words: Microalgae; *Scenedesmus* sp.; Growth-promoting bacteria for microalgae



4th **BIO IBEROAMERICA**

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ENVIRONMENTAL BIOTECHNOLOGY

Environmental Biotechnology

Byproducts as sources of phenolic compounds for functional textiles

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Introduction: The textile industry is a major global source of pollution due to its high use of energy, water and chemicals. Thus, a need has risen to move the textile industry towards a more sustainable economy. The eco-safety awareness and increased environmental concern has led to the use of green and sustainable natural dyes as the needed trend within this industry. Wastes/byproducts from the agri-food industry can serve as a natural source of functional dyes rich in phenolic compounds, being both cheap and abundant while also aiding in the reduction of waste. The main objective of this work was to study different byproducts for their potential to be used in the textile industry as functional dyes. **Methodology:** Two different green and sustainable extraction methodologies were used in four byproducts (hops, carqueja stem, lemongrass and peanut skin) from the agri-food industry. One aqueous (100% H₂O) and one hydroethanolic (70% EtOH). All extracts were characterized regarding their sugar, phenolic and flavonoid content, as well as their biological potential as antioxidants and antimicrobials. Their cytotoxicity was also evaluated against a skin cell line. **Results:** The aqueous extracts showed the highest quantity of total sugars. With the exception of hops, hydroethanolic extracts showed higher total phenolic content than the aqueous extracts, with the peanut skin extract showing the highest amount (313,1 ± 6,7 mg GAE/g of extract). The hydroethanolic extract of lemongrass had the highest amount of total flavonoid content (202,9 ± 3,1 mg CAE/g of extract) as well as the highest antioxidant activity (6279,1 μmol TE/ g extract). As for antimicrobial activity, hops extracts were able to inhibit the growth of *S. aureus* at 20 mg/mL, while no extract was able to inhibit gram-negative bacteria (*E. coli* or *P. aeruginosa*). All extracts showed some cytotoxicity against HaCaT cells except for the hops extracts at 5 mg/mL. **Conclusions:** While more studies are necessary, the results show that by-products from the agri-food industry have great potential to be used as functional dyes in the textile industry.

Key words: Textile industry, byproducts, phenolic compounds, flavonoids, antioxidant activity.

Environmental Biotechnology

Characterization of the metabolic activity of the microalgae *Scenedesmus* spp. interacting with the bacterium *Azospirillum brasilense* Cd under a biogas atmosphere

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Introduction. Biogas is a gaseous effluent that play a key role as renewable energy. This is mainly composed by methane (CH₄) and carbon dioxide (CO₂). The CH₄ present in effluent represent a valuable source of potential alternative energy. Nevertheless, presences of CO₂ in biogas reduce the calorific value of CH₄. their heating value.. From this, microalgae cultures like those belongs to the genus *Scenedesmus* have been proposed to consume the content of CO₂ from biogas through their photosynthetic activity. Interaction between microalgae and bacteria has been proposed as an strategy to improve the microalgae's metabolisms thus improving the biogas upgrade. In this sense, the bacterium *Azospirillum brasilense* has demonstrate their ability to enhance growth and metabolism of microalgae under air conditions through the exchange of key metabolites between microalga and bacterium cells. Nonetheless, production of key metabolites between both microorganisms under biogas has not been reported. From this, the objective of this work was determine the effect of biogas on IAA production by *A. brasilense* and tryptophan production by two strains of *Scenedesmus*. **Methodology.** Two species of microalgae, *Scenedesmus* CC16 and *Scenedesmus obliquus* U162, as well as the bacterium *Azospirillum brasilense* Cd, were used. All treatments (*S.* CC16 alone, *S.* CC16 / *A. brasilense*, *S. obliquus* U162 alone, and *S. obliquus* U162 / *A. brasilense*) were supplied with synthetic biogas, composed of 75% methane and 25% carbon dioxide. The effect of biogas on the microalgae alone and interacting with bacterium was determined measured photosynthetic efficiency, growth, intracellular metabolites such as lipids, carbohydrates, pigments, and proteins, as well as production of signalling molecules like tryptophan and indole-3-acetic acid. **Results.** It was found that the microalga *Scenedesmus* CC16 interacting with *A. brasilense* showed better results in terms of biomass productivity and CO₂ fixation compared to the other treatments, while *S. obliquus* U162 did not show any difference when interacting with the bacterium. In the case of microalgal growth, no significant differences were observed among the four treatments, with growth rate values ranging between 0.55 to 0.57 μ .day⁻¹. **Conclusion.** The biogas has different effect on the metabolism of microalgae depending on the species as well as if they are interacting or not with *A. brasilense*. Therefore, each microalga has a different capacity and different requirements in utilizing photosynthesis products and metabolizing CO₂ from the biogas.

Key words: *Biogas; mutualism; microalgae-bacteria; metabolism.*

Environmental Biotechnology

Potential of *Nannochloropsis oculata* biomass for obtention of nanomaterials: comparison of residual defatted biomass grown at different temperatures.

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Introduction: In recent years, microalgae gain popularity in biotechnology applications, mainly because their properties such as high growth rates and rapid accumulation of value-added compounds. Microalgae biomass has several applications in different industries, such as bioenergy, food and aquaculture. However, one recent application is the obtention of nanomaterials (nanoparticles green synthesis). This work analyses the obtention of two types of nanoparticles (silver and iron), using biomasses of *N. oculata* with different properties. **Methodology:** Three *N. oculata* biomasses were used for the obtention of nanoparticles: (1) complete biomass without any treatment, (2) residual defatted biomass, and (3) residual defatted biomass grown under stressful temperature conditions. The aqueous extracts were prepared using 500 mg of biomass mixed with 50 mL of distilled water, at 100° C for 5 min. After that, the suspension was centrifugated (6000rpm 10 min) and the supernatant was collected for the green synthesis. For that, 10 mL of the extract was mixed with 90 mL of silver nitrate (AgNO₃ 1mM), for 24 hours, in dark conditions at room temperature. For nZVI, 10 mL of extract were mixed with 90 mL of iron chloride (FeCl₃ 20 mM), for three hours at dark conditions at room temperature. The nanoparticles were separated by centrifugation (6000rpm 10 min), for later analysis by a Scanning Electron Microscope (SEM). **Results:** The nanoparticles formation were visible through change colour, (AgNPs change colour from green to brown, and iron nanoparticles a change colour from green to grey). Also, the nanoparticles were revised in SEM images, observed as spherical shape collocated in the surface on tiny dots of biomass. **Conclusion:** Complete and residual biomasses of *N. oculata* were useful for both nanoparticles obtention, representing a new promising option for these nanomaterials synthesis, mainly for the use of compounds considered as non-environmental harmful, and the low energy demands of the process, compared to the methodologies currently used.

Key words: *Nannochloropsis oculata*, microalgae, biotechnology, green synthesis, nanoparticles.

Environmental Biotechnology

***Dunaliella tertiolecta* biomass as resource for iron and silver nanoparticles synthesis.**

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Introduction: Marine microalgae are promising alternatives for different applications such as: food, medicine, aquaculture, and nanotechnology. These microorganisms present convenient properties such as: high productivities, the non-use of arable land or freshwater resources, and reduction of CO₂. The obtention of nanoparticles through green synthesis emerges as feasible proposal (using the living cells or microalgae biomass) because there are considered as eco-friendly and low-cost alternative processes. The objective of this work is the use of *Dunaliella tertiolecta* biomass (complete and defatted) for obtention of two different nanoparticles with industry relevance. **Methodology:** For green synthesis of silver nanoparticles (AgNPs) and zero-valent iron nanoparticles (nZVI), two different *D. tertiolecta* biomasses were used: (1) complete biomass, and (2) residual defatted biomass. Each biomass was used separately to obtain two aqueous extracts, using 500 mg of biomass mixed with 50 mL of distilled water, at 100° C for 5 min. Then, the suspension was centrifugated (6000 rpm 10 min) and the supernatant was collected for the synthesis. After that, 10 mL of the extract was mixed with 90 mL of silver nitrate (AgNO₃ 1mM) for 24 hours, in dark conditions at room temperature (for AgNPs). For nZVI, 10 mL of the extract were mixed with 90 mL of iron chloride (FeCl₃ 20 mM) for three hours at dark conditions at room temperature. Finally, the nanoparticles were separated by centrifugation (6000 rpm 10 min), for later analysis by Scanning Electron Microscope (SEM). **Results:** The nanoparticles formation were verified through a change colour, (AgNPs change colour from green to brown, and nZVI trough a change colour from green to grey). Also, the nanoparticles were visible in SEM images, observed with spherical shapes collocated on the surface of tiny dots of biomass. **Conclusion:** Complete and residual biomasses of *D. tertiolecta* could be used for the obtention of nanoparticles of iron and silver, offering a promising alternative for nanomaterial technologies. This approach could be considered as environmentally friendly and less resource-intensive compared to current methods, making it a sustainable option for nanoparticle synthesis.

Key words: *Dunaliella tertiolecta*, microalgae, biotechnology, green synthesis, nanoparticles.

Environmental biotechnology

ASSESSMENT OF ARSENIC REMOVAL IN WATER TREATMENT USING *Pediastrum boryanum* MICROALGAE

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Introduction: The development of a healthy environment depends on various factors, one of the main ones being the presence and quality of water in each region. However, globally, most of this resource is used only once, leading to overexploitation and lack of reuse, which generates water stress. Currently, water treatment techniques, besides being employed on a smaller scale globally, do not address all the factors necessary to improve the quality of the water output from these systems. One of the persistent issues is the presence of arsenic, which has repercussions on food, vegetation, animal life, and the health systems of each region. Therefore, the search for sustainable and efficient water treatment alternatives is of great importance. We focus on biological remediation techniques, utilizing microorganisms to transform waste and eliminate contaminants. This study evaluates the efficacy and efficiency of employing *Pediastrum boryanum* microalgae for total arsenic removal in treated water

Methodology: Experiments were conducted based on the reduction of total arsenic concentration in relation to the number of suspended cells in water samples from the effluent of a wastewater treatment system at a regional brewery, with a *P. boryanum* inoculum in exponential phase at a 1:10, 2:10 and 3:10 ratio to a concentration of 3.2×10^5 cells/mL, the water was pre-filtered with a 0.45-micron membrane to eliminate metazoans that might impede microalgae-arsenic interaction. Conditions included a temperature of 30°C, orbital agitation at 200 rpm, and a photoperiod of 12L:12D. **Results:** Reductions ranging from 10% to 40% of total As were obtained in response to different inoculum and initial concentrations of this metalloid.

Conclusion: It is necessary to study the various factors that could be altered within the treatments to optimize the response, considering their potential application at an industrial level.

Key words: P.boryanum, Arsenic, Removal, wastewater, environment.

Isolation of endophytes and ectomycorrhizal fungi from *Pinus cembroides* roots

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Introduction: Forests are a key factor in climate regulation; however, they face inadequate management due to erosion, fires and deforestation causing climate change and an increase in greenhouse gases. One of the solutions that have been used to control this problem are the implementation of reforestation programs; though, the plants have a low survival rate since they are abandoned after their establishment. In the arid zone of Mexico, we can find the species *Pinus cembroides*, which is used to repopulate forests due to its wide adaptability and resistance to adverse conditions. One of the main objectives in reforestation is to achieve survival in the field and the resilience of plants in difficult environments, which is why it is important to identify ectomycorrhizal fungi associated with this species to cultivate them and take them to seedling production greenhouse. **Methodology:** Root sampling of *P. cembroides* was carried out in 10 stands located in the mountains of Coahuila and Nuevo León. Root samples were disinfected and isolated on PDA; they were subsequently identified molecularly through DNA extraction using the amplification of the ITS region by PCR and finally, sequencing the amplicon. The obtained sequences were compared from databases of ITS fungi on GenBank portal (NCBI), considering a percentage of identity more than 97%. **Results:** A total of eight strains of endophytic fungi and five strains of ectomycorrhizal fungi were obtained. The isolated ectomycorrhizal fungi belong to the genera *Gymnopus*, *Melanogaster*, *Meliniomyces*, *Tuber* and *Russula*. Of the isolations of endophytic fungi, species of the genera *Phialocephala*, *Mycoleptodiscus* and *Podospora* were found. **Conclusions:** The obtained strains are of utmost importance for future research both *in vitro* and greenhouse cultivation as well as their use for determining survival in the field of *P. cembroides*.

Keywords: Ectomycorrhizal fungi, molecular identification, *Pinus cembroides*



Environmental Biotechnology

Optimization of culture conditions for biogas valorization as carbon source by the microalga *Chlorella* native of río Conchos-Chihuahua

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Biogas is a by-product generated from anaerobic digestion (AD) of diverse organic materials. Although biogas composition is dependent on the substrate used and operation conditions of AD process, this gaseous effluent is composed mainly of methane (65-70%) and carbon dioxide (25-30%). Today, biogas is valorized due to its high CH₄ and CO₂ content since CH₄ is considered an important energy source because of its calorific value from 20 to 25 MJ·m³, whilst CO₂ content is appreciated as a carbon source for photosynthetic microorganisms, such as microalgae. Particularly, microalgae capture CO₂ from biogas through their photosynthetic activity to convert/valorize it in biomass and high-valuable metabolites of commercial interest such as carbohydrates, proteins, lipids, pigments, among others. Subsequently, these biopolymers have incidence on bio-economy of different countries contributing to diverse economic activities, such as food and feed production, agricultural products, biofuels, and health-related products. Nonetheless, the development of biological strategies based on microalgae for CO₂ fixation from biogas is still under study and mainly focused on the following topics, for instance: (1) selecting novel microalgal strains as well as (2) optimizing culture conditions – physical, chemical, and hydrodynamic factors – to maximize the CO₂ microalgal capture; and (3) designing and scaling up suitable reactors. In this regard, the objective of this work was to determine by response superficial methodology (RSM) the optimal nitrogen concentration and light intensity to enhance the photosynthetic activity of the microalga *Chlorella* native of río Conchos-Chihuahua and boost the use of CO₂ of biogas as carbon source to produce high-valuable compounds. Our results demonstrate that 1.4 g/L of nitrogen and 341 μmol m⁻² s⁻¹ of light intensity induced the highest production of metabolites from CO₂ of biogas. Similarly, the qualitative characterization by Fourier Transform Infrared (FTIR) showed that this microalga produced mainly lipids; whilst the analysis of the lipid profile by chromatography of gases demonstrated that the hexadecanoic and octadecanoic acids were the main fatty-acids found in the microalgal biomass. Overall, these results indicate that this native microalga requires low concentrations of nitrogen generating a positive impact on the cost-benefit ratio during the production of lipids from this microalgae.

Key words: Bioprocess, Light intensity, Lipids, Nitrogen,



Environmental Biotechnology

Agave by-products as a source of polyhydroxybutyrate

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Introduction: Polyhydroxybutyrate (PHB) is a biopolymer synthesized and accumulated in cytoplasmic carbonosomes by several microorganisms. The investigation of PHB production is of current interest to the scientific community because of its physical and chemical characteristics and potential biomedical and environmental applications. In this sense, culture media for PHB production has been extensively studied for economical and viable alternatives through industrial by-products. This study aimed to evaluate media culture based on by-products from the mezcal industry for PHB biosynthesis. **Methodology:** Bagasse was dried at 60°C in a tray dryer. *Agave durangensis* leaves were cut and dried (room temperature). Media cultures were prepared with bagasse (MB), natural leaves (ML), and pre-treated leaves (MTL), supplementing NaCl and yeast extract. PHB production was carried out by *Bacillus cereus* 4N in flasks for 48 h. PHB extraction was performed according to Martínez-Herrera et al. (2021). Finally, PHB films were characterized by a differential scanning calorimeter (DSC). **Results:** PHB production was achieved by agave leaves media formulation. With ML, PHB films reached 0.29±0.42 g/L, while with MTL, 0.35±0.02 g/L were obtained. PHB production was confirmed through DSC analysis, where the temperature profile is similar to that of PHB thermograms reported elsewhere. According to thermogram results, *B. cereus* could not produce PHB with the MB formulation. However, another type of biopolymer with gum consistency was obtained (0.210± 0.04 g/L). **Conclusion:** Agave leaves-based formulations effectively stimulate intracellular PHB production.

Keywords: lignocellulosic, biopolymer, circular economy.

Insecticidal Effect of *Pseudomonas entomophila* on the Model Insect *Galleria mellonella*

Con formato: Inglés (Estados Unidos)

(Order: Lepidoptera)

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Con formato: Fuente: 10 pto, Sin Negrita

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Introduction: Pests pose a significant problem in crop maintenance and have had negative effects on global food supply and economy. While chemical insecticides have been used to control pests, their use has a considerable negative impact on ecosystems and human health. Biological control agents offer a viable and effective alternative for the control of pest insects from various orders, including the bacterium *Pseudomonas entomophila*. This bacterium produces toxins harmful to insects of the order Lepidoptera, disrupting their growth and even causing death, thus playing a key role in its ability to control insect pests. **Methodology:** The bacterium *Pseudomonas entomophila* was provided by the company Greencorp, and it was reactivated on a plate before being transferred to tryptic soy broth at 30°C, 24 hours, and 120 rpm to obtain the pre-inoculum. Three culture media were evaluated: tryptic soy broth (TSA), King B (KB), and Luria Bertani (LB), which were inoculated at an initial concentration of 1x10⁶ cells/ml, 30°C, 120 rpm. Two samples were taken, one at 24 hours and the other at 48 hours. These samples were adjusted to a concentration of 1x10⁷ cells/ml, and an insecticidal bioassay was conducted against *Galleria mellonella* larvae at the L3 stage, applying the treatment by ingestion according to IRAC methodology, where the response variable was the percentage of mortality over 7 days. **Results:** It was determined that the best culture medium was LB with a fermentation time of 24 hours, showing the highest mortality rate of 36.67% at 168 hours of evaluation on the model larvae (Tukey p=0.05). The other treatments were inferior. This mortality is attributed to the toxins generated by the bacterium *P. entomophila*. **Conclusion:** The use of biological agents like *Pseudomonas entomophila* reduces the dependence on chemical pesticides, benefiting both the environment and human health. This makes *P. entomophila* a cost-effective bacterium, generating solutions and benefiting the soil of Mexico and the world.

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Con formato: Fuente: 12 pto, Negrita, Sin subrayado, Inglés (Estados Unidos)

Con formato: Justificado

Con formato: Fuente: 12 pto, Sin subrayado, Inglés (Estados Unidos)

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Con formato: Fuente: 12 pto, Negrita, Sin subrayado, Inglés (Estados Unidos)

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Con formato: Fuente: 12 pto, Negrita, Sin subrayado, Español (España)

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Keywords: *Pseudomonas entomophila*, Entomopathogen, Lepidoptera



Environmental biotechnology

Physicochemical characterization of *Agave lechuguilla*: guishe and cogollo

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Introduction: *Agave lechuguilla* bagasse represents a high percentage of useless waste; it has been reported that it contains bioactive compounds of interest.

Methodology: A qualitative Molisch test was carried out, followed by fructan extraction by conventional and microwave methods, inulin was quantified in the extracts by reducing sugars and thin layer chromatography was performed with the extracts of guishe, cogollo, carbohydrate controls and HP inulin and FTIR was also performed. **Results:** The spot is observed in the samples of *Agave lechuguilla* extracts at the point of application, due to its high degree of polymerization, confirming the presence of inulin-type fructan, regarding the quantification of inulin-type fructans, the highest for the guishe extracted with the conventional method was the highest with 44 g/L, and cogollo by microwave with 17.33 g/L. **Conclusion:** *Agave lechuguilla* extracts are suitable for obtaining inulin-type fructans.

Keywords: fructans, reducing sugars, microwave assisted extraction, thin-layer chromatography.

Environmental Biotechnology

Regulatory mechanisms of *ASRF* gene in the mitigation of arsenic stress in *Arabidopsis thaliana*.

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Introduction: Arsenic (As) has profound implications for plant biology and agricultural systems. The negative regulatory role of the Arsenic Stress-Related F-Box (*ASRF*) gene in *Arabidopsis thaliana* under As stress has been reported. However, the molecular response of gene induction against As stress is unknown. Therefore, the present study aimed to analyze the physiological and transcriptomic changes of overexpression (OE) of *ASRF* gene in *Arabidopsis* under As stress. **Methodology:** OE *ASRF Arabidopsis* lines were generated and were grown in agar plate and soil pot experiments under As stress and compared with Col-0 as a control and with *ASRF* gene knockout (*KO*) lines. Later, RNA-seq was done followed by bioinformatic analysis. **Results:** It was observed that OE lines presented better development of rosette, root and stem compared to Col-0 and *KO* lines in the presence of As. The decreased production of reactive oxygen species and proline presence indicated the tolerance of OE lines towards As stress. At molecular level, As transporters such ABC and PHT were identified, suggesting a negative regulation of As entry and translocation in OE lines. On the other hand, up-regulation of TF such as Myb and WRKY and pathways related to the response against ROS were identified. Furthermore, the participation of cis-regulatory elements, such as TC-rich repeats and activation sequence 1, associated with oxidative stress and the activation of plant defense hormones against stress, both associated with the regulation of the *ASRF* gene were detected. Additionally, the motifs associated with signal transduction by G proteins and chromatin interacting proteins also plays a critical role in switching to a transcriptionally active state of *ASRF* gene under As stress. **Conclusion:** The results provide the basis for future investigations on the potential of the *ASRF* gene for the development of As tolerant crops.

