



UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA E
CIÊNCIA DE ALIMENTOS

JÉSSICA FERREIRA BORGES

INTEGRAÇÃO DE PROCESSOS PARA VALORIZAÇÃO DO
LICURI: PRODUÇÃO DE LIPASE, CARVÃO ATIVADO
FUNCIONALIZADO E MODIFICAÇÃO DE ÓLEO PARA
GERAÇÃO DE LIPÍDIOS ESTRUTURADOS

ITAPETINGA
BAHIA – BRASIL

2026

JÉSSICA FERREIRA BORGES

**INTEGRAÇÃO DE PROCESSOS PARA VALORIZAÇÃO DO
LICURI: PRODUÇÃO DE LIPASE, CARVÃO ATIVADO
FUNCIONALIZADO E MODIFICAÇÃO DE ÓLEO PARA
GERAÇÃO DE LIPÍDIOS ESTRUTURADOS**

Tese apresentada à Universidade Estadual do Sudoeste da Bahia como parte das exigências do programa de Pós-Graduação em Engenharia e Ciência de Alimentos para obtenção do Título de Doutora em Engenharia e Ciência de Alimentos, Área de Concentração em Ciência de Alimentos.

Orientadora: Prof. ^a DSc. ^a Cristiane Martins Veloso

ITAPETINGA
BAHIA – BRASIL

2026

DECLARAÇÃO DE APROVAÇÃO

Título: “INTEGRAÇÃO DE PROCESSOS PARA VALORIZAÇÃO DO LICURI: CARVÃO ATIVADO FUNCIONALIZADO, PRODUÇÃO DE LIPASE E MODIFICAÇÃO DE ÓLEO PARA GERAÇÃO DE LIPÍDIOS ESTRUTURADOS”

Autora: JÉSSICA FERREIRA BORGES

Orientadora: Prof.^a Dr.^a Cristiane Martins Veloso

Aprovada como parte das exigências para obtenção do Título de **DOUTORA EM ENGENHARIA E CIÊNCIA DE ALIMENTOS, ÁREA DE CONCENTRAÇÃO: CIÊNCIA DE ALIMENTOS**, pela Banca Examinadora.

Documento assinado digitalmente
 **MYLENA JUNQUEIRA PINTO BRITO**
Data: 11/05/2026 08:53:21-0300
Verifique em <https://validar.iti.gov.br>

Dr.^a Mylena Junqueira Pinbto Brito
(EMBASA)

Documento assinado digitalmente
 **MATEUS PEREIRA FLORES SANTOS**
Data: 04/05/2026 16:23:25-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. Mateus Pereira Flores Santos
(UFS)

Documento assinado digitalmente
 **ALEXSANDRA NASCIMENTO FERREIRA**
Data: 08/05/2026 12:26:47-0300
Verifique em <https://validar.iti.gov.br>

Prof.^a Dr.^a Alessandra Nascimento Ferreira
(UESB)

Documento assinado digitalmente
 **ELIZAMA AGUIAR DE OLIVEIRA**
Data: 30/04/2026 08:20:38-0300
Verifique em <https://validar.iti.gov.br>

Prof.^a Dr.^a Elizama Aguiar de Oliveira
(UESC)

Documento assinado digitalmente
 **CRISTIANE MARTINS VELOSO**
Data: 14/05/2026 14:35:55-0300
Verifique em <https://validar.iti.gov.br>

Prof.^a Dr.^a Cristiane Martins Veloso
Orientadora - UESB
Presidente da Banca

Itapetinga-BA, 29 de abril de 2026.

662.62 Borges, Jéssica Ferreira.

B731i

Integração de processos para valorização do licuri: carvão ativado funcionalizado e modificação de óleo para geração de lipídios estruturados. / Jéssica Ferreira Borges. – Itapetinga-BA: Universidade Estadual do Sudoeste da Bahia, 2026.

139 p.

Tese apresentada ao Programa de Pós-Graduação em Engenharia e Ciência de Alimentos da Universidade Estadual do Sudoeste da Bahia como requisito parcial para obtenção do título de Doutora em Engenharia e Ciência de Alimentos. Sob a orientação da Profª. D. Sc. Cristiane Martins Veloso.

