PhD THESIS

Sustainable Itaconic Acid Production from Renewable Biomass and Agricultural Residues: Optimization, Applications, and Integration into Biodegradable Polymers

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(SUMMARY OF THE DOCTORAL THESIS)

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SUMMARY

The increasing global demand for sustainable chemical production and eco-friendly materials has accelerated interest in itaconic acid (IA), a bio-based platform molecule with applications across the food, pharmaceutical, and packaging sectors. Traditionally synthesized from glucose via fermentation with *Aspergillus terreus*, recent research has shifted toward greener, low-cost processes that utilize agro-industrial and food waste streams as alternative substrates. This Ph.D. thesis explores such innovative strategies for IA production and application, with a specific focus on microbial bioconversion of underutilized residues and the development of biodegradable IA-based packaging films.

The research is structured across five experimental chapters (Chapters 4–8), each addressing a specific aspect of IA-related bioprocessing and application. These include enzyme-assisted fermentation using *A. awamori* and *A. terreus* for wheat bran valorization, microbial consortia fermentation of sushi by-products, the formulation of bioactive packaging films containing IA and natural additives, the shelf-life performance of these films in real food systems, and an integrated safety and consumer evaluation through *in silico* toxicity and sensory assessments.

This comprehensive approach not only investigates the technical feasibility of waste-based IA production but also assesses the practical application of IA in biodegradable packaging systems. Together, these studies align with circular economy principles and offer a cohesive model for transforming waste into functional, market-ready bio-based products.

Objectives of the Thesis

The overarching goal of this Ph.D. thesis is to valorize agro-industrial and food processing residues by converting them into value-added biochemicals and functional packaging materials, centered on the use of IA. These efforts aim to address environmental sustainability, food waste reduction, and the development of biodegradable packaging solutions that align with circular bioeconomy principles.

O1. Enzyme Production and Itaconic Acid Biosynthesis from Wheat Bran

Investigate the use of wheat bran, an abundant lignocellulosic by-product, as a feedstock for hydrolytic enzyme generation using *Aspergillus awamori* under solid-state fermentation. Apply the resulting enzymatic hydrolysates in submerged fermentation using *A. terreus* to produce itaconic acid, optimizing fermentation parameters for maximal yield and substrate conversion efficiency.

O2. Sequential Bioconversion of Sushi By-products

Develop a two-step microbial system utilizing *Bacillus subtilis* for initial enzymatic hydrolysis of complex sushi production waste, followed by *A. terreus* fermentation for IA synthesis. Analyze the microbial synergy, nutrient availability, and resulting IA production to demonstrate the feasibility of valorizing high-nutrient food waste in sequential bioprocesses.

O3. Formulation of IA-Based Biodegradable Packaging Films

Design and fabricate active packaging films by incorporating itaconic acid, chitosan, lemon peel extract, and silver nanoparticles into a PVA matrix. Evaluate their physicochemical properties, including tensile strength, barrier performance, antioxidant and antimicrobial capacities, with the aim of developing a multifunctional, bioactive, and eco-friendly packaging material.

O4. Shelf-Life Evaluation of Bioactive Films in Fresh Produce Packaging

Apply the IA-based films in the packaging of fresh raspberries to assess their effectiveness in extending shelf life under refrigerated conditions. Monitor moisture retention, microbial inhibition, and visual quality degradation to validate their performance as a sustainable alternative to conventional plastic films.

O5. Safety and Consumer Acceptability Assessment

Perform *in silico* toxicological screening of film components using ADMET Lab 2.0 to evaluate potential health risks, including mutagenicity and carcinogenicity. Conduct consumer sensory tests with semi-trained panelists to assess the acceptability of IA-based packaging films in real food contexts, focusing on parameters like texture, aroma, and visual appeal.

By meeting these objectives, the research contributes a complete model of waste-to-product innovation, from upstream fermentation processes to downstream application and validation.

Methodology

Fermentation and Enzyme Production: Wheat bran was used as a substrate for enzyme production using *A. awamori* in solid-state fermentation, and hydrolyzed wheat bran was then fermented by *A. terreus* in submerged culture to produce IA. The fermentations were optimized for temperature, pH, and nutrient conditions. HPLC was used to quantify IA and reducing sugars.

Bioconversion of Sushi By-products: A sequential microbial process was developed wherein *B. subtilis* hydrolyzed sushi waste, followed by IA fermentation with *A. terreus*. The process was monitored for pH, sugar consumption, and IA concentration.