Keywords: arsenic toxicity, plant stress, molecular defense mechanism



Environmental Biotechnology

Peroxidase from agroindustrial waste for biosensor used in the detection of phenolic compounds

Con formato: Inglés (Estados Unidos)

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Peroxidase (POD) [EC1.11.1.7] is an oxidoreductase enzyme produced by various plants and microorganisms. This catalyzes the oxidation of many organic compounds in the presence of hydrogen peroxide, and it is widely used in industry because it is considered one of the most thermostable. Since it is obtained mainly from plant sources, the main objective of this study was to study peroxidase extracts from different agro-industrial residues to prepare a biosensor to detect phenolic compounds.

Peroxidase was extracted from the peels of prickly pear, banana and potato using ultrasound and 0.1 M potassium phosphate buffer (pH 6.5). The peroxidase enzymatic activity of the crude extract was determined UV-VIS spectrophotometry at 470 nm in a UV-VIS at different times. The peroxidase activity for prickly pear, banana, and potato peels were 197.98 ± 15.97 , 212.89 ± 9.26 and 65.08 ± 5.77 U/mL⁻¹ respectively; this activity was also measured as a function of the ripening time and the results showed that the prickly pear peel has a greater peroxidase stability. The optimal conditions for peroxidase activity of this last were determined using a response surface methodology (MSR) for each parameter studied pH and temperature. The response surface analysis showed that the values of the parameters with the highest peroxidase activity were pH 4.0, 28.03 °C. Under these experimental conditions, it was found that the peroxidase activity was 144.8892 U/mL⁻¹. These results were used for the development of the biosensor, using a UV-VIS spectrophotometer at 439 nm it was determined that phenolic compounds (phenol, guaiacol, catechol and 4-aminophenol) can be detected by the enzyme peroxidase from cactus pear peel. It can be concluded that these prickly pear peel residues could be used for the extraction of peroxidase and the development of biosensors, adding value to this product produced in semidesert areas of the country.

Keywords: Peroxidase, enzymatic activity, prickly pear peel



Environmental biotechnology

Bioprospecting of Chemolithotrophic Microorganisms in Baños San Ignacio Natural Reserve

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Introduction: Chemolithotrophic microorganisms (QMO) derive their energy from the oxidation of inorganic compounds. Examples include anammox bacteria and autotrophic denitrifying bacteria (ADMo). Anammox bacteria oxidize ammonium to N₂, while ADMo use inorganic sources as electron donors to remove nitrogen compounds. Identifying these QMO is complex due to their unique metabolic characteristics and specific ecological niches. In this context, Baños San Ignacio Natural Reserve (BSINR) exhibit potential as a reservoir for QMO. The objective of this study was to bioprospect for QMO with applications in water pollutant removal. **Methodology:** Sediment samples were taken from three ponds within the BSINR: 1) Azul turquesa, 2) Cachorrito, and 3) Ojo de agua. DNA was isolated and purified for high-throughput sequencing of the 16S rRNA gene of Bacteria (V3–V4 region, 341F-805R) and Archaea (340F-1000R) using the Illumina MiSeq platform. Bioinformatic processing of sequences was performed using the software package DADA2 v1.6 in the R environment. Sequences were aligned with the SILVA 132 database, allowing taxonomic classification. **Results:** In the Azul turquesa and Cachorrito samples, a higher relative abundance of microorganisms from the family Firmicutes was observed (43.97% and 58.23%, respectively), whereas in the Ojo de agua samples, microorganisms from the family Crenarchaeota predominated (42.48%). At Azul turquesa and Ojo de agua the family Chloroflexi was significantly dominant (29.23% and 23.96%, respectively), while at the Cachorrito site, Proteobacteria predominated 11.65%, followed by Chloroflexi (10.23%). Similarities in various genera were found across all three sites including *Desulfobacca*, *Desulfatiglans*, *Desulfomonile*, *Planctomicrobium*, and *Pir4*, microorganisms associated with nitrate and sulfate reduction activity. **Conclusion:** The BSINR harbors microorganisms with sulfate-reducing activity (*Desulfobacca*, *Desulfatiglans*, *Desulfomonile*) and dissimilatory nitrate reduction (*Planctomicrobium* and *Pir 4*). This suggests that the site hosts a microbial community with the potential to perform anammox and autotrophic denitrification processes.

Keywords: Anammox, Nitrogen cycle, Autotrophic denitrification.



Environmental Biotechnology

Genomic characterization of some polyextremotolerant Actinomycetal isolates from Cuatro Ciénegas basin, Coahuila

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Introduction: Due to the several concurrent extreme conditions of the Cuatro Ciénegas (CC) basin, it is not surprising that we encounter microorganisms with exceptional adaptability, making them a potential source of novel metabolites. The strain collection at Laboratory 9 of the Instituto de Biotecnología (IB) from the UANL consists of approximately 700 Actinobacteria isolates from the CC basin. Some of these isolates have shown significant activity against multidrug-resistant bacteria and yeasts, but their growth conditions have posed challenges. The main objective is to evaluate genomic characteristics besides the inhibition against some pathogenic strains. **Methodology:** Selected isolates were reactivated from the strain collection. Isolates were incubated separately in aerobic conditions for 7 days (27°C in ISP2), and bacterial genomic DNA was extracted using the protocol as described by Arocha-Garza *et al.*, 2018. Libraries of each strain were prepared as required by standard protocols of each WGS sequencing platform, including sample quality testing, library construction, and library quality testing. Sequencing was performed by CINVESTAV-LANGEBIO, Irapuato, México, using the Illumina Mi-Seq 2 x 300 platform, and Novogen using Illumina Hi-seq 2 x 150 a 100x. The WGS data analysis was done partially in Google collaborative environment. Library gene assembly was performed using SPAdes or Megahit, gene annotations were achieved using the BV-BRC platform and were analysed for Biosynthetic Gene Clusters (BGCs) using ANTISMASH. BiNI was calculated as described by González-Salazar *et al.*, 2023. **Results:** Isolates PB7 and PB16 shown the highest BINi with 778.29 and 1153.1 respectively. The highest Hypothetical CDS's were detected in isolate PB16 (3,002) followed by PB7 (2,703). The range of BGCs found for the isolates was from 32-41. Isolates belonged to Streptomycetaceae and shown bioactivity against *Acinetobacter baumannii*, *Klebsiella pneumonia* or *Candida albicans*. **Conclusion:** Strains isolated from the CC basin possess a great variety of potential genes related to stress response and antibiotic resistance. Even when its growth conditions are difficult, have enormous biotechnological and pharmaceutical potential as is shown by the number of hypothetical CDS. More laboratory experimental evidence is needed to confirm predicted features.

Keywords: Bioactivity, Bioinformatics, WGS, Cuatro Ciénegas, BGCs

Environmental Biotechnology

Assessing the antimicrobial potential of actinobacteria isolated from an arid-tropical mangrove environment.

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Introduction: Antimicrobial resistance is a global issue, necessitating the development or discovery of new antibiotics and antimicrobial agents. Actinobacteria, prolific producers of bioactive compounds, offer a promising source of novel antimicrobials. This study focuses on isolating and characterizing actinobacteria from arid tropical mangrove sediments in Baja California Sur, Mexico, to evaluate their antimicrobial potential. **Methodology:** Sediment samples were collected from an arid tropical mangrove ecosystem. A total of 148 colonies were isolated and identified as actinobacteria. The antimicrobial activity of the isolates was assessed using cross-streak and well-diffusion methods against pathogens *B. cereus*, *S. aureus*, *E. coli*, *P. mirabilis*, and multi-resistant clinical isolates *E. coli* (I5), *E. faecalis* (I27), and *P. aeruginosa* (E26). The inhibitory effects of the cell-free supernatants were measured, and molecular characterization was performed to determine the taxonomic affiliations of the most potent isolates. **Results:** Among the isolates, 34 exhibited antimicrobial activity against one or more test pathogens. Notably, nine isolates (87_ML2, 105_ML2, 162_CB1, 187_CB2, 216_CB5, 219_CB5, 229_CB6, 232_CB6, and 246_CB7) demonstrated the highest inhibitory activity, with inhibition zones up to 32.03 ± 0.32 mm. Molecular characterization revealed these potent isolates belonged to the genera *Streptomyces*, *Brevibacterium*, *Nocardioides*, *Paraconexibacter*, and *Microbacterium*. This taxonomic diversity indicates a rich reservoir of actinobacterial species within the studied mangrove ecosystem. **Conclusions:** The study highlights the arid tropical mangrove sediments of Baja California Sur as a valuable source of bioactive actinobacteria with significant antimicrobial properties. The diversity of genera with potent antimicrobial activity underscores the potential for discovering new antimicrobial compounds. Future research should focus on isolating and characterizing these bioactive compounds to develop new therapeutic agents, addressing the critical need for novel antibiotics in the fight against antimicrobial resistance.

Keywords: antimicrobial activity; actinobacteria; mangrove.

Enriched PHB *Azospirillum brasilense* cells improve their maintenance and growth promoting effect on *Chlorella sorokiniana* cultures growing under biogas atmosphere

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Uses of microalgae growth-promoting bacteria (MGPB) to improve biotechnology process based in microalgae cultures have been already proposed. Nonetheless, the ability of the MPGB to growth and maintained in the culture condition used for the specific microalgae bioprocess (like biogas upgrade) is a key factor to ensure the positive effects desires for the MGPB applications. Polyhydroxybutyrate (PHB) compounds are present in some bacteria like *Azospirillum brasilense* and can ensure the survival and better adaptation of bacteria to stressful conditions. From this, the objective of the present work was determine the growth condition (culture media and atmosphere) at which *A. brasilense* cells produce and accumulates higher PHB content. Add to this; determine if cultures of *A. brasilense* enriched in PHB (ePHB) content have better effects on growth and metabolites production by microalgae cultures of *C. sorokiniana* than less enriched PHB (nPHB) *A. brasilense* cultures, when growing under biogas. Growth of *A. brasilense* on OAB-N media under biogas atmosphere allows obtaining cultures of *A. brasilense* with high PHB content ($249.36 \pm 34.69 \mu\text{g}\cdot\text{mg}^{-1}$) than other cultures media tested. On the other hand, the uses of ePHB *A. brasilense* allow a higher microalgae biomass ($0.67 \pm 0.19 \text{ gr}\cdot\text{L}^{-1}$) enriched in carbohydrates at 6 days of incubation than the microalgae cultured alone ($0.24 \pm 0.11 \text{ gr}\cdot\text{L}^{-1}$) or with nPHB bacteria ($0.28 \pm 0.08 \text{ gr}\cdot\text{L}^{-1}$). Moreover the uses of ePHB *A. brasilense* allow fast adaptation of the microalgae culture to the presences of synthetic biogas represented by the stable maintaining of the *chl a/chl b* rate. In conclusion, the uses of ePHB *A. brasilense* allows a better growth and positive effect of the bacterium to improve biomass, metabolites production and adaptation of microalgae cultures to biogas.

Key words: *Azospirillum*, Biogas, Adaptation, PHB

Genotoxicidad producida por Al, Mn, Fe, Zn, Br y Sr procedentes de la contaminación industrial depositados en árboles de encino (*quercus ssp.*)

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Introduction: Air pollution is a global problem that has been increasing in the main cities of the world due to the rapid development of industry, affecting the health of all living beings, causing imperceptible alterations in the physiology of organisms at the cellular level., Outdoor air pollution caused around 4.2 million deaths in cities and rural areas around the world due to fine particulate matter found in the atmosphere, generating cardiovascular and respiratory health problems, mutations and cancers in the population. For this study, the Allium cepa test was used in onions to evaluate cellular damage, with which the presence of chromosomal aberrations (CA) could be identified due to the presence of heavy metals from urban pollution. **Methodology:** The study begins by collecting oak leaves, which are treated by washing and centrifugation to separate the solid and liquid supernatant. The leachate is analyzed by ICP-MS and then determined for Allium cepa, cell damage. It is expressed in percentage of cellular aberration. **Results:** 21 metals were analyzed by ICP-MS, of which we will highlight the presence of Al, Mn, Fe, Zn, and Sr, which were our point of focus to associate them with the present cytotoxic damage. **Conclusion:** The results shown are preliminary data on the cytotoxic damage of liquid samples leached from oak leaves, which contain heavy metals that come from urban pollution in the city of Monterrey, specifically in the surroundings of the UANL university city. The presence of metals such as Al, Mn, Fe, Zn, Br and Sr turned out to have high concentrations compared to the rest of the metals analyzed.

Keywords: Air pollution, damage celular, heavy metals.

Environmental Biotechnology

Biogas produces changes in metabolic diversity of bacterial associated to *Scenedesmus* sp. affecting thus the behavior of microalgal cultures

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Biogas is an effluent produced by the anaerobic digestion of organic waste in biotechnological processes like wastewater treatment or even the Tequila and Sotol industry. The CH₄ present in effluent represent a valuable source of potential alternative energy. Nevertheless, presences of CO₂ in biogas reduce the calorific value of CH₄. From this, microalgae cultures have been proposed to consume the content of CO₂ from biogas through their photosynthetic activity. Nonetheless, the effect of biogas on microalgae cultures has been mainly evaluated in axenic cultures, conditions that are difficult to sustain on industry applications. Moreover, bacteria present in non axenic microalgae cultures could induces to a rapid adaptation of the microalgae cells to new culture conditions. In this sense, the objective of the present work was compare the effect of synthetic biogas (75% CH₄ and 25% CO₂) and air on the metabolic diversity of bacteria associated to *Scenedesmus* sp. cultures as well as to known the effect of this gaseous effluent on growth and metabolic composition of axenic and non axenic *Scenedesmus* sp. cultures cultured during 20 days. Biogas induces a decrement in the metabolic diversity of bacteria associated to *Scenedesmus* sp. comparing to the metabolic diversity observed when the cultures where grown under air. Moreover biomass production under biogas was 350% higher than the biomass produced under air and independent of the bacterial presence. Add to this, a higher protein and carbohydrate was observed when the microalgae cultures were growing under biogas comparing with air. Nonetheless, and independent of the atmosphere tested, axenic *Scenedesmus* sp. cultures showed a higher proteins and carbohydrate content than non axenic microalgae cultures. In conclusion, biogas induces changes in metabolic diversity of bacteria associated to *Scenedesmus* sp. without affect the growth of microalgae but affecting the production and accumulation of metabolites by the microalgae cells.

Key words: Biogas; Microalgae-bacteria interaction; Biomass production



Environmental Biotechnology

Design and construction of an electrochemical cell: quantification of hydrocarbons in contaminated soils.

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Introduction: The Municipalities of Pedro Escobedo and Huimilpan, located in the state of Querétaro, Mexico, face a significant environmental problem: soil contamination by hydrocarbons. This situation, derived mainly from activities such as fuel theft (“huachicol”) and the growth of the chemical industrial zone. In this study, the design and construction of an electrochemical cell was carried out, as well as the quantification of hydrocarbons and extraction of biocatalysts, to evaluate the bioremediation of contaminated soils. **Methodology:** An electrochemical cell was designed, constructed of acrylic, approximately 20 cm long and 7 cm in diameter, with two compartments on the sides to contain the electrolyte solutions and titanium electrodes. Soil samples were collected in the municipalities of Huimilpan, Qro. (Hui) and Pedro Escobedo, Qro., (Pe), through NOM-138-SEMARNAT/SSA1-2012. Subsequently, the extraction of hydrocarbons was carried out using the reflux extraction method (soxhlet). Previously, a standard curve of hexadecane was carried out at 280 nm. The extraction of the native biocatalysts from the soil samples was carried out through a microbiological analysis (gram stain and CFU/ml). **Results:** The construction of an electrochemical cell was obtained to be used through a low intensity electric current that induces alterations in the metabolism of biocatalysts extracted from soil. The quantification of hydrocarbons presents in the soil for the municipality of Huimilpan (Hui) was 103.09 ppm and for Pedro Escobedo (Pe) was 125.64 ppm. On the other hand, through soil microbiological analysis, native biocatalysts, gram (+) bacteria and filamentous fungi were found morphologically. For (Hui) 7.2×10^{10} CFU/g was obtained, in soil samples from (Pe) 3.5×10^{10} CFU/g was found. **Conclusion:** It has been shown that the application of a low intensity electric current induces alterations in the metabolism of microorganisms, which is why the design and construction of an electrochemical cell was achieved. With the above, the extraction of hydrocarbons and native biocatalysts from real areas contaminated by hydrocarbons is demonstrated.

Keywords: electrochemical cell, soil, hydrocarbons, biocatalyst



Environmental Biotechnology

Study and optimization of microbial bioplastics by a *Bacillus* strain using Taguchi method

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Introduction: Environmental pollution by plastics has gradually increased over the years, a trend that was further increased by the COVID-19 pandemic. In this context, the use of bioplastics derived from microorganisms is proposed as a solution to the problem. Polyhydroxybutyrate (PHB) is produced by various species of the genus *Bacillus*, which have reported superior yields compared to other bacterial genera. One way to increase PHB production is through the implementation of experimental designs such as the Taguchi method, which is notable for identifying individual optimal parameters. **Methodology:** Gram, Sudan Black B, and Nile Blue A staining were performed on a strain of the genus *Bacillus*. Additionally, PHB production was evaluated using a Taguchi experimental design, assessing 25 experimental runs where five different pH levels, agitation speeds, temperatures, and inoculum percentages were studied in GRPD growth medium. PHB extraction process consisted of cellular biomass collection, after that pellets were obtained with NaOCl and chloroform. Finally, the polymeric extract was analyzed by FTIR. **Results:** PHB extraction was observed through staining as intracellular granules to compare, and the optimal production conditions were determined to be 150 rpm, pH 7, 28°C, and 2% inoculum, according to the Minitab analysis. Subsequently, the structure of PHB was observed through FTIR, where a characteristic peak near 1720 cm⁻¹, indicative of the ester bond, was noted. **Conclusion:** The optimization of parameters allows for the determination of the optimal conditions to produce this biomaterial.

Keywords: *Bacillus*, Fermentation, Polyhydroxybutyrate, Taguchi design

Environmental biotechnology

Environmental assessment of anthropogenic activities in The Cuatro Ciénegas Basin, Coahuila, using microbial indicators.

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Introduction: Bacterial communities developing in sediments play a crucial role in biogeochemical cycles. The diversity and abundance of different taxa affect organic matter decomposition, nutrient recycling, plant diversity, and productivity. Changes in the presence or quantity of various microorganisms can be related to anthropogenic activities. Therefore, the determination of microorganisms, such as total coliforms and enterococci, serves as an indicator of fecal contamination, and as an additional indicator, the abundance of antibiotic-resistant bacteria can be included. **Methodology:** Sediment samples were taken at three locations of the Mezquite river, at four times during one year. Standard methodologies were used for *Enterococcus spp.* enumeration from soil sample by dilutions and spread plate technique. To determine the phenotypic profile of antibiotic resistance, pure cultures were isolated from soil samples and tested against seven different antibiotics (Ampicillin, Kanamycin, Cefotaxime, Chloramphenicol, Vancomycin, Tetracycline, and Ciprofloxacin) at concentrations established by the CLSI. Additionally, the physicochemical properties of soil were determined according to standard methodologies. **Results:** A pattern was observed in the number of multidrug-resistant strains isolated depending on the sampling season, ranging from 14-21-77-6, as well as significant differences in *Enterococcus spp.* counts depending on the sampling time and site (3.21 to 4.33 LogCFU/gr). Statistical analysis did not show a correlation between the tourist activity and the presence of enterococci. On the other hand, the number of multidrug-resistant bacterial isolates can be influenced by tourist influx. These microbiological indicators prevail in arid conditions, sandy, alkaline soils, and oligotrophic conditions, where concentrations of phosphorus and organic matter, according to Mexican official standards, are considered low. **Conclusion:** The use of microbial indicators, particularly of multidrug-resistant bacteria, can be useful in assessing the influence of tourist activities on preserving the Cuatro Ciénegas Basin. This highlights its potential use as a microbiological indicator for monitoring anthropogenic impact.