1. Licuri – Valorização – Processamento de resíduos. 2. Casca de licuri – Processamento – Carvão ativado. 3. Desenvolvimento sustentável – Bioeconomia – Resíduos regionais. I. Universidade Estadual do Sudoeste da Bahia - Programa de Pós-Graduação de Doutorado em Engenharia e Ciências de Alimentos, *Campus* de Itapetinga. II. Veloso, Cristiane Martins. III. Título.

CDD(21): 662.62

Catálogo na Fonte:

Cláudia Aparecida de Souza – CRB 1014-5ª Região
Bibliotecária – UESB – Campus de Itapetinga-BA

Índice Sistemático para desdobramentos por Assunto:

1. Licuri : Valorização
2. Licuri : Processamento de resíduos
3. Casca de licuri : Bioeconomia
4. Carvão ativado
5. Resíduos regionais

A Deus

A minha mãe

*A todas aqueles que encontraram nos estudos a possibilidade de ser alguém na vida, e com
isso, tornaram-se instrumentos de transformação da realidade*

Dedico!

SUMÁRIO

LISTA DE TABELA	9
CAPÍTULO 3	9
LISTA DE FIGURAS	10
RESUMO	12
ABSTRACT	14
CAPÍTULO 1	15
1. INTRODUÇÃO	16
2. REVISÃO BIBLIOGRÁFICA	19
2.1. Valorização de resíduos agroindustriais na bioeconomia	19
2.2. Produção e funcionalização de carvão ativado	20
2.2.1 Funcionalização de carvão ativado	23
2.3. Imobilização enzimática	24
2.4. Enzimas e sua obtenção por fermentação em estado sólido (FES)	27
2.4.1 Lipases	29
2.5. Produção de lipídeos estruturados	30
3. REFERÊNCIAS	31
CAPÍTULO 2	37
OBJETIVO GERAL	38
OBJETIVOS ESPECÍFICOS	38
CAPÍTULO 3	39
ARTIGO 1	39
Enzymatic interesterification of regional oils: advances in lipase immobilization and perspectives for structured lipid production - A Short Review	39
ABSTRACT	40
1. Introduction	41
2. Fundamentals of Enzymatic Interesterification (EIE)	42
2.1. Principles of reaction and role of lipases	42
2.2. Mechanisms of EIE: acidolysis, transesterification, and intramolecular interesterification	44
3. Immobilized lipase in interesterification: Strategies and Supports	48
4. Types of Structured Lipids: Technological and Functional Applications	51
4. Challenges and Future Perspectives in Structured Lipid Research	54
6. Conclusion	55
7. References	56
CAPÍTULO 4	70
ARTIGO 2	70

Valorization of Licuri Coconut Shell Waste into Functional Activated Carbon for Enzyme Immobilization and Biocatalysis.....	70
ABSTRACT	71
1. Introduction	72
2. Materials and methods	74
2.1. Materials and reagents.....	74
2.2. Synthesis of activated carbons.....	74
2.2.2. Activated carbon synthesis.....	74
2.2.3. Optimization of activated carbon synthesis.....	75
2.3. Functionalization of the activated carbon	75
2.3.1. Modification with genipin	75
2.3.2. Modification with iron salts and chelating	75
2.4. Supports characterization.....	76
2.5. Lipase immobilization on carbon supports	77
2.5.1. Immobilization on activated carbons from experimental design.....	77
2.5.2. Immobilization on functionalized supports under optimal conditions.....	77
2.5.3. Lipase activity assay	78
2.6. Statistical analysis.....	78
3. Results and discussion.....	78
3.1 Lignocellulosic Composition and Ash Content of Licuri Shells.....	78
3.2 Optimization of Activated Carbon Synthesis	79
3.3 Textural and surface properties of optimized and functional carbons	82
3.4 Effect of surface functionalization on carbon surface chemistry	83
3.5 Immobilization of lipase and activity assay	92
4. Conclusion.....	95
5. References	95
CAPÍTULO 5	108
ARTIGO 3.....	108
Sustainable Valorization of Licuri Residues into Functionalized Carbon Supports for Lipase Immobilization and Licuri Oil Modification	108
2. MATERIAL AND METHODS	112
2.1. Reagents and raw materials.....	112
2.2. Microorganisms and inoculum preparation.....	112
2.3. Solis-state fermentation for lipase production	112
2.4. Experimental design and statistical analysis	113
2.4.1. Doehlert design.....	113
2.4.2. Rotatable Central Composite Design (RCCD)	113
2.5. Extraction of crude enzyme	114