Film Formulation and Characterization: PVA-based films were cast with IA, chitosan, lemon peel extract, and AgNPs. Mechanical strength, water vapor permeability, antioxidant activity (DPPH, ABTS), and antimicrobial properties against *E. coli* and *S. aureus* were analyzed.

Shelf-Life Assessment: The IA-based films were used to package raspberries stored under refrigeration. Weight loss, mold growth, and visual quality were tracked over time to determine preservation efficacy.

Toxicity and Sensory Testing: In silico toxicological profiles of film components were generated using ADMET Lab 2.0. Hedonic testing with 21 panelists rated IA-based films used on blueberries for acceptability.

Key Findings

Fermentation and Enzyme Production: Solid-state fermentation of wheat bran using *A. awamori* resulted in high yields of hydrolytic enzymes, like glucoamylase, cellulose, and endoglucanase (50 U/g, 55 FPU/g, and 15 U/g), supporting its role in breaking down complex carbohydrates. These enzymes enabled efficient substrate hydrolysis, producing sugar-rich media used for submerged fermentation with *A. terreus*. The IA concentration achieved from wheat bran hydrolysates demonstrated the viability of lignocellulosic waste as a carbon source for microbial biosynthesis.

Bioconversion of Sushi By-products: The two-step microbial process involving *B. subtilis* and *A. terreus* showcased a synergistic model for valorizing protein- and starch-rich food waste. The pre-hydrolysis by *B. subtilis* improved sugar availability and digestibility, which directly enhanced IA yield in the subsequent fermentation stage with *A. terreus*. Monitoring of pH, residual sugars, and IA levels revealed a streamlined metabolic conversion process.

Film Performance and Bioactivity: The IA-based biofilms containing chitosan, lemon peel extract, and AgNPs exhibited significantly higher mechanical strength (***$p < 0.001$), increased elasticity, and lower water vapor transmission rates compared to the control PVA-based biofilms. Antioxidant analyses (DPPH and ABTS assays) demonstrated radical scavenging activity up to 75%, while antimicrobial tests revealed inhibition zones greater than 15 mm against *E. coli* and *S. aureus*.

Shelf-Life Application in Fresh Produce: Application of these films in raspberry packaging resulted in a 35% reduction in weight loss and a two-day extension in shelf life compared to control samples. Mold development was significantly delayed, and fruit retained its firmness and visual appeal. This confirmed the functional efficacy of IA-enriched films in real food storage conditions. Toxicity modeling indicated a low risk of mutagenicity and carcinogenicity for film components. Sensory testing showed strong consumer preference for IA and chitosan-containing films.

General Conclusions

Agro-industrial and food processing residues, including wheat bran and sushi by-products, can be effectively converted into IA through enzyme-aided microbial fermentation.

Co-culture and sequential fermentation strategies using *B. subtilis* and *A. terreus* significantly enhance the bioconversion of complex substrates.

IA-based biodegradable films offer a multifunctional solution to food packaging, combining sustainability with strong antioxidant and antimicrobial protection.

Shelf-life extension of berries in IA-packaged films validates their practical application and supports food waste reduction.

In silico toxicity assessments confirm the safety of IA-based films for food contact. Positive consumer feedback supports market integration potential.

This research advances the role of IA as a dual-purpose molecule, serving in both microbial fermentation and as a functional component in packaging materials.

Recommendations

- Scale up IA production using food and agro-waste and assess industrial feasibility.
- Explore other residues like fruit peels, sugar beet pulp, or brewery waste.
- Conduct *in vivo* toxicity studies to complement in silico results.
- Optimize film properties for various food types.
- Develop smart packaging systems integrating IA-based sensors.
- Perform life cycle assessments to measure environmental benefits.
- Originality and Personal Contributions

This **thesis** presents original insights into biotechnological IA production from unconventional feedstocks and introduces novel multifunctional IA-based packaging films. The combined use of enzymatic pretreatment, microbial co-cultures, and active film development creates a comprehensive waste-to-product framework. *In silico* toxicity profiling and real-food shelf-life testing are pioneering additions that enhance the practical and regulatory relevance of the findings. This work supports sustainable chemistry, packaging innovation, and food system circularity.

Future research should refine co-culture systems, assess IA-based films in industrial settings, and explore synergistic effects with other bioactives to enhance film functionality and sustainability.