Keywords: Assessment, Multidrug-resistant, Indicators, Soils, Anthropogenic-activities



Agricultural & Food Biotechnology

Screening of epibiont and endobiont bacteria and fungi producers of ACC desaminases and potassium solubilizing associated with *Ulva lactuca* of the Colombian Caribbean

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Introduction: Climate change has intensified periods of drought, putting food security at risk due to the decline in agricultural production, therefore, a search was carried out for microorganisms associated with *Ulva lactuca*, since this is a potential source of bacteria and fungi with the ability to counteract the effects of drought. In this work, the microbial communities associated with *Ulva lactuca* are searched for microorganisms that have the potential to make plants tolerate drought conditions. **Methodology:** The collection of *Ulva lactuca* was carried out on the rocky coast in Punta de la Loma of the Caribbean of Colombia (Magdalena), and the isolation of microorganisms was carried out using traditional cultivation methodologies. 71 morphotypes of microorganisms were isolated, which were evaluated in Aleksandrov culture for the solubilization of potassium and in the culture of minimum salts DF for the production of ACC deaminases. Atomic absorption spectroscopy was used to quantify potassium solubilization. **Results:** 39 isolates were positive for potassium solubilization and 33 for the production of ACC deaminases. Genera such as *Pseudomonas*, *Bacillus*, *Pseudoalteromonas*, *Brochothrix*, *Glutamicibacter*, *Rahnella*, *Rhodotorula* and *Prieta* were noted for their ability to both solubilize potassium and also produce ACC deaminases. Potassium solubilizing activity was highlighted in *Brochothrix thermosphacta*, which solubilized 110.25 mg/L of potassium and in *Pseudomonas* sp who recorded 93.9 mg/L of soluble potassium. **Conclusion:** In conclusion, endobiont and epibiont microorganisms associated with *Ulva lactuca* may represent a viable solution to mitigate the effects of drought on various crops.

Keywords: *Ulva lactuca*, endobiont microorganisms, epibiont microorganisms, potassium solubilization, ACC deaminase.



Environmental Biotechnology

Characterization of a marine bacterial lytic polysaccharide monoxygenase (LPMO)

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Introduction. Lytic polysaccharide monoxygenase (LPMO) is an auxiliary copper-dependent enzyme (CAZyme AA10); recently, LPMO was described as an alternative to chemical hydrolysis in processes for the bioconversion of recalcitrant polysaccharides such as lignocellulose or chitin. LPMO catalyze the oxidative cleavage of glycosidic bonds in the chitin chain. In the genome of the marine bacterium *Pseudoalteromonas* P80D-2, with chitinolytic activity, the putative gene for LPMO is present. Detailed studies are required on the production and the catalytic mechanism of this novel marine LPMO. The objective of this study is to increase the production of native and recombinant LPMO at fermentation level by rational design biostatistics, and molecular techniques for biochemical characterization. **Methodology.** The strain *Pseudoalteromonas* P80D-2 was cultivated in marine media. LPMO activity was evaluated by colorimetric assay (Brander *et al.*, 2021) and analytical techniques. **Results.** The LPMO activity was increased at low concentrations of carbon source and salts with a protein secretion pattern in the extract concentrated and dialysated. **Conclusion.** The strain P80D-2 from Gulf of Mexico produces LPMO with potential for degradation of polymeric substrates.

Key words: LPMO activity, *Pseudoalteromonas*, production.

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“Temporal Interplay of Microbial Diversity and Phytochemical Accumulation in *Agave lechuguilla guishe* Juice”

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Introduction: The *Agave lechuguilla*, integral to local economies in northern Mexico for its fiber, also produces bagasse, better known as *guishe*, a byproduct harboring microbe that synthesizes valuable compounds. This study investigates the microbial profiles in *guishe* juice and their relation with phytochemical accumulation through metagenomic and phytochemical analyses.

Methodology: *Guishe* samples were collected from the Cosme shared land in Ramos Arizpe, Coahuila. Following extraction using a hydraulic press, the juice underwent 15 days of fermentation, with aliquots sampled every 24 hours for phytochemical analysis. Semiquantitative techniques, including DNS, Folin-Ciocalteu, and Flavonoids, were employed to characterize sugars, polyphenols, and flavonoids. Four different samples were collected for DNA extraction and microbial characterization. **Results:** *Guishe* juice exhibited color changes from green to brown throughout fermentation, culminating in a dark brown hue. Characterization unveiled higher concentrations of essential components in the 15 days samples pivotal for various industrial applications' antioxidant processes. A consistent linear trend was noted in sugar and polyphenol concentrations, accompanied by an uptick in flavonoids. Regarding the microbial composition, an abundance of bacteria, bacilli and cocos. The four samples collected were used to get total DNA for metagenomic analysis. These observations enabled the establishment of a DNA extraction and amplification process at 4 points in the kinetics, revealing defined bands from day 0 to day 15, despite the lower integrity and quality compared to ideal standards.

Conclusion: The sample demonstrated superior concentrations across all characterization techniques. The specimens showed sugars and polyphenols decreased from the first day and increased flavonoids. Concentration variations emerged in subsequent days for acetic acid quantification, with the highest accumulation observed on one of the intermediate days (9). Therefore, the extraction of genetic material was instrumental for metagenomic analysis in observing the predominance of microorganisms present concerning acetic acid accumulation. This holistic understanding of *guishe* juice composition offers valuable insights for leveraging its industrial potential, indicating promising avenues for future research and biotechnological advancements.

Keywords: *Agave lechuguilla*, *guishe* phytochemical, microbial, DNA.

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Environmental biotechnology

Valorization of tarbush leaves (*Flourensia cernua*) for the accumulation of biomolecules through ultrasound-assisted extraction and its antioxidant activity

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Introduction: *Flourensia cernua* is an endemic plant species that grows in semi-arid areas and has been investigated for its bioactive properties. These bioactivities are due to compounds present in the plant that have been extracted by conventional techniques, which have disadvantages, such as the large number of solvents required and the long extraction times. On the other hand, emerging techniques offer higher extraction yields with shorter extraction times; however, it is necessary to consider the type of solvent and other conditions to maximize the effects. That is why the objective of this work was to perform an optimization of such extraction using a central composite design with five central points. Where the factors to be evaluated remained the same (solvent concentration, temperature, and time).

Methodology: Optimization factors (solvent concentration, temperature, and time) were carried out under a central composite design. Subsequently, total polyphenol and flavonoid contents were determined by Folin-Ciocalteu and aluminium chloride, respectively. Finally, antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) methods.

Results: In this work, the optimum point between temperature and solvent was between 50 % ethanol with a temperature of 50 °C. It was observed that extraction time is not significant. Furthermore, the interaction between both factors (i.e., ethanol concentration and temperature ratio) was significant, indicating that the effect of ethanol concentration is not the same with all temperatures. For the case of polyphenol yield, up to 24 mg/g were obtained. On the other hand, 163.98 mg/g of flavonoids were obtained. Finally, for antioxidant activity determination, values of 58.42 and 90.36 were obtained for DPPH and ABTS, respectively.

Keywords: Tarbush, biomolecules, allelopathy



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FRONTIER IN BIOTECHNOLOGY

Endosymbiont engineering: Metabolic, transcriptomic and genomic analysis of aromatic compound production under nitrogen limitation in *Kluyveromyces marxianus* associated with an endosymbiotic bacterium

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Introduction: Mezcal, an emblematic drink of Mexican culture, seeks to be standardized using Indigenous yeasts such as *Kluyveromyces marxianus* to optimize resources, time, and quality. This study analyzes two strains of *K. marxianus* isolated from fermentations in Durango, Mexico, highlighting their ability to produce industrial aromatic compounds. **Methodology:** Genomic and transcriptomic studies show that the production of 2-PE and 2-PEA in the presence of phenylalanine comes from both the Ehrlich pathway and the activation of the Shikimate pathway, while in the absence of phenylalanine, production is exclusively de novo. **Results:** Twenty-one aromatic molecules were identified, including esters, higher alcohols, and aldehydes, with strain ITD0211 being more efficient in producing essential compounds such as 2-phenylethanol (2-PE) and 2-phenylacetate (2-PEA), even under limited nitrogen conditions. The differences in the production of these compounds can be attributed to the genetic diversity and the adaptation of the strains to their environment. A significant finding is the presence of an endosymbiotic bacteria in *K. marxianus*, which seems to supply ammonium by fixing environmental nitrogen, thereby reducing the need for external nitrogen sources. This symbiosis could be a crucial factor in the strain's ability to produce a variety of aromatic compounds without the need for expensive nitrogen supplements, highlighting the complexity of the process and the potential for further research. The overproduction of 2-PE and 2-PEA in *K. marxianus* is linked to the overexpression of key enzymes in the Ehrlich pathway and modifications in the Shikimate pathway, as well as to the strain's genomic composition. **Conclusions:** These results suggest that *K. marxianus* is not only an efficient model for the production of industrial aromas but also a cost-effective one. By leveraging its symbiosis with an endosymbiotic bacteria, it minimizes production costs by eliminating the need for nitrogen supplements. This potential for cost savings makes *K. marxianus* an attractive option for Mezcal production.

Key words: Rational engineering, optimization, fermentation, endemic microorganisms, yeast endosymbionts.

Endosymbiont engineering: strategy for detection, description and exploration of endosymbiotic bacteria in yeast

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Introduction: The impact of endosymbionts has been investigated using molecular tools and technologies that have allowed us to gather information from different perspectives to understand their influence on metabolic processes associated with endosymbiotic interaction. This study proposes a strategy for detecting, describing, and exploring endosymbionts in the yeast *Kluyveromyces marxianus* isolated from the mezcal fermentation process in Durango, Mexico. **Methodology:** The bacteria associated with the yeast was identified by polymerase chain reaction (PCR) using specific oligonucleotides. We assessed the diversity and abundance of bacteria based on the needs of the yeast through 16S amplicon sequencing analysis under conditions of presence and absence of nitrogen sources. In addition, we observed these bacteria using brightfield microscopy. **Results:** Molecular analyses confirmed the presence of bacteria, actinobacteria, and archaea in *K. marxianus*. Although bacterial diversity was consistent, the abundance of specific genera, such as Bacteroides, Alistipes, and Barnesiella, increased in response to the metabolic needs of yeast, especially under nitrogen starvation conditions. Bacteria were observed in the vacuole and the cytoplasm of yeast, with a notable increase in their abundance under nutritional stress conditions. **Conclusion:** These results suggest that the endosymbiotic interaction in *K. marxianus* is highly adaptive, impressively critical for its survival and metabolic functionality in unfavorable environments. This symbiosis could offer new opportunities to optimize industrial and biotechnological processes in which this yeast participates, highlighting the importance of endosymbionts in metabolic regulation and their potential application in industry.

Keywords: Rational engineering, directed endosymbiosis, yeast endosymbionts, molecular detection, diversity and abundance bacteria

The Heparin Revolution: Enzymatic Swine Liver Extraction for Next-Level Hemodialysis

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Introduction: The prevalence of chronic kidney disease (CKD) has been rising steadily over the years, posing an escalating public health challenge. Heparin is a high molecular weight sulphated glycosaminoglycan, which exerts its anticoagulant effect by inhibiting the activity of thrombin and other proteins involved in blood coagulation. This property makes heparin essential in medical procedures that require anti-coagulation, such as haemodialysis in CKD. Hereupon, heparin was enzymatically extracted from the swine liver to functionalize chitosan-based membranes for hemodialysis application. **Methodology:** The liver was crushed, and enzymatic hydrolysis was carried out using alcalase 1% (W/V) during 6h. The hydrolysed solution was subsequently processed through a filtration system equipped with a 10 kDa cut-off membrane and quantified by HPLC. A hybrid chitosan-based membrane with polyvinyl chloride (PVC) was prepared in a ratio of 1:2, respectively. A proof-of-concept phase for hemodialysis, focusing on the semi-permeability parameters was performed using urea and albumin indicators (urea 37 mg/dL and albumin 8 g/dL). A membrane with 6x1 cm was used in Gilson Minipuls 3 peristaltic pump. The membrane was functionalized with heparin (6.67 mg/mL) by dipping procedures for further dialysis studies. **Results:** This work proposes a green and sustainable method of heparin extraction from swine liver and developed heparin-functionalized membranes for greener and biological dialysis systems. The membranes permeated 70% urea and retained 100% of albumin. **Conclusion:** This method demonstrates an efficient approach for heparin extraction from liver tissue and shows its potential in a vital biomedical application.

Keywords: Liver, heparin, chitosan, membrane, hemodialysis

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Frontier in Biotechnology

Integrating Artificial Intelligence in Protein Modeling: Advancements and Biotechnological Implications in *Helicobacter pylori* Research

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Introduction: Protein modeling has been revolutionized by artificial intelligence, especially by DeepMind's AlphaFold, which predicts structures with high precision. *Helicobacter pylori*, a bacterium associated with gastric diseases, uses its type 4 secretion system to inject effector proteins into host cells. The CagX protein is crucial in this system. Modeling CagX with AI has significant biotechnological implications, such as the design of specific inhibitors, vaccine development, secretion system engineering, and evolutionary studies. These technologies enhance our understanding of pathogenesis and offer new avenues for treatments and biotechnological applications. **Methodology:** The sequence of CagX from *H. pylori* 26695 was downloaded from NCBI and its secondary structure was analyzed using PSIPRED. Utilizing AlphaFold3 and hybrid modeling methods, the tertiary structure was obtained, validated using tools such as MolProbity and PDBsum. ChimeraX was employed to assemble a 14-subunit CagX multimer, validated by PDBsum. The multimer was then integrated into the complete T4SS. **Results:** The use of AlphaFold3 and hybrid methods has enhanced the accuracy in modeling the CagX protein from *H. pylori* 26695, validated by MolProbity and PDBsum. ChimeraX facilitated the assembly of a 14-subunit CagX multimer, validated by PDBsum, and its successful integration into the type 4 secretion system. **Conclusion:** These findings suggest the functional viability of the multimer and underscore the importance of protein modeling with artificial intelligence when complemented by experimental methods. Moreover, significant biotechnological implications are highlighted, including the design of specific inhibitors and the development of vaccines against diseases associated with *H. pylori*.

Protein modeling; Artificial intelligence; *Helicobacter pylori*; Secretion system type IV

Note: Next page shows an example of abstract

Alternative high-quality protein ingredients as key nitrogen source for probiotics

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Introduction: Microbiological culture media are essential for studying microbial growth in laboratory settings. A key and costly component of these media is the nitrogen source which is crucial for supporting bacterial metabolism and growth. Nowadays, the valorization of food by-products and the use of new sustainable protein sources (e.g. insects) is a priority. Animal by-products are routinely dumped in landfills without adequate treatment, resulting in environmental impact. Recent research suggests that proteins, hydrolysates, and peptides can boost probiotic development. This study aimed to characterize and evaluate the potential of alternative protein hydrolysates as substitutes for the traditional protein used in microbiological culture media.

Methodology: Three protein hydrolysates were obtained from pork (PH) and fish (FH) by-products and black soldier fly larvae (IH). These hydrolysates were characterized regarding protein content (Kjeldahl method), peptide profile (HPLC-SEC), free amino groups (TNBS), free amino acids (HPLC) and mineral content (ICP-OES). Bioactive properties were also evaluated, such as antioxidant activity by ABTS and ORAC. The growth analysis of probiotics *Lactobacillus casei* 01 and *Lactocaseibacillus rhamnosus* LGG was studied for three concentrations of the protein hydrolysates (0.5, 1 and 2.5% w/v) replacing traditional protein used in Man Rogosa Sharpe broth (MRS), i.e. peptone and yeast extract (2.5% w/v). **Results:** The hydrolysates had a high crude protein content between 60-90%, consisting mainly of peptides <10 kDa and a high amount of free amino acids, generated during the hydrolysis process. The most abundant minerals in all hydrolysates were potassium, phosphorus, sodium and calcium. Hydrolysates have promising antioxidant activity, highlighting FH and especially IH, which obtained ABTS values of $126.5 \pm 3.1 \mu\text{mol eq. Trolox/g}$ and ORAC of $530.7 \pm 37.8 \mu\text{mol eq. Trolox/g}$. The investigated media demonstrated similar or better growth of the probiotics strains, when compared with the reference. Notably, comparable growth was achieved by using smaller amounts of protein hydrolysate, specifically, 1% for FH and 0.5% for IH. **Conclusion:** The exploration of a new class of alternative high-quality protein ingredients proves to be an interesting approach to the traditional prebiotics and protein used in culture media.

Keywords: Protein hydrolysates; Alternative protein sources; Industry by-products; Microbiological nutrient media; probiotics

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Frontiers in Biotechnology

Endosymbiont engineering: isolation, sequencing, genomic exploration and biotechnological bioprospecting of a yeast endosymbiotic bacterium

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Introduction: *Kluyveromyces marxianus* is an industrially significant yeast often isolated from fermentation processes. It has the ability to assimilate lactose, is thermotolerant, and produces volatile metabolites with organoleptic properties. In this study, a close interaction between *K. marxianus* and a group of endosymbiotic bacteria, which play a crucial role in its physiological functioning, has been identified. These bacteria have various application, and those retaining their ability to grow freely can have the potential to be applied in food production, antibiotic generation and especially in the production of secondary metabolites. The objective of this work is to describe the genomic-metabolic characteristics and biotechnological potential, with a special focus on a strain of *Pseudomonas asiatica*, an endosymbiotic bacterium of *K. marxianus* isolated from artisanal fermentation in Durango, Mexico. **Methodology:** Endosymbiotic bacterias from *K. marxianus* were isolated using microbiological techniques. DNA was extracted, genomes were sequenced, and a genomic-metabolic analysis was performed using bioinformatics tools to reveal genes potentially involved in the production of secondary metabolites of biotechnological interest. **Results:** Twelve genomes were annotated using the BV-BRC tool, which enabled a bioprospecting process where metabolic pathways involved in the production of secondary metabolites were identified. The genome of *P. asiatica* was then selected for in-depth investigation to elucidate biotechnologically relevant biosynthetic pathways. The analysis of operons reveals, in theory, the progression of alginate metabolite production up to its transport out of the cell. **Conclusions:** The results of this study provide valuable insights into the metabolic pathways and key genes involved in the production of secondary metabolites by *P. asiatica*, an endosymbiont of *K. marxianus* isolated in Durango, Mexico. The identification of operons and genes such as *algR*, implicated in the biosynthesis and transport of alginate, highlights the potential to leverage these endosymbiotic interactions to enhance biotechnological processes. These findings open new opportunities for the application of endosymbionts in the optimization and development of biotechnological products, offering an innovative approach to the sustainable production of high-value industrial metabolites.

Key words: Endosymbionts, bacterial bioprospecting, genomic exploration, endemic microorganisms.



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HUMAN HEALTH & BIOTECHNOLOGY

Hydrothermal preparation of Selenium-doped carbonated hydroxyapatite with anticancer and antibacterial activity

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Introduction: Carbonated hydroxyapatite (CHA) is similar in composition to biological apatite, making it highly suitable for various biomedical applications, particularly in hard tissue engineering. **Methodology:** In this study, selenium-doped carbonated hydroxyapatite (CHA-Se) was synthesized using the hydrothermal method to explore its potential anticancer and antibacterial applications. The synthesis was conducted in a Teflon-lined autoclave at 180°C for 6 hours using selenium tetrachloride as the selenium source. The product was centrifuged, washed to pH 7, and dried at 80 °C. Selenium doping was achieved by maintaining molar ratios of Se/(P + Se) ranging from 0.01 to 0.20. The synthesized products were characterized using X-ray diffraction (XRD) to assess crystallinity and phase purity, Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) to identify functional groups, and dynamic light scattering (DLS) to determine hydrodynamic particle size. Anticancer activity was evaluated using the MTT assay on lung and cervical cancer cells over 24 hours, and antibacterial activity was assessed on *S. epidermidis*. **Results:** XRD analysis revealed that pure phase carbonated hydroxyapatite were synthesized and there was a decrease in crystallinity and crystallite size corresponding to an increase in doped selenium concentrations. FTIR-ATR analysis confirmed the formation of B-type carbonated hydroxyapatite, with increased transmittance in the phosphate group region as selenium concentration increased. DLS results corroborated the XRD findings, showing a reduction in particle size with higher selenium content. In vitro anticancer tests demonstrated that CHA-Se exhibited anticancer properties against cervical and liver cancer cells, with enhanced effects at selenium concentrations of 500, and 1000 ppm. **Conclusion:** Selenium substitution in carbonated hydroxyapatite via the hydrothermal method reduced its crystallinity and particle size but enhanced its potential for biomedical applications, particularly in anticancer and antibacterial treatments.

Keywords: Carbonated hydroxyapatite, anticancer, antibacterial, selenium

Human Health & Biotechnology

Evaluation of the Adhesion of *Levilactobacillus brevis* and *Lactiplatilbacillus rhamnosus* to Caco-2 Cells: Probiotic Potential and Future Study in Murine Models of Depression

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Introduction: Major depressive disorder significantly impacts mood, as well as neurodegenerative, cognitive, and psychomotor functions, highlighting the need for innovative therapies such as microbiota modulation through probiotics. For a microorganism to be considered a probiotic, it must meet certain criteria, notably its ability to adhere to intestinal cells. This study evaluated the adhesion capacity of two probiotic bacteria, *Levilactobacillus brevis* and *Lactiplatilbacillus rhamnosus*, to Caco-2 cells, a human colon adenocarcinoma cell line that differentiates into mature enterocytes under standard culture conditions. This evaluation is crucial for future research in an *in vivo* model of depression.