2.6.	Lipase activity assay	114
2.7.	Enzymatic characterization	115
2.7.1.	Determination of optimum temperature and pH	115
2.7.2.	Enzyme stability	115
2.7.3.	Determination of kinetic parameters	115
2.8.	Immobilization of the crude enzymatic extract.....	116
2.9.	Enzymatic interesterification.....	116
2.10.	Rheological analysis.....	117
2.11.	Thermal characterization by Differential Scanning Calorimetry	117
3.	RESULTS AND DISCUSSION	117
3.1.	Lipase production and process optimization by solid-state fermentation	117
3.2.	Effect of pH on lipase activity	121
3.3.	Effect of temperature on lipase activity	122
3.4.	Thermal stability of the enzyme	123
3.5.	Enzyme kinetic parameters	124
3.6.	Immobilization of crude extract lipase on activated carbon: effect of support type and contact time	125
3.7.	Rheological behavior of interesterified licuri oil using immobilized lipase	128
3.8.	Thermal characterization by Differential Scanning Calorimetry (DSC)	130
4.	Conclusion	132
5.	References	132
	CAPÍTULO 6	137
	CONSIDERAÇÕES FINAIS	137

LISTA DE TABELA

CAPÍTULO 3

Table 1. Vegetable oils modified by lipases through enzymatic interesterification.

Table 2. Enzymatic interesterification using immobilized lipases: support materials, immobilization methods, applications, and effects on triacylglycerol composition.

CAPÍTULO 4

Table 1. Immobilization capacity and enzyme activity (U) of lipase activated carbons synthesized under the conditions defined by the Rotatable Central Composite Design (RCCD).

Table 2. Parameters of the quadratic model were estimated for enzyme activity and immobilization capacity. X_1 time (min) and X_2 impregnation ratio (%w/w).

Table 3. Textural properties of activated carbons.

CAPÍTULO 5

Table 1. Experimental levels (coded and real values) of fermentation time and inducer ratio in the Doehlert design.

Table 2. Experimental results of lipase production by solid-state fermentation according to the Doehlert design.

Table 3. Analysis of variance (ANOVA) for lipase production by solid-state fermentation using licuri coconut cake as substrate based on the Doehlert design.

Table 4. Experimental results obtained from the central composite rotational design (CCRD) for lipase production.

Table 5. Kinetic parameters obtained from the Michaelis–Menten model for the crude enzymatic extract.

Table 6. Rheological parameters (consistency index, K, flow behavior index, n, and apparent viscosity) of crude and enzymatically modified licuri oil samples.

Table 7. Thermal properties of crude licuri oil and enzymatically interesterified samples determined by differential scanning calorimetry (DSC).

LISTA DE FIGURAS

CAPÍTULO 1

Figura 1- Esquema representativo da conversão de resíduos agroindustriais de licuri (*Syagrus coronata*) em carvão ativado. Imagem elaborada com BioRender.

Figura 2- Esquema ilustrativo dos principais métodos de imobilização enzimática.

Figura 3- Principais características desejáveis em materiais de suporte para imobilização enzimática. Imagem elaborada com BioRender.

CAPÍTULO 3

Figure 1. Comparison between chemical and enzymatic interesterification of lipids, emphasizing catalyst type, selectivity, operating conditions, industrial applicability, and advantages.

Figure 2. Schematic representation of the intramolecular enzymatic interesterification mechanism catalyzed by lipases.

Figure 3. Schematic overview of lipase immobilization strategies and support materials applied in enzymatic interesterification, highlighting their advantages and limitations.