Methodology: First, 20-hour growth kinetics were performed to determine growth times. For the adhesion assay, bacteria cultured overnight were centrifuged and resuspended in PBS at 10⁸ CFU/mL (*E. coli* 11 EPEC as control). Caco-2 cell monolayers were washed with PBS and then exposed to the bacterial suspension (1:100 ratio) for 1 hour at 37°C. After washing to remove non-adherent bacteria, cells were lysed with 0.01% SDS. Serial dilutions were plated on MRS agar for CFU counting and calculation of the adhesion percentage. Additionally, Giemsa staining was used for microscopic observation of adherence. **Results:** The adhesion assays demonstrated a strong adhesion capacity in both bacteria, with an adhesion percentage of 24.67% for *L. rhamnosus* GG and 12% for *L. brevis* WLP672. The control strain, *E. coli*, also exhibited its adhesion capacity along with the characteristic lesion associated with these strains. **Conclusion:** The adhesion assay revealed that the bacteria possess a good adhesion capacity to Caco-2 cells, corresponding to what is reported in the literature. The investigated strains meet this criterion, making them promising for evaluation in the modulation of the intestinal microbiota with potential antidepressant treatment in an *in vivo* model.

Key words: *Levilactobacillus brevis*, *Lactiplatilbacillus rhamnosus*, probiotics, depression, Caco-2 cells, adhesion.

Human Health & Biotechnology

Antioxidant and antitumor activity of extract from *Parthenium hysterophorus* obtained by solid-state fermentation with *Aspergillus niger*

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Introduction: *Parthenium hysterophorus* is a Mexican native plant used in traditional medicine to treat health issues and which could be a source of phenolic compounds with possible antioxidant and antitumor activity. Hence, this work was designed to evaluate the phytochemical profile, antioxidant and antitumor activity of the extract from *P. hysterophorus*. **Methodology:** The flowers of *P. hysterophorus* were subjected an extraction by solid-state fermentation (SSF) with *Aspergillus niger*, during 72 h. Subsequently, the extract was recovered and evaluated for their phenolic composition and antioxidant activity by DPPH and ABTS assay. In addition, the antitumor capacity of the extract on *in vitro* human cervical (HeLa), colon (CaCo-2), liver (HepG2) and breast (MCF-7) cancer cell lines were determined by MTT assay. **Results:** The extract of *P. hysterophorus* showed the presence of phenolic compounds (57.12 ± 2.72 mg GAE/g) and flavonoids (9.52 ± 1.18 mg QE/g). In addition, the extract inhibited radicals in DPPH (6.19 ± 2.13 % a 74.57 ± 0.13 %) and ABTS (1.06 ± 0.07 a 74.83 ± 0.77 %) assays. Finally, the extract decreased the viability of HeLa (IC₅₀: 787.60 ± 12.28 µg/mL), CaCo-2 (IC₅₀: 567.62 ± 23.32 µg/mL), HepG2 (IC₅₀: 730.20 ± 14.42 µg/mL) and MCF-7 (IC₅₀: 148.51 ± 1.27 µg/mL) cell lines. **Conclusion:** The *P. hysterophorus* extract obtained by SSF exhibited the presence of phenolic compounds and flavonoids with antioxidant activity and antitumor capacity on human cancer cell lines, exhibiting a major effect on breast cancer cells.

Keywords: *Parthenium hysterophorus*, antioxidant activity, antitumor capacity.



Human Health & Biotechnology

Physicochemical characterization and evaluation of the antioxidant potential of tunite extracts (*Opuntia cochenillifera*)

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Introduction: The tunita (*Opuntia cochenillifera*) is a cactus widely used in the traditional gastronomy of the Huasteca Potosina. It is a xerophytic plant with an expansive tree structure, multiple ascending branches and trunks that can reach up to 20 centimeters in diameter and a height among 3 to 4 meters. Its growth occurs between the months of September-April and is cultivated in warm and dry climates. The tunitas, a product of this cactus, have a green stem with long reddish stamens. The objective of this study is focused on the physicochemical, phytochemical and antioxidant characterization of tunita extracts. **Methodology:** The recollection of samples of tunita was in the locality of Palo Viejo in Ciudad Valles, S.L.P. Organic solvents such as ethanol and methanol were used to evaluate the physicochemical properties in fresh (moisture, ash, pH, acidity, soluble solids, proteins, reducing sugars, ascorbic acid and fiber), phytochemical profile and antioxidant activity (DPPH•, ABTS•, total polyphenols and flavonoids). **Results:** The physicochemical analysis showed the following results: moisture 88.99±3.34%, ash 0.153±0.017%, pH 5.33±0.024, acidity 1.267±0.125%, ascorbic acid (negative) and soluble solids 0.867±0.125° Brix. For the antioxidant determinations in total polyphenols were obtained: 79.2126±0.71 mg EAG/L in ethanol extract and 83.2930±1.81mg EAG/L in methanol, for flavonoids 174.2692±4.66mg CatEq/L in ethanol extract and 161.1556±3.08 mg CatEq/L in methanol. In the phytochemical profile positive results were obtained in the test of Wagner, unsaturations, Salkowski, cumarins, tannins and oxalates. Regarding the DPPH• radical inhibition assays, the results were 48.6959±2.66% in ethanol extract and 74.7969±3.91% in methanol, for ABTS• they were 48.7912±4.49% in ethanol extract and 47.5212±2.63% in methanol. **Conclusion:** The physicochemical analysis indicates that the tunita sample has a high protein and fiber content. High levels of polyphenols and flavonoids, especially in the methanol extract, suggest a considerable antioxidant potential, corroborated by the significant inhibition of DPPH• and ABTS• radicals. These properties could be used in the development of nutraceutical products that help the prevention and/or treatment of diseases related to oxidative stress.

Keywords: *Opuntia cochenillifera*, phytochemical, antioxidant, polyphenols, tunita.

Human Health & Biotechnology

Mexican mango seed: Research for its use as a nutraceutical.

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Introduction: Mango seed is a waste from mango processing that may have applications in human health. The objective of this work was to obtain a mango seed extract of the Ataulfo variety rich in pentagalloylglucose (PGG), the major compound. **Methodology:** The extract was partially purified, characterized for hydrolyzable tannins (HT), condensed tannins (CT) and PGG and analysed by *in vitro* assays for antioxidant (DPPH and ABTS), anti-diabetic (*in vitro* – α -amylase inhibition and *in silico* - molecular docking), anticancer (*in vitro* - MTT assay), cytotoxic (*in vitro* - MTT assay) and antiviral (*in silico* - molecular docking) potential. **Results:** The results indicated a high amount of HT (313.82 ± 46.28 mg GAE/g) and TC (22.83 ± 2.89 mg CE/g) and PGG (564.75 ± 36.92 mg/g). Antioxidant assays exhibited high activity against DPPH (IC_{50} : 28.35 ± 0.67 μ g/mL) and ABTS (IC_{50} : 41.19 ± 3.66 μ g/mL), superior to Trolox (DPPH: 126.14 ± 2.04 μ g/mL and ABTS: 148.81 ± 9.07 μ g/mL). The antidiabetic assay showed a high α -amylase inhibition capacity (IC_{50} : 173.97 ± 1.33 μ g/mL). The anticancer potential was tested in a human colon cancer line (HCT-116), the results revealed effects at 100 and 200 μ g/mL reducing cell viability by more than 30%. Cytotoxicity was determined in mouse fibroblasts (L929) and human peripheral blood mononuclear cells (PBMC). The results showed that the extract was cytotoxic only at 200 μ g/mL for the L929 line. The PBMC line did not show cytotoxicity at any concentration tested (1.56-200 μ g/mL) so the extract was considered safe. *In silico* studies demonstrated that PGG and hexagalloylglucose are responsible for the inhibition of α -amylase, furthermore, PGG showed a high potential to inhibit the Mpro protein of SARS-CoV-2, implying a therapeutic potential against COVID-19. **Conclusion:** These results demonstrate the high potential of mango seed extract for the development of a nutraceutical product.

Keywords: Antioxidant, Bioactive Compounds, Bioavailability, Human Health.

Human Health & Biotechnology

Evaluation of solid-state fermentation conditions from pineapple waste (*Ananas comosus* L.) by *Aspergillus niger* spp., for the release of polyphenols

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Introduction: The polyphenolic compounds are secondary metabolites found in different parts of plants, leaves, fruits, stem, etc. [1]. The polyphenols or tannins are classified in condensed tannins (CT) and hydrolyzable tannins (HT), which have been of great interest due to different applications in food and biotechnology industry [2]. It has been proven that pineapple has tannins and to release uses different alternative methods. The objective of the present work was to evaluate the conditions of the solid-state fermentation (SSF) process using *Aspergillus niger* spp. for the release of polyphenolic compounds from pineapple waste.

Methodology: An exploratory Plackett-Burman design was carried out to determine which were the best factors in the SSF. Temperature (°C), humidity (%), inoculum (spores/g), NaNO₃ (g/L), MgSO₄ (g/L), KCl (g/L) and KH₂PO₄ (g/L) were evaluated. Once the fermentation time had elapsed, an extract was obtained with 15 mL of 50 % ethanol and the quantification of CT by HCl-Butanol and HT by Folin-Ciocalteu was carried out. Subsequently, the determination of antioxidant activity was carried out by ABTS and DPPH methods. Finally, the compounds were identified by HPLC-MS analysis.

Results: For *A. niger* HT3, the best treatment was 6, reaching up to 86.91 mg/g of CT and 17.46 mg/g of HT. For *A. niger* Aa20, the best treatment to CT was 4, reaching up to 92.03 mg/g. The best treatment to HT was 7, reaching up 8.81 mg/g. The fermented extracts showed higher antioxidant activity compared to the unfermented samples. Treatment 6 for both strains showed the highest percentage inhibition for the DPPH assay; treatments 7 and 6 (*A. niger* HT3 and *A. niger* Aa20, respectively) showed the highest percentage inhibition in the ABTS assay. Compounds from the families of curcuminoids, hydroxycinnamic acids, methoxycinnamic acids, flavonols, lignans, catechins and anthocyanins were found.

Conclusions: The SSF using *Aspergillus niger* spp. from pineapple waste as substrate are effective for higher release of tannins compounds. The tannins present high antioxidant activity and this activity can be attributed to some families found as hydroxycinnamic acid and anthocyanins among others.

Keywords: condensed tannins, hydrolyzable tannins, Plackett-Burman, Antioxidant activity

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Analysis and Assessment of the Biological Activity of the TA-PAsp Polymer

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Introduction: Polymers have been studied for their biodegradable and biocompatible characteristics and for their physicochemical properties. Currently, great importance has been given to polymer derivatives produced from modifications, to optimize their qualities for greater benefit. The synthesis of two polymers with potential anticancer, antibacterial activity and low hemolytic effects was carried out. **Methodology:** They were obtained through the reaction of sodium polyaspartate (PAspNa) and modified with two quaternary ammonium salts, which were chemically grafted to the macromolecular chains of sodium polyaspartate with an acid-base reaction. The chemical structures of the two polymers were analyzed and confirmed by FT-IR and ¹H NMR. The corresponding thermal stability was analyzed by thermogravimetric analysis (TGA). Hemolysis tests were carried out on isolated human erythrocytes. Subsequently, *in vitro* tests were carried out against a cancer cell line (MCF-7) as well as four different bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The antibacterial activity of the TA-PAsp derivative was determined by measuring the minimum inhibitory concentration and the minimum bactericidal concentration. Finally, cell proliferation was evaluated by MTT assay after 24, 48 and 72 h of treatment. **Results:** The polymer TA-PAsp (New molecule) were synthesized and characterized. The chemical modifications in the structure of the salts were confirmed by NMR and the addition of PAspNa to the modified polymer. It was confirmed that both modified polymers are thermally stable by thermogravimetric analysis. In the case of the TA-PAsp polymer, it was slightly hemolytic at concentrations of 12 and 25 ppm. It was observed that the polymer only had a cytotoxic effect in MCF-7 cells only at a concentration of 1800 µg/mL at 24 h. The TA-PAsp polymer had an effect on *Escherichia coli* and



Pseudomonas aeruginosa at 2000 µg/mL with a growth reduction of 50 and 27% respectively. **Conclusion:** The polymer developed in this work has a better potential as an antibacterial, confirming the decrease in the microbial load. Its possible antiproliferative effect on cancer cells is ruled out.

Key words: polymers, sodium polyaspartate, ammonium salts, biological activity.



Antimicrobial effect of *Capsicum annuum* on pathogens causing foodborne diseases

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Introduction: Nutritional quality and food safety are two aspects that have a direct impact on the health and life quality of populations, therefore foodborne diseases (FBD) are a problem that is considered in a technological, economic and social environment, this leads us to seek new ways for food to have a longer shelf life without compromising the quality, composition and characteristics of products. The use of extracts from different sources such as plants, fruits or vegetables is a great option to substitute chemical additives used in food. That is the case of plants belonging to the *Capsicum* genus, which have been reported to have antimicrobial activity when extracts of different species such as *C. chinense*, *C. frutescens*, *C. pubescens*, among others, are used; however, there are still few studies on *C. annuum* (bell pepper) regarding its biotechnological use. **Methodology:** The *Capsicum annuum* extract was made by macerating the bell pepper sample in isopropyl alcohol and then evaporating it with a modified Soxhlet and finally concentrating it with a rotary evaporator. The inhibition test of *Capsicum annuum* extract was performed by the modified Kirby Bauer method using *Salmonella Typhi*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Cronobacter sakazakii*. **Results:** A yield of 1.6% was obtained from the bell pepper extract. Inhibition halos with a diameter between 7 and 13 mm were recorded in the strain corresponding to *Bacillus cereus*. **Conclusion:** *Capsicum annuum* extract has inhibitory action against *Bacillus cereus*.

Key words: *Capsicum annuum*, extract, inhibition, *Bacillus cereus*.

Human Health & Biotechnology

Physicochemical and proximate characterization of aguamiel from the species *Agave salmiana* and *Agave atrovirens* for the subsequent evaluation of its hypoglycemic effect in a type 2 diabetes-induced murine model.

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Introduction: Mead has various health benefits attributed to it, among them the potential to lower blood glucose concentration. Two of the most commonly used species for mead production are *Agave atrovirens* and *A. salmiana*. The objective was the characterization and proximal analysis of mead from both species to select the one with the best characteristics for its subsequent administration in an induced murine model with type 2 diabetes. **Methodology:** Mead of both species was pasteurized at 80°C for 10 minutes and subsequently stored at -80°C. The following physicochemical parameters were analyzed: density (densitometer), viscosity (viscometer), pH (potentiometer), alcohol content (Gay Lussac alcoholmeter), turbidity (turbidity meter), color (colorimeter), brix (refractometer), titratable acidity (titration with NaOH) and the following parameters were determined in the proximate analysis: Moisture (AOAC 925.45), protein (Kjeldahl), crude fiber (AOAC 962.09), fat (AOAC 945.16), ash (AOAC 920.181) and total sugars (Antrona). **Results:** The mead from *A. atrovirens* obtained higher results in the parameters of viscosity (1.35 mPa.s), brix degrees (14), ash (12.57%) and protein (1.43%) with respect to the mead from *A. salmiana*: Viscosity (1.35 mPa.s), brix degrees (14), ash (12.57%) and protein (1.43%) concerning the mead from *A. salmiana*: viscosity (1.35 mPa.s), brix degrees (10), ashes (2.55%) and protein (1.06%), while it has a lower percentage of moisture (84.55%) than that of *A. salmiana* (90.57%). Both samples had 0 alcohol content. **Conclusion:** *A. atrovirens* is the most suitable for administration in a murine model to evaluate its possible hypoglycemic effect due to its better nutritional content and higher sugar content.

Key words: Agave, mead, characterization.

Antimicrobial potential of *Eysenhardtia texana*: a medicinal plant from arid zone of Mexico

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Introduction. The use of medicinal plants continues to be a great contribution to the health of the world's population, especially for populations with fewer resources and access to modern medicine. The *Eysenhardtia* genus includes 14 species that have been used in traditional medicine for the treatment of kidney and bladder infections. It is worth, there is little scientific evidence that exists about this genus. Therefore, the objective of the present study was to obtain and characterize extracts of *E. texana* and evaluate their antimicrobial activity. **Methodology.** Aqueous extracts (Ac), and hydroalcoholic extracts (HA) Ethanol:water 50:50 and 70:30% in leaves (H) and branches (R) were evaluated for antioxidant activity (%RSA). **Results.** The highest yields were observed in the H-HA50:50 treatments with $15.17 \pm 0.86\%$ and in H-HA70:30 with $12.91 \pm 0.53\%$, with the lowest yield being all the branch extracts. Regarding the content of total phenols (CFT), higher values were presented in R-Ac, R-HA50:50 and R-HA70:30 (548.89, 554.04 and 598.68 mg AG/g extract respectively). For the antioxidant capacity by ABTS, differences were presented between the R-Ac treatment with a higher content with 84.0 IC₅₀ mg/mL and a lower content in H-HA70:30 of 38.5 IC₅₀ mg/mL. On the other hand, the antioxidant capacity by the DPPH method was higher in H-HA50:50, H-HA70:30 and R-HA70:30 (70.2, 72.2 and 69.1 IC₅₀ mg/mL, respectively). In antifungal activity against *Fusarium oxysporum*, 100% inhibition was achieved in the extracts, H-HA50:50, H-HA70:30 and R-HA70:30 in concentrations from 2000 mg/mL, in contrast to the aqueous ones. On the other hand, the antibacterial activity evaluated against the strain of clinical interest *E. coli* did not show an inhibitory effect. **Conclusion.** this research offers new knowledge on *E. texana*, showing encouraging results for the control of phytopathogenic fungus that causes diseases in several plants, causing great losses in agriculture.

Key words: *Eysenhardtia texana*, antimicrobians, antioxidants.

Prospective study of tick-borne bacteria of clinical interest in locations of Chihuahua, Mexico.

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Introduction: Chihuahua is one of the states with the highest number of cases of Rocky Mountain Spotted Fever (RMSF) and other rickettsiosis. These diseases are transmitted by ticks, which are considered important vectors of human and animal pathogenic microorganisms. Due to the increase in cases of tick-borne diseases, a “One Health” approach can support the control of these pathogens. Therefore, the objective of this work was to preliminarily evaluate the prevalence of Spotted Fever Group (SFG) and Typhus Group (TG) *Rickettsia* spp., *Borrelia burgdorferi* and *Ehrlichia canis* associated with ticks from communities in Chihuahua. **Methodology:** The collection of brown dog ticks (*Rhipicephalus sanguineus*) from municipalities of Chihuahua was carried out from households with clinical cases of suspected or confirmed Rocky Mountain Spotted Fever (RMSF) reported in 2022. The identification of *Rickettsia* of the SFG and the TG, *Borrelia burgdorferi* and *Ehrlichia canis* was carried out by PCR. **Results:** A total of 1,961 ticks were collected, mainly from areas of Nuevo Casas Grandes and Ciudad Juárez. In the analysis, a general prevalence of 12% of SFG *Rickettsia*, 7% of *B. burgdorferi* and 2% of *E. canis* was found. Dogs were the main hosts with a 23% overall pathogen prevalence. The minimum range of total infection for SFG *Rickettsia* was 2.49%, for *B. burgdorferi* it was 2.10% and for *E. canis* it was 0.6%. Of 35 tick samples from homes with confirmed clinical cases of RMSF in Juárez, 8.57% were positive for SFG *Rickettsia*. **Conclusion:** This work provides a preliminary overview of the prevalence of SFG and TG *Rickettsia*, *B. burgdorferi* and *E. canis* in two major localities of Chihuahua, in addition to identifying possible risk factors for the transmission of pathogens by ticks, which may contribute to local public health policies.

Key words: Ticks, One Health, Rickettsia.



Human Health & Biotechnology

Rational design of antigens as molecular tools in the epidemiological control of zoonotic diseases.

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Introduction: The rational design of antigens has become a molecular tool for the epidemiological control of different diseases. This work aims to analyze the mechanism of viral fusion proteins using bioinformatic techniques. This allows us to know the properties of pathogens to develop biotechnological tools that help us predict the behavior of these zoonotic diseases from an epidemiological perspective. **Methodology:** This approach is based on integrating bioinformatics tools to optimize biological antigens based on the structural knowledge of these viruses, which will allow us to increase the effectiveness of rational designs by preserving immunogenic characteristics. **Results:** A bioinformatic analysis generated perspectives on the structural biology of the RHDV VP60 protein, the DENV E protein, and the EBOV GP protein. **Conclusion.** The structural analysis of etiological agents generates important information for the rational design of recombinant tools that help to epidemiologically predict the zoonotic behavior of diseases.

Keywords: Antigen-design, structural-biology, zoonoses, viral-fusion-proteins.



Human Health & Biotechnology

Vocal Biomarkers for detection and monitoring of emotional state

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Introduction: The human voice, beyond a means of communication, reflects an individual's emotional and physical state. Over the last decade, vocal biomarkers have emerged as promising tools for detecting illnesses and mental disorders. These biomarkers reveal valuable information about an individual's health, from physical conditions like Parkinson's and Alzheimer's to mental disorders such as depression and anxiety. This non-invasive early detection capability allows for timely interventions and personalized treatments, thereby enhancing patients' quality of life. This project aims to explore the intersection of human voice and emotions, promoting the use of innovative tools for early detection and management of emotional and behavioral disorders among students at the Universidad Autónoma de Coahuila. **Methodology:** A cross-sectional analytical study will investigate the relationship between vocal characteristics and the presence of anxiety or depression. Screening instruments, including the PHQ-9 and GAD-7 questionnaires, alongside voice analysis by artificial intelligence (three responses, each lasting a minimum of 20 seconds), will be utilized. Data will be analyzed using descriptive statistics. **Results:** Possible associations between vocal characteristics and anxiety/depression will be identified. Descriptive statistics will reveal symptom prevalence. Recommendations for seeking professional attention will be provided for identified cases. **Conclusion:** The study aims to validate the utility of vocal biomarkers for early detection of emotional disorders.

Keywords: Vocal biomarkers, depression, anxiety.



Human Health & Biotechnology

Biosynthetic Potential of Extreme-Tolerant Actinobacteria from Cuatro Ciénegas, Coahuila for Antimicrobial Compounds against Drug-Resistant Clinical Isolates

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Introduction: The global crisis of antimicrobial resistance is alarming, and it is estimated that in 25 years it will cause the death of more than 20 million people. To offer an alternative that mitigates the crisis, actinobacteria known for their natural products and isolated from the extreme and unexplored environment of Cuatro Ciénegas have been studied. **Methodology:** The growth of actinobacteria isolates under extremophilic conditions (pH, salt, and temperature) was evaluated. Subsequently, using the OSMAC (One Strain Many Compounds) approach, those with antimicrobial activity were identified, and their biosynthetic potential was determined using genomic mining. **Results:** A total of 107 actinobacteria isolates were evaluated, of which 97 isolates grew under alkalotolerant conditions (pH >9), 28 isolates were light halotolerant (2-5% NaCl), 77 moderate halotolerant (5-20% NaCl), and 3 thermotolerant (>45°C). Of the total actinobacteria, 89 showed antimicrobial activity against at least one of the evaluated pathogens, and 9 had activity against all pathogens. The genomes of the isolates with the highest inhibition were sequenced and identified as *Streptomyces_CHC01*, *Streptomyces_CHC02*, and *Streptomyces_CHC03*. Predictive search for biosynthetic gene clusters (BGCs) revealed the presence of 52, 39, and 30 groups for *Streptomyces_CHC01*, *Streptomyces_CHC02*, and *Streptomyces_CHC03*, respectively. Among the BGCs found, most belong to the PKS and NRPS groups, which form enzymatic complexes involved in antibiotic assembly and can generate different types and conformations. Lastly, >80% of the BGCs found had no significant similarity with reported data, suggesting that these isolates could be producers of compounds with novel conformations and, therefore, new therapeutic agents. **Conclusion:** The set of our results reaffirms the biotechnological potential that actinobacteria from Cuatro Ciénegas, Coahuila have for the production of new molecules with antimicrobial activity against clinical drug-resistant pathogens.

Keywords: Actinobacteria, OSMAC, Antimicrobial, Cuatro Ciénegas,

Stimulation in the growth of *Lactobacillus reuteri* by enriching the MRS culture medium with different prebiotic substrates

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Introduction: *L. reuteri* ATCC®55730 can generate metabolic products with benefits on the biological functions of the host because of modulation in gut microbiota elimination of infections and attenuate the gastrointestinal symptoms of enteric colitis, used as an antibiotic-associated diarrheal in inflammatory bowel disease. It is considered a possible functional food in preventing or treating oral diseases. *L. reuteri* converts naturally in the human intestine, ferments glycerol as an energy source and produces a non-protein compound called reuterin (74.08g/mol). The main objective was to evaluate the growth of *L. reuteri* when different prebiotic substrates (glucose, fructose, sucrose, fructooligosaccharides (FOS) and high molecular weight fructans from agave (FAPM), lactose, glycerol, rhamnose, cellobiose, raffinose, arabinose, mannose, xylose and galactose), are added as carbon sources as well as prebiotic effect to the MRS-broth. **Methodology:** *L. reuteri* was cultured according to ATCC specifications. The inoculum was adjusted to an $Abs_{595nm}=0.125$, equivalent to a 1.5×10^8 CFU/mL. The stimulation in the growth of this probiotic bacterium was evaluated by Optical Density at 595 nm for 24 hours in MRS-broth, replacing the prebiotic substrate with 1% of the respective carbohydrate. The test was performed in triplicate on 96-well plates in a final volume of 200 μ L and incubated at 37°C. Standard deviation was calculated with the average data obtained, and growth curves were made. Then, the exponential phase's specific growth rate (μ) was calculated. **Results:** As denoted in the growth curves, *L. reuteri* degraded each type of substrate very diversely. The presence of FOS, FAPM, lactose, and glycerol showed a slow adaptation (lag phase) of 13.75 ± 3.3 h. An extensive exponential phase was observed for cultures with glucose, fructose, FAPM, glycerol, and galactose at 7.5 ± 0.5 h. Specific growth rate was varied $\mu_{sucrose}=0.603 \pm 0.01$ h⁻¹, $\mu_{glucose}=0.46 \pm 0.01$ h⁻¹, $\mu_{fructose}=0.33 \pm 0.01$ h⁻¹, $\mu_{glycerol}=0.172 \pm 0.06$ h⁻¹. **Conclusion:** This study allowed us to know the development of *L. reuteri* in the presence of different prebiotic substrates. The results are promising, and the biological characterisation of this probiotic will continue to be explored by analysing metabolites produced during the degradation of substrate yields and exploring therapeutic applications from *L. reuteri*, reuterin as another metabolite and functional food.

Keywords: *Lactobacillus reuteri*, Specific growth rate, Prebiotic substrate, Degradation, Metabolic products.



Human Health & Biotechnology

Phytochemical screening and antioxidant activity of *Fluorensia cernua*: A chemical based analysis.

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Introduction: Traditional medicine, which includes the use of plant extracts and teas, is widely employed against infectious diseases and serves as a framework for scientists in new drug development. The objective of this study is to check the phytochemical profile, chemical characterization and antioxidant potential of *Fluorensia cernua*. **Methodology:** In this study *Fluorensia cernua* of semi desert Region of Coahuila was analyzed for the phytochemical content, and antioxidant potential. Ultrasound and maceration assisted extraction were performed using ethanol and water as a solvents. HPLC were carried out for the presence of compounds. **Results:** The crude extract of plant in the current study have good source of phytochemical with flavonoid content of 18.16 ± 0.84 mgEqCAT/g plant. Similarly hydrolysable tannin in *F. cernua* was 76.30 ± 12.10 mgEqGAE/g respectively. Few important antioxidant and antimicrobial compounds were detected in *Fluorensia cernua* are Caffeic acid 4-O-glucoside, 6,8-Dihydroxykaempferol, Scopoletin, Kaempferol, 3-Feruloylquinic acid and Rosmanol. **Conclusions:** The crude extract of *Fluorensia cernua* used in this study is a rich source of phytochemical contents and antioxidant. Presence of different compound in these plant can be a good source of antioxidant, antimicrobial, and anticancer agents.

Keywords: Phytochemical, Antioxidant, Chemical analysis.

Agricultural & Food Biotechnology

Nutritional and techno-functional properties of *Ferocactus Pilosus* inflorescence.

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Introduction: Many cactus species in the arid and semi-arid regions are edible. Mexico houses lots of undervalued, under-cultivated and underutilized cacti, without information about their composition. This study evaluated the proximal composition, mineral contents and techno-functional properties of *Ferocactus pilosus* inflorescence for the first time. **Methodology:** *F. pilosus* inflorescences were collected from three locations in Mexico [Gomez Farias (Coahuila), Cienega de Roca Montes (Zacatecas) and Nuevo Leon]. The samples were cleaned, dehydrated, grounded into flour, and sieved to obtain a homogenous powder. Furthermore, the proximal and mineral compositions, as well as the techno-functional properties of the flour were determined using standard methods. **Results:** The inflorescences showed high total crude protein, ash and fiber contents in the ranges of 9.92-11.56%, 6.17-7.50% and 17.67-19.33%, respectively. Low levels of total lipids were found in all the samples (1.5-1.67%). A wide range of mineral contents was detected in the samples (Ca, K, Mg, Cl, Zn and Fe). All the inflorescences had high techno-functional properties (water and oil holding capacity). **Conclusion:** Based on the results obtained, *F. pilosus* is a good source of nutrients and a potential material in the food industries for functional food formulation.

Keywords: Minerals, proximal composition, fat content, oil holding capacity.



Human Health & Biotechnology

From nature to the laboratory: meta-analysis of spider silk as a biomaterial with potential for high societal impact resulting from synthetic biology and bioprocess engineering.

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Introduction: Spider silk stands out as a biomaterial with great potential due to its unique mechanical and biological properties, such as high strength, biocompatibility, and low toxicity. However, large-scale production of natural spider silk is unfeasible, which has prompted the development of recombinant methods for its production. **Methodology:** A literature review was carried out to evaluate different approaches for recombinant spider silk production using different hosts such as *Escherichia coli* and other biotechnological systems. The properties of natural silk were compared with recombinant silk and other materials. In addition, production costs and environmental impact were analyzed. **Results:** The meta-analysis found that high yields of protein can be produced that can be used as biomaterial, however, these yields are associated with high production costs and high carbon dioxide emissions, highlighting the need for more sustainable methods. **Conclusion:** The design of biomaterials based on spider silk has great potential for biomedical applications, thanks to its outstanding mechanical and biological properties. Despite the challenges in recombinant production, technological advances could improve efficiency and reduce costs, making spider silk a key resource for biomedical applications and other industries.

Keywords: Spider-silk, biomaterial, recombinant-production.



Human Health & Biotechnology

Effect from Mexican avocado peel and seed phenolic extracts on breast cancer cells *in vitro*

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Introduction: Due to the high consumption of the Mexican *Hass* avocado, there has been an increase in the production of residues, mainly peel and seed, which has generated the need to take advantage of these residues to reduce the environmental impact and promote its recovery. These residues are rich in polyphenols, of which there are reports that generate anticancer, antiviral, antihypertensive effects, among others. Therefore, the objective of the work is to investigate the antiproliferative effect of these residues on breast cancer cells *in vitro*. **Methodology:** Aqueous and ethanolic extracts from waste of the *Hass* avocado (peel and seed) were used to evaluate the effect on cell viability in a 4T1 and MDA-MB-231 breast cancer cells due to the high incidence of this disease worldwide. The extraction was performed by microwave assisted extraction a 600 W, for seed were 75 °C, 15 min with 58.5% ethanol, and for peel they were 66 °C, 1 min and 42% ethanol, and same conditions with water. Bioactive compounds were identified by HPLC-ESI-MS. Breast cancer 4T1 and MDA-MB-231 cells were cultured at 37 °C with 5% CO₂ and 10% FBS. Cell viability was evaluated with peel and seed extract concentrations of 0.05-10 mg/mL of extracts using the alamar blue technique and absorbance at 560/590 nm was measured. **Results:** The results obtained showed that the ethanolic extracts and the aqueous extract of avocado peel showed a lethal concentration 50 (LC₅₀) at 0.2 mg/mL while the aqueous extract of seed reached the LC₅₀ at a concentration of 1 mg/mL. Viability in MDA-MB-231 cells decreased almost entirely (<1%) at concentrations of 5 and 10 mg/mL with the four extracts. **Conclusion:** These results showed that avocado residue extracts have a cytotoxic effect on highly metastatic breast cancer cell models *in vitro* at 24 hours of treatment.

Keywords: avocado, agroindustrial waste, breast cancer.



Human Health & Biotechnology

Metformin improves the long-term memory in diabetic mice

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Introduction: Diabetes mellitus and Alzheimer's disease share common mechanisms that warrant investigation of the effects of hypoglycemic agents on learning. This study focused on evaluating the effects of metformin at different concentrations on long-term memory in mice with type 2 diabetes mellitus. (DM2).

Methodology: The study was conducted on 27 female mice of the BALB/c strain, between 8 and 12 weeks old, divided into four groups: a control group without treatment and three groups treated with metformin at doses of 50 mg/kg, 150 mg/kg and 250 mg/kg. Metformin was administered orally once daily for one month. The study was carried out in accordance with the NOM-062-ZOO-1999 standard and approved by the Ethics Committee of the Faculty of Chemical Sciences (UAdeC, TMC-22-09-23-2). Long-term memory was assessed using the object recognition test. In addition, blood glucose levels and body weight were monitored weekly throughout the study.

Results: Initial results showed that untreated DM2 mice had poorer long-term memory than those treated with metformin. Among the treated groups, the group receiving the highest dose (250 mg/kg) showed the greatest improvement in learning. After four weeks, the control group continued to show long-term learning problems associated with elevated blood glucose levels. **Conclusion:** The metformin-treated groups showed improvements in long-term learning that were proportional to the dose administered.

Keywords: Diabetes Mellitus, Alzheimer's, Metformin, Long-term memory.



Human Health & Biotechnology

Evaluation of the Loop-amplification method using bentonite-activated carbon to improve the detection of *Vibrio mimicus* in oyster samples

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Vibrio mimicus is an emerging pathogen that can cause intestinal diseases, mainly due to the consumption of contaminated fish products. Therefore, there is an interest in preventing infections caused by this microorganism by establishing frequent and permanent surveillance that allows its timely detection. The main problem to identify *V. mimicus* through conventional microbiological methods are the high costs and long times for diagnosis. The purpose of this research was to develop a loop-mediated isothermal amplification method (LAMP) using activated carbon with bentonite (ACB) for inhibitor removal and improving the sensitivity of the test.

For the identification of *V. mimicus* in oyster samples, the methodology proposed by Luan & Levi (2010) was followed. It consists of adding 50 g of samples in flasks containing 450 mL of Peptone Water with different pH values (6, 7, 9) and 23 g of activated carbon with bentonite. Different contact times were evaluated to eliminate reaction inhibitors.

The results showed that LAMP sensitivity method was improved when samples were placed in a pH 6 solution and with a contact time of 15 min with activated carbon and bentonite. LAMP sensitivity, negative predictive value, positive predictive value, and LAMP specificity were equal or greater than 90% for all samples tested. Therefore, LAMP method could be an alternative to identify *V. mimicus* in oysters due to a faster application, higher sensitivity and lower cost than standard method.

Key words: food safety, pathogen, rapid identification.



Human Health & Biotechnology

Medicinal plants from the Mexican semi-desert that promote intestinal health: proximal and phytochemical characterization

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Introduction: In Mexico, there is a high incidence of infectious diseases caused by the consumption of water and/or food contaminated by pathogenic microorganisms. Many of these diseases are related to gastrointestinal problems, as they are disorders that affect the organs associated with digestion. The importance of the desert region in northern Mexico lies in the diversity of plants that have acquired the ability to grow under extreme climatic conditions, using a variety of chemical compounds with potential biological effects. The objective of this work is to evaluate the bioactive compounds in infusions obtained from *Artemisia ludoviciana* Nutt (stafiate), *Flourensia cernua* (hojasén) and *Phoradendron californicum* (desert mistletoe) that promote intestinal health. **Methodology:** The phytochemical profile of the plants was determined as alkaloids, hydrolyzable tannins, condensed tannins, and flavonoids. The plant material's chemical-proximal characterization (moisture, fats, fiber, protein, ashes, and carbohydrates) was carried out. **Results:** A statistical analysis of mean comparisons was conducted using the Tukey test ($p < 0.05$). Hojasén demonstrated the highest concentration of flavonoids (26.2 ± 0.57 mg/100g). Estafiate obtained the best results for hydrolysable tannins with 14.47 ± 0.06 mg/100g and presented the highest concentration of condensed tannins (39.11 ± 0.11 mg/100g). Regarding the alkaloid content, desert mistletoe obtained the highest value with 0.061 ± 0.006 mg/100g. In the proximate analysis, desert mistletoe showed a high content of proteins, fiber, and ash. Hojasén showed a higher lipid content. **Conclusion:** All the studied plants demonstrated the presence of bioactive compounds, to which their biological properties can be attributed. The proximate analysis exhibited the nutritional content of each plant.

Keywords: Gastrointestinal diseases, intestinal microbiota, medicinal plants, bioactive compounds.

Phytochemical and antioxidant determination of bioactive compounds present in purple maguey leaves (*Rhoeo discolor*)

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Introduction: Medicinal plants represent a globally recognized therapeutic alternative, especially in regions with extreme poverty and developing countries. The purple maguey (*Rhoeo discolor*) is an herbaceous plant with almost straight leaves that are purple on top and green underneath. It grows mainly in Central and South America. Traditionally used for the treatment of rhinitis, ulcers, mycosis, and cancer. It has been studied for its biological properties, being the antioxidant action one of the most significant. **Methodology:** The sample was collected in Cd. Valles, S.L.P. The extraction of bioactive compounds was carried out with ethanol and methanol in a 1:4 ratio of purple maguey leaves: solvent by means of ultrasound-assisted extraction; subsequently, phytochemical and antioxidant determinations were performed, such as: polyphenols, flavonoids, DPPH• and ABTS• radical inhibition. **Results:** For the phytochemical analysis of the methanol and ethanol extracts, a positive result was obtained in the Wagner, sesquiterpelactones, flavonoids, saponins, coumarins, phenolic oxidriles, tannins, anthocyanins, quinones and oxalates tests, and negative in the Mayer test in both extracts; the insaturations test was positive for the ethanolic extract and negative in the methanolic. For the antioxidant activity the flavonoid content of the methanolic extract was: 245.8076 ± 5.4392 mgCat/ 100 gr, and the ethanolic was: 216.3076 ± 0.3807 mgCat/ 100 gr; polyphenol content of the ethanolic extract was: 71.5272 ± 3.1701 mgEAG/100 g and the methanolic was: 71.5878 ± 2.2733 mgEAG/100 g. Finally, for the inhibition of DPPH• radical in the ethanolic extract was: 77.735 ± 0.9263 %, and the methanolic was: 66.2 ± 0.6505 %. The determination of ABTS• shows that the methanolic extract was: 67.0588% and the ethanolic extract was: 62.8958%. **Conclusion:** Both solvents were soluble for the phytochemical tests allowing the identification of bioactive compounds, however, for polyphenols and flavonoids a better concentration was obtained in methanol and a higher free radical inhibition of DPPH• and ABTS•.

Keywords: secondary metabolites, biological properties, oxidation, antioxidant.

Human Health & Biotechnology

In vitro biological evaluation of *Syzygium aromaticum* essential oil and their major compounds against *Trypanosoma cruzi* epimastigotes

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Introduction: *Trypanosoma cruzi* is a flagellated protozoan responsible for American trypanosomiasis also known as Chagas disease, which is considered neglected by the World Health Organization (WHO). The treatment is currently based on the use of the chemotherapeutics Nifurtimox and Benznidazole, however, these medications do not meet the criteria established by the WHO, both compounds are characterized by being toxic and having adverse effects such as allergic dermatitis, anorexia, or dose-dependent peripheral sensory neuropathy in addition to the various reports of resistance developed by parasites, which is why alternatives to plant-derived products have been sought for the development of drugs against *T. Cruzi*. The objective of this study was to evaluate the in vitro activity of the essential oil (EO) *Syzygium aromaticum* and its major compounds against epimastigotes of *T. cruzi*. **Methodology:** EO, eugenol and β -caryophyllene were obtained commercially and characterized by GC-MS and were evaluated on *T. cruzi* epimastigote forms for five days. Epimastigotes (1×10^6 cells/mL) were incubated at 28°C for five days in the absence or presence of different concentrations (100 to 400 $\mu\text{g/mL}$) of clove essential oil, eugenol and β -caryophyllene. The results were expressed as a percentage of inhibition. The average of at least two independent experiments was used to calculate IC₅₀ /24 h (50% inhibition of parasite growth). **Results:** The composition of EO eugenol (81.41%) and β -caryophyllene (11.01%) were identical. EO and the compounds inhibited the growth of epimastigotes depending on the concentration; at higher concentrations (400 $\mu\text{g/ml}$) there was 100% inhibition at 48 h in the case of EO with an IC₅₀ / 24 h of 64.5 $\mu\text{g/ml}$. ml while for eugenol (24 $\mu\text{g/ml}$) an inhibition of $95.04 \pm 2.1\%$ was observed at 72 h and β -caryophyllene (24 $\mu\text{g/ml}$) inhibited $99.94 \pm 0.08\%$ at 120 h. **Conclusion:** It is suggested that the major components and EO affect the viability of epimastigotes and are promising agents that require further studies that seek therapeutic alternatives against *T.cruzi*.

Key words: *T.Cruzi*, Epimastigotes, Essential oils, GC-MS

Human Health & Biotechnology

Assessment of *Bacillus cereus* from food samples: Prevalence, Toxigenic Profile and Antibiotic resistance response.