Figure 4. Overview of the main applications of structured lipids in food and pharmaceutical systems, highlighting their role in improving functionality, nutritional quality, and physicochemical properties.

CAPÍTULO 4

Figure 1. Response surface with optimization of activated carbon production for better immobilization condition (A) and (B) response surface with optimization of activated carbon production for better recovery condition of enzyme activity.

Figure 2. Point of zero charge of activated carbon (AC), functionalized with genipin (AC-GF), and activated carbon functionalized with iron particles and chelator agent (AC-MF).

Figure 3. FTIR of the shell of the licuri coconut, activated carbon (AC), activated carbon, functionalized with genipin (AC-GF), and activated carbon functionalized with iron ion particles and chelator (AC-MF).

Figure 4. SEM images of AC (493x;9.79kx) (A), AC-GF (501x;9.07kx) (B), and AC-MF (1.02kx; 6.89kx) (C).

Figure 5. Elemental composition of activated carbon unfunctionalized (AC), functionalized with genipin (AC-GF), and functionalized with iron and chelating agent (AC-MF) by EDX analysis.

Figure 6. Diffractogram of activated carbon (AC) (A) and activated carbons modified with iron ion particles and chelator (AC-MF) (B).

Figure 7. Thermogravimetric (TG), differential thermal (DTA), and derivative thermogravimetric (DTG) curves of unfunctionalized activated carbon (AC) (a), genipin-functionalized carbon (AC-GF) (b), and iron/chelating agent-functionalized carbon (AC-MF) (c).

Figure 8. Immobilization efficiency (IE) and relative activity recovery (RA) of *Candida rugosa* lipase immobilized on different supports: activated carbon (AC), genipin-functionalized activated carbon (AC-GP), and iron-chelated activated carbon with iminodiacetic acid (AC-MF). Different uppercase letters indicate statistically significant differences ($p < 0.05$) in RA according to Tukey's test.

CAPÍTULO 5

Figure 1. Contour surface(A) Response surface (B) of *Penicillium roqueforti* lipase activity as a function of the effect of fermentation time (h) and inducer ratio (%).

Figure 2. Effect of pH on the enzymatic activity of lipase

Figure 3. Effect of temperature on the enzymatic activity of lipase

Figure 4. Thermal stability of the enzymatic extract at different temperatures (30, 40, 50, and 60 °C) over time (0–120 min). Residual activity was determined under optimal conditions and expressed as relative activity (%).

Figure 5. Effect of immobilization time (2, 4, and 6 h) and support type (AC, AC-GF, and AC-MF) on the enzymatic activity of immobilized lipase. Error bars represent standard deviation

Figure 6. Apparent viscosity as a function of shear rate for crude and enzymatically interesterified licuri oil samples.

RESUMO

BORGES, J.F. **Integração de processos para valorização do licuri: produção de lipase, carvão ativado funcionalizado e modificação de óleo para geração de lipídios estruturados.** Itapetinga – BA: UESB, 2023.140 p. (Defesa – Doutorado em Engenharia e Ciência de Alimentos). *