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Introduction: Access to safe food is crucial to ensure food safety, public health and population's confidence in food ingesta. It is important to guarantee food safety of these foods, especially of those microorganisms whose tropism is towards dairy products and starchy foods such as *B. cereus*, which causes two syndromes: diarrhoeal syndrome and emetic syndrome; that is why this work aims to detect the toxigenic profile of *B. cereus* isolates from samples of raw rice and pasteurised milk marketed in different places in the CDMX, as well as to determine the antimicrobial resistance profile.

Methodology: From 50 samples of raw rice and 50 samples of pasteurised milk, the isolation and identification of *B. cereus* was carried out using the methodology described in chapter 14 of the FDA Bacteriological Analytical Manual. For the detection of the toxigenic profile of each isolate, the genes *hblACD* (haemolysin), *nheABC* (non-haemolytic), *cytK* (cytotoxin K), *ces* (cerulide), *entFM* (FM enterotoxin) and the *tE* (toxin E) were amplified by PCR. Antimicrobial susceptibility profiling was performed using the Kirby Bauer method, described in the Clinical and Laboratory Standards Institute (CLSI) manual, testing 12 antibiotics: trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), ciprofloxacin (CIP), penicillin (PEN), gentamicin (GEN), cephalothin (CF), furazolidone (FUR), vancomycin (VA), fosfomicin (FO), oxacillin (OX), clindamycin (CC), erythromycin (EM). **Results:** Of the total number of samples analysed, 56% (28/50) from raw rice and 82% (41/50) from pasteurised milk were positive, with a total of 68 isolates identified. Regarding the toxigenic profile, of the isolates obtained, the *hblA* gene was detected in 75% (51/68), *hblC* in 67.6% (46/68), *hblD* in 64.7% (44/68), *nheA* in 45.5% (31/68), *nheB* by 67.6% (46/68), *nheC* by 94.1% (64/68), *cytK* by 83.8% (57/68), *ces* by 10.2% (7/68), *entFM* by 33.8% (23/68) and *tE* by 27.9% (23/68). In relation to the resistance profile, antibiotics were grouped according to antimicrobial activity, with FUR (100%), OX (91.7%), CF (85.2%), PEN (85.2%), GEN (73.5%) and VA (54.4%); antibiotics with intermediate sensitivity are TET (57.3%) and SXT (48.5%) and finally, sensitive antibiotics are FO (83.8%), CIP (75%), EM (54.4%) and CC (50%). **Conclusions:** The consumption of this type of food represents a risk for the consumer, due to the presence of *B. cereus* isolates with more than one gene coding for emetic and diarrhoeal syndrome toxins, in addition, they show a resistance profile to antibiotics that can be used for the treatment of infections.

Keywords: *Bacillus cereus*, toxin profile, antimicrobial resistance, pasteurized milk, rice raw, food safety



Human Health & Biotechnology

***In vitro* anticancer activity of enzymatically modified quercetin on HeLa and MCF7 human cancer cells.**

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Introduction: Currently, cancer is a high incidence disease throughout the world and one of the main treatments to destroy cancer cells is chemotherapy. However, non-tumoral cells also get damaged or their growth turns slow, causing severe side effects that reduce patient's life quality. Several reports focus on anticancer analysis of new molecules such as quercetin, a well-known anticancer agent largely consumed in human diet. However, it has low bioavailability due to its poor solubility, inactive metabolic products and short half-life. Thus, it is necessary to develop strategies to improve quercetin anticancer effects avoiding severe damage to non-tumoral cells. In this work we evaluated whether enzymatically modified quercetin (EMQuer) preserves or improves higher cytotoxic activity against human cancer cells than against non-tumoral cells.

Methodology: Quercetin (Quer) was subjected to an enzymatical reaction with extracted laccase from cultured white rot saprotrophic fungus *Trametes sanguineus*. The resulting material (EMQuer) characterization was carried out by FT-IR, UV and NMR spectra. HeLa cervix cancer cells (ATCC-CCL-2TM) and MCF7 breast cancer cells (ATCC-HTB-22) and non-tumoral Vero cells (ATCC-CCL-81) were treated with different concentrations of Quer and EMQuer for 72 h, followed by flow cytometry viability assay using the BD Cell Viability Kit, BD Pharmingen FITC Annexin V Apoptosis Detection Kit and FxCycle PI/RNase Staining Solution, to determinate cytotoxicity, induced apoptosis and cell cycle alterations, respectively. Each experimental condition was run six times. **Conclusion:** Instrumental analysis seems to suggest a partial oxidation of EMQuer, due to a mainly ionic product with phenolates with hydroxy groups organic oxidation that have lost hydrogen and have not been transformed into carbonyl groups. This could lay the foundations for a possible complete oxidation mechanism of quercetin via ionic and non-radical pathway. A possible structure of EMQuer is also proposed. The results suggest that EMQuer has a similar concentration-dependent inhibitory effect on non-tumoral Vero cells, when compared with Quer, and it improves its cytotoxic activity against HeLa cervix cancer cells and MCF7 breast cancer cells. Better efficiency of EMQuer than Quer on suppressing cancer cells growth might come from the fact that enzymatical modification such as laccase-catalyzed oxidation is expected to improve bioavailability of quercetin. Further studies are necessary by using derivatives on different tumoral cell lines and cancer animal models.

Keywords: Quercetin, bioavailability, cytotoxic activity, laccase, enzymatical modification, cancer cells.

Prebiotic and antioxidant potential of *Agave salmiana* waste

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Introduction: *Agave salmiana* is one of the magueys used to obtain aguamiel and co-products such as pulque, honey and mezcal; however, the lignocellulosic wastes generated in the scraping of the plant are discarded into the environment, wasting a material rich in insoluble fiber, polysaccharides and bioactive compounds with prebiotic potential. However, the high content of bioactive compounds could be considered as anti-nutrient compounds and affect the prebiotic effect. In this work, the objective was to evaluate the antioxidant activities and their relationship with the prebiotic potential of bioactive compounds from *Agave salmiana* bagasse in crude extract against probiotic microorganisms. **Methodology:** Agave bagasse treated with soxhlet extraction with 70% ethanol (ABT) and without any treatment (AB) was evaluated. Extractions of the treatments were performed from 100 mg of sample in 1 mL of methanol-water (80:20 v/v). The supernatant was dried, and the dried crude extract was saved for subsequent analyses. The antioxidant activities of the extracts were determined by DPPH, ABTS, and FRAP methodologies, and then the prebiotic bacteria (*Lactobacillus paracasei* and *Enterococcus faecium*) were evaluated in MRS broth, for this purpose the treatments were diluted in 8% saline water. The following treatments were prepared (10 mL): MRS broth (control), and MRS broth with 20 g/L of each of the treatments. They were sterilized and inoculated at a concentration of 2% of each inoculum. They were fermented at 37°C for 24 and subsequently seeded on MRS agar by the pour-plate method. Plates were incubated at 37°C for 24 hours for CFU counting. **Results:** For the DPPH and FRAP assay, a decrease was observed in the treated sample (DPPH 97.08±8.09 and 43.46±6.98 mg TE/g; FRAP 68.49±3.10 and 34.79±1.60 mg TE/g), while ABTS showed no significant differences (146.89±24.52 and 155.89±8.09 mg TE/g). For the prebiotic potential, an increase was observed in the treated sample (Lp 9.24 Log₁₀ CFU/mL and Ef 9.03 Log₁₀ CFU/mL) and a decrease in the untreated sample (Lp 9.08 Log₁₀ CFU/mL and Ef 8.78 Log₁₀ CFU/mL), which is related to the decrease in antioxidant activities. **Conclusion:** This indicates that the antioxidant compounds in agave have prebiotic potential in moderate amounts and may have a positive effect on intestinal health by improving the growth and diversity of the gut microbiota.

Key words: Agave waste, Antioxidant activities, Potential prebiotic.



Human Health & Biotechnology

Analysis of the crosstalk between Nischarin and Insulin in a breast cancer cell model: role of IRS-1.

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Introduction: Breast cancer is the second most common neoplasm in our country, surpassed only by cervical cancer, and it is one of the leading causes of death among women aged 30 to 65. Nischarin, a protein with antitumor properties, and insulin, a hormone regulating cell proliferation, have shown evidence of inverse regulation, suggesting potential cross-communication between them. **Methodology:** This study evaluated the viability of MCF-7 breast cancer cells through viability assays to assess the effects of nischarin overexpression. Furthermore, qPCR analyses were conducted to measure the expression levels of the Insulin Receptor (IR) and nischarin. Molecular docking studies were performed to investigate potential binding sites of nischarin on Insulin Receptor Substrate 1 (IRS-1). **Results:** Viability assays revealed that overexpression of nischarin significantly reduced the viability of MCF-7 cells, demonstrating its antitumor effects. qPCR results indicated an inverse relationship between nischarin and INSR expression, supporting the hypothesis of mutual regulation. Docking studies identified specific binding sites for nischarin on the terminal region of IRS-1, suggesting a mechanism for their interaction. **Conclusion:** These findings suggest that nischarin can modulate insulin signaling in breast cancer cells, influencing cell proliferation. The inverse regulation between nischarin and the insulin receptor, combined with specific binding interactions, underscores the potential for developing targeted therapies leveraging nischarin's antitumor properties in breast cancer treatment.

Key words: Breast cancer, nischarin, insulin receptor, IRS-1, MCF-7 cells



Human Health & Biotechnology

Evaluation of a baked product with fermented cassava dough

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Introduction: The glycemic index (GI) is a tool that compares the area under the curve of the glycemic response after consuming a food under study against a reference. Foods such as bread are widely consumed in the world, however, they have high rates, with low rates being those related to benefits for the prevention of chronic non-communicable metabolic diseases. Therefore, the objective of this research was to evaluate the sensory and functional properties through the GI in apparently healthy volunteers of a white bread added with yucca fermented dough. **Methodology:** Cassava flour fermented dough with *Lactobacillus plantarum* was used as a starter culture for 32 hours at 35°C. White bread was made using a traditional recipe with an addition of this fermented dough at 15% (w/w). The sensory evaluation was carried out with 60 semi-trained panelists. The evaluated parameters were color, texture, hardness, flavor, aftertaste and general acceptability. In the *In vivo* study, twenty-eight volunteers participated and only 15 met the stipulated standards. Subsequently, using glucose as a reference, the capillary glycemia curve was obtained under the official protocol ISO 26642:2010. This project was approved under registration number TDCYTA-20-10-22-1. **Results:** The sensory evaluation showed better results in the sourdough bread compared to a commercial version, in addition there was a significant reduction in the glycemic index (40 ± 6.33). **Conclusion:** The substitution of CFD in the formulation of a wheat-based bread presents sensory properties similar to its commercial version, so it may be a viable option for the substitution of wheat flour.

Keywords: Cassava, Fermented dough, Bread, Glycemic index.

Anticancer activity of fermented lychee peel

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Introduction: Cancer is one of the most common diseases worldwide, being lung cancer and colon cancer the main causes of death by cancer. Several authors have reported that in lychee peel, pulp and seed some compounds such as polyphenols have demonstrated biological activities among which are antioxidants, anti-inflammatory, anticancer, antiviral, among others, that is why the objective of this work was to evaluate the anticancer activity of lychee peel extracts obtained by fermentation in solid state. **Methodology:** The optimal fermentation conditions were 25°C, 60% humidity and a pH of 7 with the fungal strain *Aspergillus niger* GH1, with antioxidant activity as a response variable. The extract's fractions were separated by chromatography with XAD-16 amberlite resin, from which the aqueous fraction and ethanol fraction were obtained, which were subsequently dried at 45°C for 24 and 48 hours. To evaluate the cytotoxicity of the fractions, cell lines L929 corresponding to healthy mouse fibroblasts and HCT-116 corresponding to human colon cancer were used, evaluated at a serial concentration of 6.25-200 µg/ml. **Results.** A higher antioxidant activity was presented for fermented extracts compared to non-fermented extracts. For the evaluation of cytotoxicity, according to ISO 10993-5;2009, no extract was cytotoxic for the L929 cell line with an IC₅₀ <200 µg/ml and for the HCT-116 cell line the ethanol fraction was the one that reduced in higher percentage the cell viability presenting cytotoxicity at a concentration of 200 µg/ml with an IC₅₀ of 145.1 µg/ml. **Conclusion:** Solid-state fermentation of lychee peel yielded an extract rich in compounds with high antioxidant activity and promising potential for inhibiting cancer cell lines.

Keywords: Lychee, solid-state fermentation, biological activities



Human Health & Biotechnology

Antigenic determinants for developing structural biology perspectives for developing vaccines and new generation diagnostic systems.

diseases.

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Introduction: Through an integrative structural biology approach, perspectives can be generated that give us knowledge about the antigenicity of bacteria and viruses, allowing us to develop more effective vaccines and diagnostic systems. **Methodology:** In this case study presented, pilot tests were carried out to create protein crystals of the RBD protein of the SARS-CoV-2 virus for analysis by X-ray crystallography and its *in-vitro* biological characterization. The protein was produced and purified for the crystallization tests by evaluating 5 *Escherichia coli* lines characterized by SDS-PAGE. The vapor diffusion method (sitting/hanging drop) was used, evaluating various conditions to obtain crystals. **Results:** In preliminary tests, in 3 of 4 experiments, a metastable area was reached for crystal growth, and in the 4th, an amorphous precipitation was obtained. Using the Western Blot technique for detection, the RBD was used as the primary antibody, serum from immunized patients. The development of the gel showed a band at 24 kDa, demonstrating that an antigenic response capable of being recognized was preserved. **Conclusions:** The tests from a structural biology point of view of the case study presented allow us to develop strategies that are tropicalized to a country like Mexico for the development of new diagnostic strategies and vaccines.

Keywords: Structural-Biology, Antigenicity, Receptor-Binding-Domain (RBD).

Human Health & Biotechnology

Inhibition of α -Amylase Activity by Polyphenols Obtained from Mexican Medicinal Plants

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Introducción: Medicinal plants are a rich source of natural compounds with pharmacological properties, and their therapeutic use is a promising alternative for the treatment of chronic diseases such as diabetes. It is estimated that at least 800 plants are used to treat diabetes in Mexico, although there is little scientific evidence to support their hypoglycemic activity. There are several drugs that reduce hyperglycemia, but they have side effects for people who consume them. The aim of the present work was to evaluate the inhibition of α -amylase activity using polyphenols from medicinal plants such as capulin (*Ardisia compressa Kunth*), Mexican firecracker (*Hamelia patens*), mohuite (*Justicia spicigera*) and wereke (*Ibervillea sonora*). The plant material was washed and dried at 50°C for 24 hours, then pulverized in an industrial blender. The extracts were obtained by ultrasound, using ethanol as solvent in a ratio of 1:10 and filtered by gravity. The highest possible ethanol content was then removed from the extract using a rotary evaporator and then purified through a column filled with XAD-16 Amberlite. The fraction was dried in an oven at 50 °C for 24 hours. Total polyphenols were quantified using the Folin-Ciocalteu, obtaining for capulin 1.262 \pm 0.38 mg GAE/mL, Mexican firecracker 1.578 \pm 0.24 mg GAE/mL, mohuite 0.455 \pm 0.35 mg GAE/mL and wereke 0.038 \pm 0.015 mg GAE/mL. The percentage of plant yield was 1.9, 3.3, 2.2 and 4.5% for capulin, Mexican firecracker, mohuite and wereke, respectively. Enzyme inhibition was evaluated by the Bernfeld method (1995) with DNS using starch as the reducing carbohydrate. The Tukey test was performed to compare the effect of the four plants on enzyme inhibition. The results show that the mohuite plant has a better effect on the inhibition of the enzyme since its IC₅₀ value (0.414 \pm 0.15 μ g/mL) is lower, followed by Mexican firecracker (0.627 \pm 0.01 μ g/mL), wereke (6.698 \pm 4.06 μ g/mL) and capulín (36.29 \pm 4.42 μ g/mL). The four plant materials were able to inhibit the activity of porcine pancreatic α -amylase.

Key words: α -amylase, polyphenols, medicinal plants, inhibition.



Human Health & Biotechnology

Enumeration of total and faecal coliforms in beverages marketed in Casco de Santo Tomás (Mexico City) and the susceptibility of *Escherichia coli* to the antimicrobial activity of Kombucha tea.

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Introduction:

Natural drinks and fresh juices are widely consumed among the Mexican population; they are made from water, fruit and sugar. Due to their composition, they are the ideal substrate for the proliferation of microorganisms such as coliforms (indicator group for determining the microbiological quality of both water and food), including *Escherichia coli*, which can cause Foodborne Diseases (FBD).

On other hand, Kombucha tea (*Manchurian fungus*) is a non-alcoholic artisanal drink that is presumed to be a potential antagonist against this microorganism despite its high resistance to various antibiotics.

It is therefore necessary to assess the microbiological quality of this kind of food to avoid possible outbreaks of FBD and evaluate the susceptibility to the antimicrobial activity of Kombucha tea.

Methodology: Detection of total, faecal coliforms and isolation of *Escherichia coli* in beverages was carried out by the modification of the methodology described in the Mexican Official Norm (NOM) 210. The inhibitory effect of *M. fungus* on the isolates of *Escherichia coli* was carried out through a kombucha tea disc inhibition test during seven days of fermentation.

Results: A total of 50 samples were obtained from different stalls in “Casco de Santo Tomás” located in Mexico City, Mexico. The collection was performed weekly from March 2024 to July 2024. A total of 31 colonies with characteristics of *Escherichia coli* were phenotypically identified based on the colonial morphology on Eosin Methylene Blue Agar (EMB) as described in the NOM 210. We identified a total of 20 isolates with biochemical identification IMViC and MUG test.

Key words: *Escherichia coli*, Foodborne illness, beverage, Kombucha, Food safety.



Agricultural & Food Biotechnology

BioIberoamerica is a biannual event that strengthens the bonds between Latin America and the Iberian Peninsula and the international forum most important in this region.

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Introduction: BioIberoamérica 2024 will take place from 3-6 September 2024 in Monterrey, one of the most dynamic cities in the world, located in the northeast of Mexico, a region bordering the USA, characterized by a dynamic strategy for economics, social development, innovative, with educative institutions recognized globally and a great biotechnological sector including the main biotech cluster in Mexico. The main objective is to get together all the scientific community interested in biotechnological developments, including outstanding scientific leaders. **Methodology:** The event proposes a broad program of activities with oral presentations, poster sessions, round tables, workshops, and parallel technical sessions, to bring together experts to promote the exchange of experiences among the different actors of the biotechnology sector, to identify the demands of research, innovation and technology transfe. **Results:** Bioiberoamerica has become one of the leading biotech events in Iberoamerica, being the perfect setting for forging new partnerships, scientific and academic collaboration, and strengthening existing business relationships in the biotechnological global sector. **Conclusion:** BioIberoamerica 2024 will bring together more than 500 participants working in health, sustainable agri-food, and climate change solutions and biotechnological innovations. The event is attended by professionals, students, exhibitors, and investors.

Keywords: BioIberoamerica, Mexico, biotechnology.



Human Health & Biotechnology

Enumeration of total and fecal coliforms in beverages marketed in Miguel Hidalgo (Mexico City) and the susceptibility of *Escherichia coli* to the antimicrobial activity of Kombucha tea.

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Introduction: Miguel Hidalgo is located in the central part of the city and is considered one of the most important and influential towns. Natural drinks and fresh juices are widely consumed among the Mexican population; they are made from water, fruit and sugar, the ideal substrate for the proliferation of microorganisms such as coliforms, including *Escherichia coli*, which can cause Foodborne Diseases (FBD). Kombucha tea (*Manchurian fungus*) is a non-alcoholic artisanal drink that is presumed to be a potential antagonist against this microorganism. Hence, is necessary to assess the microbiological quality and evaluate the susceptibility to the antimicrobial activity of Kombucha tea.

Methodology: Detection of total, faecal coliforms and isolation of *Escherichia coli* in beverages was carried out by the modification of the methodology described in the Mexican Official Norm (NOM) 210. The inhibitory effect of *M. fungus* on the isolates of *Escherichia coli* was carried out through a kombucha tea disc inhibition test at seven days of fermentation.

Results: A total of 45 samples were obtained from different stalls in Miguel Hidalgo. Total coliforms (CT) were present in 53.3% of samples, and fecal coliforms (FC) in 28.8%. Fresh juices showed highest prevalence (71.4%). A total of 21 isolates were analyzed, 13 from CT and 8 from CF, where 14 isolates were identified as *E. coli* by IMViC and MUG test. The 14 isolates were subjected to inhibition test, using Kombucha tea to observe the susceptibility.

Conclusion: The presence of total and fecal coliforms was detected in fresh waters and juices marketed in Miguel Hidalgo, potentially indicating poor hygienic practices during processing

Key words: *Escherichia coli*, Foodborne illness, beverage, Kombucha, Food safety.



Human health & Biotechnology

Formulation of topical application containing chloroquine as a therapeutic alternative for cutaneous Leishmaniasis

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Key words: Topical formulation, Leishmaniasis, Tropical diseases

Introduction: Cutaneous Leishmaniasis (CL) is an infectious, non-contagious disease caused by protozoa of the genus *Leishmania*, which causes ulcers on the skin and mucous membranes. Characterized as a public health issue in 85 countries, with cases reported in all regions of Brazil, making it one of the nine countries that account for approximately 85% of CL cases. In recent decades, the treatment used has been marked by a scarcity of therapeutic options and the use of drugs that cause significant toxicity. Given these observations, the objective was to reposition the drug Chloroquine, used in the treatment of malaria transmitted by *Plasmodium vivax*, as a topical application alternative for CL. **Methodology:** The formulation was prepared in the Biotechnology Laboratory for Natural Products at IFRO. Different proportions and concentrations of water, Carrageenan, Locust bean gum, Propylene glycol, Potassium chloride, and Chloroquine were tested. To assess the formulation's ability to release the active ingredient and to analyze the amount released over a specific period, an *ex vivo* skin permeation test was conducted. Following 12 hours of this assay, a skin retention test was performed. The substance used in the study (Chloroquine diphosphate salt) was obtained from Sigma-Aldrich, at a concentration of 3.219443 mg/cm². **Results:** It was observed that approximately 54% of the substance incorporated into the formulation was released after 12 hours, equivalent to a release of 1.75 µg/cm² on pig skin. The results demonstrated that the amount of chloroquine retained in the skin was 3.25%, equivalent to 104.7 µg/cm². **Conclusion:** From a drug repositioning perspective, the use of chloroquine shows promising *ex vivo* results for skin permeation and retention. These findings suggest new *in vivo* research approaches to evaluate the effects and confirm the potential of repositioned chloroquine in topical formulations as an alternative therapy for CL.

Human Health & Biotechnology

Nutritional Status And Obesity Perception Of Parents With School Age Children In Saltillo

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Introduction: Obesity and overweight have been one of the critical health issues in Mexico. The perception of obesity by the parents with school age will have impacts on their children. This study was carried out to determine the nutritional status and obesity perception of parents with school age children in Saltillo

Methodology: A simple random sampling method was used to select two hundred willing and ready parents with children in elementary schools in Saltillo Coahuila. Information on the socio-economic characteristics were obtained through pretested Structured Questionnaires. Nutritional status of the parents was determined through their weight and height measurement and were classified into their Body Mass Index (BMI) according to the World Health Organization (WHO) standard. The parents' perception was assessed with the use of Validated Structured Questionnaires. Data were analyzed using Statistical Package for Social Science Version 25.

Results: The Results of the Socio-economic characteristics showed that majority (91%), of the respondents were men, higher percentage were married (69.7%), and 10% were divorced. More than half (52.7%) of the subjects had University education while 26.9% had postgraduate education. Body Mass Index of the subjects indicated that 32% were overweight, 19% had obesity grade 1, 6% had Obesity grade 2 and 3% had morbid obesity. The results of the obesity perception revealed that only few of the parents had wrong perception of Obesity ($p < 0.05$)

Conclusion: Few of the parents were obese and there is a negative correlation between Obesity perception and the nutritional status of the parents.

Key words: Obesity, perception, Nutritional status and children.

Anthropometric Indices and Mental Health of workers in health sector in Saltillo Coahuila

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INTRODUCTION

Mental health issues are growing problem among workers in health due to conditions such as work environment, job requirements, and the complexity of medical care. This was carried out to determine the anthropometric indices and mental health of workers in the health sector in Saltillo Coahuila.

METHODOLOGY

A simple random sampling method was used to select two hundred and fifty workers from the health sectors in Saltillo. Anthropometric measurements such as weight, height, waist circumference (WC), Hip circumference (HC) and mid upper arm circumference (MUAC) were determined using World Health Organization Standard (WHO). Mental health was assessed using validated structured Depression Anxiety and Stress Scale (DASS-42). Data were analyzed using Statistical Package for Social Science Version 25.

RESULTS

The mean results of the anthropometric characteristics indicated that the mean weight was (73.49±18.59), height (1.64±0.09), MUAC (29.05±9.7), WC (89.89±17.64), and HC (103.17±16.03). DASS 42 results indicated that 7% had extreme depression, 10% extreme anxiety; and 5% had extreme stress.

CONCLUSION

Few of the workers had extreme depression, anxiety and stress. The study showed a significant relationship between anthropometric parameters and mental health studied.

Key words: Depression, Anxiety, Stress, and anthropometric characteristics.

Cardiometabolic Risk Factors and Dietary Diversity Score of female workers in Health sector Saltillo Coahuila

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INTRODUCTION

The majority of the burden in Mexico's advanced epidemiological shift can be attributed to chronic non-communicable diseases and a shift from traditional diets that are diverse. This study was designed to determine the cardiometabolic risk and women dietary diversity score.

METHODOLOGY

A validated structured interview guide was used to solicit information from 122 randomly selected willing women in health sector. Body mass index (BMI) ≥ 30 kg/m² and waist circumference (WC) ≥ 80 cm in women (WCF) with Systolic blood pressure ≥ 140 (SBP) and Diastolic blood pressure ≥ 90 (DBP) are reference cardiometabolic risk markers used. Food and Agriculture Organization of the United Nations (FAO) pre validated structured questionnaire for Women dietary diversity (WDDS) was adapted to the Mexican context with little modification. Data on dietary diversity were collected using both quantified and unquantified 24-h open recall. Data were analyzed using Statistical Package for Social Science Version 25.

RESULTS

The mean results of the BMI, WC, SBP and DBP were 27.39 ± 6.06 , 88.15 ± 16.32 , 112.08 ± 16 and 75.84 ± 12 respectively. Cardiometabolic risks among the women were 23.77% (BMI), 59.83% (WCF), SBP 7% and DBP 7%. The dietary diversity score among the women were 9.07 ± 2.7 while only 3% of the study group had low WDDS.

CONCLUSION

The cardiometabolic risks among health workers were high. There is a positive relationship between the DDS and cardiometabolic risk ($p < 0.045$). There is a critical need for health education for the health workers.

Key words: cardiometabolic risk, Women, Dietary diversity and Health.



Human Health & Biotechnology

Anticancer and Antibacterial Properties of Novel Xanthan gum Derivatives

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Introduction: Xanthan gum (XG) is an exopolysaccharide made up of a main chain of D-glucose and side chains made up of mannoses with acetyl or pyruvic groups and glucuronic acids. It is obtained by aerobic fermentation from a carbon and nitrogen source with bacteria of the genus *Xanthomonas*. It participates mainly in the food, pharmaceutical, and cosmetic industries thanks to its physicochemical properties. However, modifications are required to enhance or develop new biological properties for biomedical applications. **Methodology:** Two quaternary ammonium salts were synthesized: 1-(2-aminoethyl) pyridinium bromide (PYB) and 1-(2-aminoethyl) trimethylammonium bromide (TAB), through quaternization reactions. These salts were chemically grafted onto xanthan gum, previously oxidized, to form Schiff bases. Subsequently, the products obtained were characterized by Fourier Transform Infrared Spectrometry (FTIR) and Nuclear Magnetic Resonance (NMR), in addition to carrying out Thermogravimetric Analysis (TGA) and evaluating the hemolytic, antibacterial, and anticancer properties of the products. **Results:** FTIR revealed some characteristic peaks corresponding to O-H and C=O attributed to xanthan gum and C=N attributed to ammonium salts. With NMR, it was determined the monosaccharides and methyl groups of XG and the carbons of trimethylammonium, pyridinium, and aminoethyl groups that correspond to the ammonium salts. TGA determined the degradation point for XG-PYB (246 °C) and for XG-TAB (385 °C). The hemolytic test showed no toxicity on erythrocytes. XG didn't show cytotoxicity on breast cancer cells, but XG-PYB showed toxicity at 3 000 µg/mL and XG-TAB decreased cell viability from 100 µg/mL. XG-TAB inhibited nearly 100 % of *Escherichia coli* and *Staphylococcus aureus* growth and XG-PYB also inhibited *Staphylococcus aureus*. **Conclusions:** It was obtained two novel derivatives of xanthan gum since characteristic peaks of functional groups of the molecules were observed based on the vibrations of their bonds and the protons of the carbons that make up the molecules, in addition to demonstrating that XG-TAB is more resistant to temperature, both compounds proved to be non-toxic in human blood, XG-TAB revealed to be toxic on breast cancer cells at low concentrations, besides both derivatives showed antibacterial properties.

Key words: Xanthan gum, ammonium salts, biological properties.

Human Health & Biotechnology

Exploring the antioxidant potential of *Sphenarium purpurascens* grasshopper protein extracts

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Introduction: Obesity, affecting over 30% of the Mexican population, is linked to severe conditions like diabetes, hypertension, and cancer. Preventive measures emphasize healthy diet, exercise, and functional food consumption. Bioactive peptides derived from proteins have diverse health benefits, including antimicrobial, antihypertensive, and antioxidant properties. Insects, particularly grasshoppers, are emerging as valuable sources of bioactive peptides for food and pharmaceutical applications. **Methodology:** Alkaline protein extraction from *Sphenarium purpurascens* was performed, followed by hydrolysis using Alcalase 2.4L. Peptide isolation was performed by fast protein liquid chromatography (FPLC) with a Superdex 30 increase column using dual absorbances at 280 nm and 214 nm. Antioxidant activities were assessed using DPPH and ABTS assays. **Results:** A hydrolysis degree of 17.19% was achieved. The initial protein concentration was 12.11 ± 0.99 mg/mL, which decreased to 1.63 ± 0.05 mg/mL post-hydrolysis. Antioxidant assays demonstrated that *S. purpurascens* extracts exhibit competitive or superior antioxidant activities compared to other insect species. DPPH assay results showed values ranging from 30.93 to 60.93 $\mu\text{g/mL}$ Trolox equivalents, while ABTS assay results ranged from 20.78 to 48.57 $\mu\text{g/mL}$ Trolox equivalents. These outcomes highlight the diverse antioxidant potential present in different peptides from *S. purpurascens*. **Conclusion:** This comprehensive investigation underscores the promising potential of *S. purpurascens* extracts in developing functional foods and pharmaceuticals. Future research should focus on identifying and characterizing specific peptides responsible for these bioactivities, facilitating targeted applications and enhancing their functional properties. This study contributes to the growing body of knowledge on the potential of insect-derived bioactive peptides in addressing obesity-related health challenges.

Key words: *Sphenarium purpurascens*, bioactive peptides, antioxidant, hydrolysis,



Human Health & Biotechnology

Validation of the AccXible screening and monitoring system for depression and anxiety in the community of the Autonomous University of Coahuila

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Introduction:

About 2.5 million young people in Mexico suffer from depression, while 1 in 3 experience anxiety. The impact of the COVID pandemic on the prevalence of anxiety and depression has doubled, especially in Mexico, the United Kingdom and the United States. The Accessible system allows the detection of mental diseases through voice analysis. Currently, AI models related to mental health (depression, anxiety) have been developed. Accessible models look at both “what you say” and “how you say it.” The combination of voice and language analysis provides an accuracy of 90% in detection in the early stages of the disease. “When something is wrong in your head, it is likely to show in your voice”. Vocal biomarkers have emerged as promising tools for detecting illnesses and mental disorders and valuable information about an individual's health, from physical conditions like Parkinson's and Alzheimer's to mental disorders such as depression and anxiety. This non-invasive early detection capability allows for timely interventions and personalized treatments, thereby enhancing patients' quality of life. This project aims to explore the intersection of human voice and emotions, promoting the use of innovative tools for early detection and management of emotional and behavioral disorders among students at the Autonomous University of Coahuila. **Methodology:** Explore the validity of the spontaneous discourse analysis system “AcceXible” for the correct screening of depression and anxiety in the university population of the Autonomous University of Coahuila, including and comparing with the results of the PHQ-9 and GAD-7 questionnaires. **Results:** Possible associations between vocal characteristics and anxiety/depression will be identified by AI and a genetic study to research for a possible genetic marker. Descriptive statistics will reveal symptom prevalence. Recommendations for seeking professional attention will be provided for identified cases. **Conclusion:** The study aims to validate the utility of vocal biomarkers for early screening of emotional disorders as depression and anxiety.

Keywords: Vocal biomarkers, AI, mental disorders, depression, anxiety.

Human Health and Biotechnology

***In silico* screening of the antiviral activity of Mexican oregano (*Lippia origanoides*) against the Hepatitis B virus**

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Introduction: Hepatitis B is an infection caused by the Hepatitis B virus. The WHO estimates that, in 2022, 254 million people in the world suffered from a chronic infection. The disease can be transferred through contaminated organic fluids, such as blood, saliva, vaginal secretions, and semen. Treatments focus on chronic infection, but they are expensive, prolonged treatments that can have serious side effects in patients; For this reason, alternative treatments are being sought to assist current treatments or to replace them. Recently, the Mexican oregano (*Lippia origanoides*) has been studied with respect to its antiviral activity, thanks to the fact that its main bioactive compounds, Thymol and Carvacrol have shown good effects against other viruses, so it is proposed to use the bioactive compounds of this plant in *in silico* analysis to determine if they present a possible antiviral activity against the Hepatitis B virus. The *in silico* study is a computational test in which the possible interactions that take place between two molecules are analyzed, it is commonly used because it is easy and quick to perform. **Methodology:** It is intended to use an online database to extract the molecules of the proteins of the Hepatitis B virus, the compounds Thymol and Carvacrol, and the molecules of certain drugs that will serve as a control in the study. The interactions that take place between the bioactive compounds and the proteins will be compared with the interaction of the drugs and the same proteins in which they act, to determine if they have such antiviral activity.

Key words: Hepatitis B, *in silico*, Mexican oregano, Thymol, Carvacrol



Human Health & Biotechnology

Obtaining decellularized plant structures with possible biomedical applications

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Introduction: Plants transport nutrients and waste throughout their structure through the network of capillaries that make up their vascular system. The decellularization of plant structures is a process that involves the isolation of the structure (cellulose) of the leaf, this process has been used in medical research and tissue engineering to create scaffolds for growing artificial cells and tissues. The objective of this work was to obtain decellularized plant structures with possible biomedical applications. **Methodology:** Healthy leaves of *Psidium guajava* and *Iresine herbstii* were selected. Two treatments of ionic solutions were tested, without heat (25°C) and with heat (60°C). The decellularized plant structures were used as cellular scaffolds for NIH-3T3 fibroblast cells. Cell viability was carried out using the TOX1 kit (Sigma-Aldrich). The morphology of the membranes was determined by using a scanning electron microscope (SEM). **Results:** The two methods evaluated allow obtaining decellularized plant structures, a 3D structure and the detailed architecture of the decellularized structures were observed. The three-dimensional shape and organization of the scaffold obtained functions as a support environment that favors the biochemical signaling necessary for the growth and development of NHI-3T3 cell cultures. **Conclusion:** T1 treatment is a replicable methodology that allows obtaining manipulable decellularized plant structures that can be used as cellular scaffolds for the growth of NIH-3T3 cells. This work demonstrates the potential use of decellularized *Psidium guajava* structures as a cellular scaffold that can be useful in the development of new structures to perform 3D cytotoxicity tests or tissue development.

Key words: 3D structure, cellular scaffold, decellularization, tissue engineering, cellulose.

Cosmetic Preparation with Antioxidant, Photoprotective, and Repellent Action Using Nanoencapsulated Actives

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Introduction: The skin, being the outermost organ of the body, is constantly exposed to ultraviolet (UV) radiation, which can cause inflammation, photoaging, and even skin cancer. Antioxidants can prevent this damage by slowing the oxidation of substrates. Chemical sunscreens absorb UV radiation, while physical sunscreens block it mechanically. However, natural active ingredients are chemically unstable, leading to rapid degradation. Nanoencapsulation, involving encapsulating actives in polymeric membranes, can protect these components and prolong their effectiveness. Therefore, the objective of the present study was to prepare a cosmetic formulation using natural products from the Amazon region that have photoprotective and antioxidant action with the application of nanotechnology. **Methodology:** The methodology included the nanoencapsulation of the active ingredients, incorporation into the cream vehicle, evaluation of zeta potential, size, and encapsulation efficiency, cytotoxicity analysis on L929 cell line, antioxidant activity assessment, preliminary in vitro repellency tests, determination of the sun protection factor (SPF), and *ex vivo* skin permeation and retention assays. **Results:** The developed cosmetic formulation maintained physical and chemical stability over 60 days and exhibited moderate antioxidant activity. The SPF was 18, indicating medium sun protection. *Ex vivo* assays showed slow permeation, with 9.04% of the essential oil and 30.99% of the crude extract permeating after 12 hours, and skin retention of approximately 5% and 25%, respectively. The formulation did not prevent *Aedes aegypti* mosquito landing but was effective against engorgement. The encapsulation efficiency was 99.99% for the essential oil and 99.8% for the crude extract, and the zeta potential of -32.9 mV indicated electrostatic stability. **Conclusion:** The developed formulation demonstrated stability, moderate antioxidant activity, medium sun protection, and a controlled permeation profile, indicating its potential as a multifunctional cosmetic product.

Key words: Nanoencapsulation, Photoprotection, Antioxidant.



Biotechnology

MODIFICATION OF NOPAL PECTIN TO GIVE IT ANTIBACTERIAL, ANTICANCER AND BIOCOMPATIBILITY PROPERTIES.

Modality: Cartel

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Introduction: Biopolymers have been of great importance, which is why their physical and chemical characteristics have been studied. Among biopolymers, pectin stands out because it has many applications in industry, but it is very prone to bacterial contamination, which is why modifications to its structure are required to improve its biological properties. In this work, two ammonium salts (1-(2-aminoethyl) trimethylammonium bromide and 1-(2-aminoethyl) pyridinium bromide) were synthesized using quaternization reactions and subsequently grafting these salts to pectin previously oxidized with periodate of sodium. These new pectin derivatives were characterized by FTIR (Fourier Transform Infrared Spectroscopy) where the functional groups of the molecules could be appreciated and complemented with the NMR (Nuclear Magnetic Resonance) technique. The products obtained were also evaluated for their hemolytic, antibacterial and anticancer properties. For the antibacterial tests, the following strains were used: S.aureus ATCC 6538, S.aureus ATCC33592, E.coli ATCC 11229, P. Aeruginosa ATCC 15442, Enterobacter and C.albicans, Fecalis, obtaining an antimicrobial material. For anticancer tests, we worked with 3T3 and MCF7, demonstrating that these pectin derivatives inhibit cell proliferation of these cancer cell lines. Finally, the results of hemolytic tests demonstrated that these materials are biocompatible with human blood erythrocytes.

Keywords: pectin, quaternary salts, antibacterial, anticancer.



Human Health & Biotechnology.

Applying genomic biotechnology to molecular epidemiology, bioengineering with a social impact, using zoonosis as a case study.

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Introduction: Recent years have witnessed substantial technological advancements in life sciences, i.e. Monoclonal Antibodies for chronic diseases. Nevertheless, these innovations have not been uniformly applied to infectious diseases, particularly those often neglected. The primary challenge lies in adopting a comprehensive approach with industry collaboration for effective commercial solutions. Highlighting the importance to implement these technologies in the management of infectious diseases of a zoonotic nature. The emphasis should shift towards developing next-generation rationally designed chimeric proteins via scalable systems.

Methodology: This process starts with in-silico bioinformatic analysis, followed by synthetic biology, molecular biology, genetic engineering, and bioprocess development, culminating in pilot plant production. Our primary goal is to optimize integrated platform solutions, primarily for biomolecules and diagnostics. **Results:** Our research, includes antibodies with biological recognition, effectively identified in-silico-designed epitopes and upscaling production up to 150 mg*L⁻¹ to pilot plant levels, yielding promising results. **Conclusion:** The integration of advanced technologies, such as the development of next-generation chimeric proteins through scalable systems, is crucial for addressing neglected infectious diseases.

Key words: Rational-design-protein, ScFv, Bioprocess-development, Recombinant-protein.

Phenethyl Isothiocyanate from Watercress By-Products: A Novel Green Extraction and Its Potential Health Benefits

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Introduction: Cruciferous vegetables are gaining popularity as “superfoods” containing exceptionally high phytochemicals content, particularly isothiocyanates, that bring health-promoting effects beyond basic nutrients. Phenethyl isothiocyanate (PEITC) is one of the most interesting isothiocyanates, showing antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. This makes PEITC suitable for nutraceuticals and functional foods as an adjuvant against oxidative and inflammatory-related disorders, especially in the gastrointestinal tract, which is increasingly burdening the healthcare system globally¹. Furthermore, as a widely consumed vegetable, crucifers are highly wasted in the supply chain process. PEITC can be obtained from watercress by-products. This commitment to reducing food waste can help achieve circularity and sustainability in agricultural production². Extracting isothiocyanates traditionally involves harmful solvents. Microwave gravity hydrodiffusion (MHG) is a promising solvent-free extraction technique. Following this idea, this work is innovative as it is the first to apply MHG to extract PEITC. **Methodology:** MHG extraction of PEITC was developed and optimized. The phenolic profile of MHG extracts and their bioactivities, including antioxidant, anti-diabetic, and antihypertensive (IC₅₀) and antimicrobial activities against gastrointestinal pathogens, were evaluated. Furthermore, this work begins investigating the concept of hormesis applied to the PEITC and its effects on intestinal cells (Caco-2 and HT29-MTX), including anti-inflammatory activity and the ability to neutralize ROS. **Results:** MHG successfully extracted the PEITC (1818 µg/g watercress DB). The optimized MHG extraction was also applied to turnip. This innovation resulted in a patent application where freezing is the key novelty. One obtained extract was a stable suspension with a zeta-potential of -12.6 mV. Ferulic acid was one of the main phenolic compounds. Besides, the extracts provided antioxidant, antihypertensive, and anti-diabetic activity and inhibited the growth of pathogens. Low concentrations of PEITC inhibited cellular metabolism compared to higher concentrations in the first few hours, but higher concentrations exhibited cytotoxic effects after 24h. Besides, PEITC reduced IL-8 secretion cells and the basal level of ROS in the cells. **Conclusion:** This study demonstrates that MHG is a green technique for extracting PEITC with potential bioactivities for enhancing gastrointestinal tract health. Besides, it highlights the importance of upcycling vegetable waste from the agricultural industry to create high-value-added products.



Keywords: Phenethyl isothiocyanate (PEITC); Microwave gravity hydrodiffusion (MHG); Eco-friendly extraction; Watercress by-products; Health benefits

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Human Health & Biotechnology

Gentamicin-loaded electrospun membranes based on Kefiran and Schizophyllan for application in biomedicine

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Introduction: Skin wound healing is a complex process that takes time, during which it is susceptible to external biological attacks from bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Advanced dressings are added with drugs capable of interacting with cells in the skin wound, including infectious agents. Electrospinning has been widely used to produce nanofibers with specific surface area and high porosity with applications such as active compound delivery. These can be from natural sources such as kefir and components like antibiotics can be added to provide antimicrobial activity and antioxidants to aid the healing process. **Methodology:** Kefiran was obtained from the kefir microbial consortium. For this, the ultrasound-assisted ethanolic extraction methodology was used, and the polymer was lyophilized for storage. Polymer solutions were prepared using PVA as a guide polymer, obtaining a final concentration of PVA at 8% v/v, kefir at 1% v/v and schizophyllan at 1% v/v. The above solutions were electrospun following a 2³ factorial experimental design with center point, varying flow conditions from 0.1 to 2.0 mLh⁻¹, voltage from 10 to 25 kV and needle-collector distance from 10 to 20 cm. The fibers were visualized using an optical microscope to verify the absence of beads or clumps of solution. Contact angle was determined for the membranes. **Results:** The yield obtained from kefir was 0.61%. Furthermore, the optimal conditions obtained for the formation of fibers were: 0.1 mLh⁻¹, 22 kV and 20 cm between needle-collector. The contact angle of the membrane was of 53.63°, which indicates a hydrophilic nature. **Conclusion:** It was determined that the optimal conditions for kefir extraction were heating at 50 °C and subsequent treatment with ultrasound. Similarly, it was found that the conditions for obtaining electrospun fibers without the presence of beads from a solution of PVOH 8%-Kefiran 1%-Schizophyllan 1% w/v are a flow of 0.1 mLh⁻¹, 20 cm of distance between needle-collector and 22 kV of applied voltage. The contact angle of the electrospun membranes was obtained, classifying them as hydrophilic materials.

Keywords: electrospinning, biomaterials, kefiran.

Potential of caprylic acid to replace β -propiolactone in the inactivation process of the rabies virus for equine immunization

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Introduction: Rabies, caused by the rabies virus, is characterized as acute and lethal progressive encephalitis. The prophylactic approach to rabies is carried out through pre- and post-exposure vaccination, as well as the application of anti-rabies serum. FUNED's anti-rabies serum is produced by hyperimmunizing horses with the rabies virus replicated in cell culture and inactivated with β -propiolactone (β -PL). β -propiolactone is potentially carcinogenic and brings biosafety concern. Furthermore, β -PL is difficult to acquire in Brazil. Therefore, the objective of this project is to investigate alternative products to replace β -PL in the rabies virus inactivation process for equine immunization. **Methodology:** Two batches of viruses were divided into 4 aliquots and inactivated with β -propiolactone and 1%, 0.75% and 0.5% caprylic acid, respectively. Residual activity assay was performed *in vitro* and *in vivo* to confirm viral inactivation. Next, 3 groups of rabbits were immunized with the rabies virus inactivated with β -propiolactone and 1% and 0.75% caprylic acid, mimicking the immunization cycles (doses and intervals) used for horses. Blood samples were taken throughout the immunizations to monitor the development of the immune response by ELISA and the level of neutralizing antibodies by the RIFFT assay. **Results:** After inactivation, the residual activity assay showed that only 0.5% caprylic acid was not able to completely inactivate the rabies virus. The evaluation of IgG production after immunization of rabbits with the different inactivated virus preparations showed a high antibody titer regardless of the groups. The serum neutralization test showed that all antigen preparations were capable of inducing the production of neutralizing antibodies; however, the group immunized with rabies virus inactivated with β -propiolactone gave a much higher titer compared to those with caprylic acid. **Conclusion:** Caprylic acid was shown to be capable of inactivating the rabies virus and inducing neutralizing antibodies after immunizations of rabbits. However, acid concentration conditions and immunization schedule need to be improved to achieve higher neutralizing antibody levels.

Keywords: rabies virus inactivation, caprylic acid, neutralizing antibodies.



Human Health and Biotechnology

Anthropometric Indices and Mental Health of workers in health sector in Saltillo Coahuila

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Mental health issues are growing problem among workers in health due to conditions such as work environment, job requirements, and the complexity of medical care. This was carried out to determine the anthropometric indices and mental health of workers in the health sector in Saltillo Coahuila. A simple random sampling method was used to select two hundred and fifty workers from the health sectors in Saltillo. Anthropometric measurements such as weight, height, waist circumference (WC), Hip circumference (HC) and mid upper arm circumference (MUAC) were determined using World Health Organization Standard (WHO). Mental health was assessed using validated structured Depression Anxiety and Stress Scale (DASS-42). Data were analyzed using Statistical Package for Social Science Version 25. The mean results of the anthropometric characteristics indicated that the mean weight was (73.49±18.59), height (1.64±0.09), MUAC (29.05±9.7), WC (89.89±17.64), and HC (103.17±16.03). DASS 42 results indicated that 7% had extreme depression, 10% extreme anxiety; and 5% had extreme stress. Few of the workers had extreme depression, anxiety and stress. The study showed a significant relationship between anthropometric parameters and mental health studied.

Key words: Depression, Anxiety, Stress, and anthropometric characteristics.



Human Health & Biotechnology

A Machine Learning Approach For Predicting Antibiotic Resistance in *Escherichia coli*

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Introduction: One of the greatest challenges facing humanity is the severe crisis of antibiotic resistance. Multidrug-resistant bacteria, ineffective treatments for infectious diseases, and a significant financial burden on health systems are issues arising from this resistance. Among the main pathogenic bacteria is *Escherichia coli*, notable for its prevalence and evolutionary adaptability. In light of this situation, the search for innovative approaches to predict antibiotic resistance becomes indispensable. Machine learning emerges as a powerful resource for biomedicine, as it allows for the analysis of large datasets and the detection of complex patterns. One of the key research areas in its application focuses on the prediction of antibiotic resistance. The primary objective of this work is to find a machine learning model that can predict the resistance phenotype from the genomic information of the strain.

Methodology: Genotypes and resistance phenotypes of 6,582 *E. coli* isolates were analyzed using data from the NDARO (National Database of Antibiotic Resistant Organisms). Three machine learning models were employed: Support Vector Machine (SVM), K-Nearest Neighbors (K-NN), and Extra Trees, to predict resistance to various antibiotics, including fluoroquinolones, aminoglycosides, and amoxicillin-clavulanic acid. **Results:** During the model development, it was found that the SVM and Extra Trees models performed better than the KNN model. The accuracy of the SVM model was 0.98, that of Extra Trees was 0.93, and that of KNN was 0.81. The SVM and Extra Trees models outperformed previously reported state-of-the-art models. **Conclusion:** SVM and Extra Trees proved to be effective tools for predicting antibiotic resistance in *E. coli*, demonstrating their potential for practical applications in the monitoring and management of bacterial resistance.

Key words: Resistance, antibiotics, prediction, genome.



Human Health and Biotechnology

PRESENCE OF SALMONELLA SP IN FOODSTUFFS OF POPULAR CONSUMPTION IN MEXICO CITY

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INTRODUCTION: Foodborne diseases are mainly originated by food consumption elaborated from meats and vegetables. It is estimated that one of ten persons gets sick after consuming contaminated food and that 420 000 persons die each year with children under 5 years being the most affected with 125 000 deaths per year. Among the bacteria related to foodborne illness of medical importance and mandatory notification is *Salmonella*. Therefore, the objective of this study was to determine the presence of total coliform organisms and *Salmonella* genus in food sold on the streets of Mexico City. **METHODOLOGY:** Fifty samples were analyzed (25 of tacos al pastor and 25 of tacos de suadero) obtained from different establishments located in the Cuauhtemoc district. The methodology described in the BAM FDA and NOM-113-SSA1-1994 and NOM-210-SSA1-2014 was used. **RESULTS:** From 100% of the samples analyzed, 86% showed the presence of coliform organisms in a range of 1 000 to 13 000 000 CFU/g, while *Salmonella* sp was found in 12% of the samples analyzed. **CONCLUSION:** The presence of *Salmonella* sp and coliform organisms show that food sold on public streets in Mexico City is prepared with poor quality and hygiene measures during its preparation and to be a threat to consumers' health.

Keywords: Salmonella, meat, establishment



Human Health and Biotechnology

MICROBIOLOGICAL QUALITY ON READY-TO-EAT SALADS IN MEXICO CITY

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INTRODUCTION: Nowadays ready-to-eat salads consumption has increased. This means potential risk of foodborne diseases transmission due to lack of hygiene practices during preparation. In the case of vegetables irrigation water, the use of untreated organic fertilizers, soil, rain, post-harvest operations have been considered as potential contamination factors. Indicator organisms search allows microbiological quality evaluation on this kind of food. Therefore, the objective of this study was to isolate indicator organisms and foodborne pathogens in 336 samples of ready-to-eat salads commercialized in different points of sale in Mexico City. **METHODOLOGY:** Microbiological quality was carried out following the methodology described on the bacteriological analytical manual of Food and Drugs Administration (BAM-FDA). **RESULTS:** We detected the presence of coliforms (CO) in 27.97% (94/336) by the plate count enumeration; fecal coliforms by MPN in 32.44% (109/336); 32.73% (110/336) of mesophilic aerobics (MA); moulds 12.20% (41/336) and yeast 11.90% (40/336). Regarding foodborne pathogens, *Salmonella* was isolated in 2.08%, *Staphylococcus aureus* 7.14%, *Listeria monocytogenes* 2.38%, *Bacillus cereus* 9.52% and *Escherichia coli* 29.16%. **CONCLUSION:** Our data show the potential risk of consuming these products regardless their origin, which means possible harm to the consumer's health due to the presence of foodborne pathogens.

Keywords: indicator group, safety, salads, vegetables

LPFG biosensor for IL-6 detection related to the ischemic process in a murine model.

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Introduction: Today, several disciplines are working together to develop reliable bio-detector devices. that allow for the timely detection of diseases related to the main causes of mortality worldwide. These devices named biosensors are composed of three main components: the bioreceptor, transducer, and output device. The bioreceptor element chosen in the biosensor design provides the specificity and affinity for joining the analyte. In this study, a biosensor device specific to IL-6 was developed because this protein was related to the inflammation that occurs after an ischemic cerebral vascular event and the sequences of the disease as well as the survival of the patient. **Methodology:** The biosensors were assembled by the self-assembled monolayer technique before this process Long Period Fiber Grating (LPFG) was recorded at 20 points by applying an electric arc. After being put together, the biosensor devices were tested on samples of ischemic rats that were killed at various post-ischemic times. IR spectroscopy, transmission spectroscopy, and electronic microscopy were used to characterize the assembly and detection processes, and PCA of the experimental data was then performed. **Results:** In data of transmission spectroscopy, changes in the transmission power due to the change in the refraction index at the various stages of assembly and detection were observed. Bands associated with the bonds corresponding to the different molecules linked on the surface of the optic fiber were observed when performing characterization by IR spectroscopy. Using the data from the two spectroscopic techniques, PCA was carried out obtaining clustering and discriminating of the data from the assembly and detection at various post-ischemia hours. Surface morphological alterations were seen in the micrographs, which distinguished between each stage of the device assembly. **Conclusion:** According to the results of the LPFG biosensor which was assembled and characterized by various approaches it was feasible to identify IL-6 in blood serum samples from ischemic rats and to differentiate its presence at different times following the induction of the disease.

Key words: LPFG biosensor, ischemia, IL-6.



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Industrial Biotechnology.

Characterization of extremotolerant filamentous fungi with chitosanolytic activity isolated from arid environment.

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Introduction. Filamentous fungi who inhabit extreme environments usually don't have optimal development, these organisms are known as extremotolerant. The habitat that conjunct various extreme conditions is an arid environment. Despite these, filamentous fungi have been reported growing in arid environments with adaptations that allow them to grow and feed, related to their enzymatic activity. This work aims to characterize the growth in solid media with different nutrients, incubation temperatures, and salinity of fungi with chitosanolytic activity isolated from an arid environment from Baja California, Mexico. **Methodology.** Three growth media were used to observe the effect of the nutrients on mycelial growth: PDA, YPD, and minimum media (MM). To get the effect of salinity and incubation temperature on mycelial growth the solid media with the best growth were incubated at 4, 28, 37, 45, and 50°C. About the effect of salinity stress, NaCl was added at three different concentrations: 2.5, 3.8, and 5.2 M. Finally, the hydrolytic activity was evaluated in PDA with chitosan at 1, 3, and 5 mg/mL. The selection criteria was the observation of a hydrolysis halo. **Results.** There was no difference in the mycelial growth between YPD and PDA. The best temperature to support growth of the isolates was 37°C, but two of the filamentous fungi can grow from 4 to 45 °C. The 60% of the isolates tolerated 2.5 M NaCl in the growth media showing a growth reduction and mycelial morphology changes. 80% of the isolates showed chitosanolytic activity in solid media. **Conclusion.** Fungi with chitosanolytic activity were nitrogen-promoted with a larger mycelial growth. Nutrients, incubation temperature, and salinity affect mycelial growth, morphology, conidiation, and pigmentation.

Keywords: Extremotolerant fungi, salinity resistance, temperature, chitosan.



Industrial Biotechnology

Lipase production by *Yarrowia lipolytica* strain 14v: Optimization and Evaluation

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Introduction: Lipases, important in lipid degradation, find diverse biotechnological applications. Focus on *Yarrowia lipolytica* lipase production, particularly strain 14v, aims at optimal substrate selection and lipase production. Besides enzymatic efficacy, substrate specificity and concentration are crucial. Lipases show promise in oil transformation for biotechnological purposes. This research highlights lipases' versatile role and their lipase activity. **Methodology:** Gompertz kinetic model was developed for strain 14v. Yeast-Tryptone medium with 10 g/L emulsion (olive oil or oleic acid) was used to screen the best carbon source using lipase activity as response. Growth conditions were maintained at 30°C for up to 96 h and kinetic parameters were determined. As a screening method for C/N and emulsion concentration at which an optimal value for lipase activity is achieved, different levels for these factors were assayed. Screening results determined the boundaries for a consecutive box-Behnken design, which consist in 3 factor blocked design (agitation speed, Carbon and Nitrogen). **Results:** Oleic acid showed to be the best carbon source. Lipase activity measurement highlighted peak activity at 72 hours in the presence of oleic acid, Optimization by surface response methodology revealed that 10 g/L of emulsion with C/N ratio of 5 are optimal for lipase production. Further optimization showed that a concentration of 4.327 g/L of tryptone and 4.064 g/L of yeast extract, with an agitation of 133 rpm, yield the most favourable conditions for growth and lipase production. These findings significantly contribute to understanding and optimizing lipase production, with strain 14v of *Yarrowia lipolytica* showing promise. The efficacy of oleic acid as a carbon source and the identified optimal growth conditions provides crucial insights for future biotechnological applications.

Keywords: *Lipases, Yarrowia lipolytica, Optimization*

Industrial Biotechnology

Effect of the components of the culture medium on the production of rhamnolipids produced by *Burkholderia thailandensis*

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Introduction: The metabolic activity of fungi, yeasts, and bacteria synthesizes biosurfactants. They are composed of both a hydrophilic and a hydrophobic part in a single molecule, when the hydrophilic part is a carbohydrate and the lipophilic part is a carbon chain, the biosurfactant is a glycolipid. One type of glycolipids are Rhamnolipids (RL). RL contain one or two rhamnose units linked to each other and to a carbon chain (hydrophobic moiety) that can vary in length (typically from C8 to C18). This represents a great possibility of combinations to obtain a variety of species. These species can reduce the surface and interfacial tension like synthetic surfactants but with the advantage of being biodegradable, biocompatible, eco-friendly. Finally, *Burkholderia thailandensis* is a known non-pathogenic microorganism that can biosynthesize RL. **Methodology:** Using a Plackett Burman design, the effect of the concentration of meat peptone, meat extract, NaNO₃, glycerol, trace elements, inoculum, and pH of the culture medium on the production of rhamnolipids by *B. thailandensis* E264 was evaluated. The RL were recovered from the supernatant after acid precipitation and ethyl acetate extraction. The product was analyzed using thin-layer chromatography (TLC), FTIR, HPLC-ELSD, and surface tension measurements. **Results:** Based on the statistical analysis, it was found that except peptone and meat extract, the rest of the factors had a significant effect on the production of RL; glycerol being the most important variable, followed by trace elements and the pH of the medium. On the other hand, the use of NaNO₃ and the level of inoculation negatively affect the synthesis of RL. In TLC, similar retention factors were observed between treatments. The FTIR analysis showed the characteristic bands for the OH stretching (3300 cm⁻¹), CH, CH₂, CH₃ (2900 cm⁻¹) and COO (1700 cm⁻¹). The RL of the treatment with the high levels of the factors reduced the surface tension of the water from 72 mN/m to 44.8 mN/m at 143 ppm. **Conclusion:** NaNO₃, glycerol, trace elements, inoculum, and pH of the culture medium affect the production of rhamnolipids by *B. thailandensis* E264. Glycerol is the most important factor, followed by trace elements and pH of the medium.

Key words: Rhamnolipids, Biosurfactants, *Burkholderia*

Industrial Biotechnology

**Effect of carbon and nitrogen source on the production of polyhydroxyacanoates by
*Burkholderia thailandensis***

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Introduction: Polyhydroxyalkanoates (PHA) are a family of polyhydroxyesters synthesized by various microorganisms as carbon and energy storage compounds under nutrient-limited conditions. PHA possess properties similar to synthetic thermoplastics such as polypropylene. This makes them useful for multiple applications. However, high production costs limit commercial applications of PHA. The objective of this work was to evaluate the effect of different carbon and nitrogen sources on the growth and PHA production by *Burkholderia thailandensis* E264. **Methodology:** Fermentation was carried out in 250 mL baffled Erlenmeyer flasks with 50 mL of culture medium. The medium was inoculated with 5% (v/v) of a 24 h bacterial culture and incubated with constant shaking (150 rpm) at 30 °C for 7 days. Samples were taken every 24 h. Biomass concentration was measured by dry weight and PHA concentration was analyzed by a spectrophotometric method. Four carbon sources (glucose, sucrose, xylose and glycerol) and four nitrogen sources ((NH₄)₂SO₄, NaNO₃, urea and peptone) were evaluated at a concentration of 20 and 2.5 g/L, respectively. Finally, the effect of the carbon source concentration (10 - 80 g/L) on the growth and PHA production was evaluated. **Results:** *B. thailandensis* grew and produced PHA from glucose, sucrose and glycerol as carbon sources, but not from xylose. The highest biomass concentration (5.6 ± 0.5 g/L) and PHA concentration (4.6 ± 0.4 g/L) were obtained at the end of the culture using glycerol as carbon source and urea as nitrogen source. Under these conditions the product/biomass yield ($Y_{P/X}$) was 0.91 ± 0.16 g PHA/g biomass. No significant effect of carbon source concentration was observed in the range of 10 to 40 g/L, but growth inhibition was observed at 80 g/L glycerol. **Conclusion:** *B. thailandensis* is a versatile microorganism that can grow and produce PHA using different carbon and nitrogen sources. The kinetic description of the growth and product formation will allow the design of efficient processes for the production of PHA by this microorganism.

Key words: Bioplastics, PHA, Carbon source, Nitrogen source, *Burkholderia thailandensis*