A valorização de resíduos agroindustriais tem ganhado destaque como estratégia sustentável e tecnológica, especialmente em regiões com elevado potencial produtivo e escassa industrialização, como o semiárido baiano. Este trabalho propõe a integração de processos biotecnológicos, utilizando diferentes resíduos do processamento do licuri (*Syagrus coronata*), visando ao desenvolvimento de produtos de alto valor agregado para a indústria de alimentos. A casca foi utilizada na otimização da síntese de carvões ativados por meio de planejamento experimental. O carvão ativado otimizado foi posteriormente funcionalizado com genipina (AC-GF) ou com íons ferro, em conjunto com um agente quelante (AC-MF). Diferentes técnicas de caracterização, incluindo FTIR, MEV, EDX, DRX, análise térmica e pHpzc, confirmaram o sucesso dos processos de ativação e funcionalização. Os parâmetros otimizados foram tempo de carbonização de 107,3 minutos e razão de impregnação de 2,53 g·g⁻¹. Os carvões ativados produzidos foram utilizados como suportes para imobilização da lipase de *Candida rugosa*, alcançando eficiência de imobilização superior a 85% em todos os materiais avaliados. O suporte AC-GF apresentou a maior recuperação de atividade (~65%). A torta de licuri foi utilizada como substrato na produção de lipase por fermentação em estado sólido, com *Penicillium roqueforti*. A otimização do processo permitiu alcançar uma elevada atividade enzimática (até 197,73 U mL⁻¹), evidenciando o potencial do resíduo como fonte de baixo custo para a produção enzimática. A enzima obtida apresentou características compatíveis com aplicações industriais, incluindo atividade ótima em condições moderadas de pH e temperatura. A lipase produzida foi imobilizada no suporte previamente desenvolvido e aplicada à interesterificação enzimática do óleo de licuri. A análise reológica indicou alterações nas propriedades do óleo após o tratamento enzimático, com transição do comportamento pseudoplástico para comportamento próximo ao newtoniano, sugerindo rearranjos moleculares nos triacilgliceróis. Esses resultados foram corroborados pela análise térmica (DSC), que evidenciou modificações no perfil de cristalização e fusão do óleo, confirmando alterações na organização estrutural dos lipídios. Os resultados demonstram a viabilidade de um sistema biotecnológico completo, que conecta a produção do suporte, a obtenção do biocatalisador e sua aplicação em processos de modificação lipídica. O estudo contribui para o avanço da bioeconomia ao propor alternativas sustentáveis para o aproveitamento de resíduos regionais, agregando valor às cadeias produtivas do semiárido brasileiro.

Palavras-chave: *Syagrus coronata*; Bioeconomia; Valorização de resíduos; Biocatálise; Lipídeos estruturados.

ABSTRACT

The valorization of agro-industrial residues has emerged as a sustainable and technological strategy, particularly in regions with high productive potential and limited industrialization, such as the semi-arid region of Bahia, Brazil. This study proposes the integration of biotechnological processes based on licuri (*Syagrus coronata*) to develop high value-added products for the food industry. Licuri shells were used to produce activated carbons, whose synthesis was optimized through experimental design. The optimal conditions were a carbonization time of 107.3 min and an impregnation ratio of 2.53 g·g⁻¹. The activated carbon obtained under optimal conditions was further functionalized with genipin (AC-GF) or iron ions combined with a chelating agent (AC-MF). Characterization techniques, including FTIR, SEM, EDX, XRD, thermal analysis, and pH_{pzc}, confirmed the effectiveness of the activation and functionalization processes. Lipase immobilization from *Candida rugosa* showed high efficiency (>85%) across all supports, with AC-GF achieving the highest activity recovery (~65%), likely due to favorable surface interactions and improved enzyme orientation. Licuri cake was used as a substrate for lipase production via solid-state fermentation using *Penicillium roqueforti*. Process optimization resulted in high enzymatic activity (up to 197.73 U mL⁻¹), demonstrating the potential of this residue as a low-cost substrate. The enzyme exhibited suitable characteristics for industrial applications, including optimal activity under moderate pH and temperature conditions. The produced lipase was immobilized on the developed support and applied in the enzymatic interesterification of licuri oil. Rheological analysis revealed structural modifications, with a transition from pseudoplastic to near-Newtonian behavior, indicating molecular rearrangements in triacylglycerols. Overall, the results demonstrate the feasibility of an integrated biotechnological system linking support production, biocatalyst development, and lipid modification. This work contributes to the advancement of the bioeconomy by proposing sustainable strategies for the valorization of regional residues and adding value to production chains in the Brazilian semi-arid region.

Keywords: *Syagrus coronata*; Bioeconomy; Waste valorization; Biocatalysis; Structured lipids.

CAPÍTULO 1

INTRODUÇÃO GERAL

REFERENCIAL TEÓRICO

OBJETIVOS

1. INTRODUÇÃO

A busca por soluções tecnológicas que conciliem inovação e sustentabilidade tem incentivado a exploração do potencial de subprodutos agroindustriais, especialmente aqueles abundantes em regiões historicamente negligenciadas do ponto de vista econômico e científico (Alves et al., 2023). No semiárido brasileiro, há grande disponibilidade de biomassa lignocelulósica com pouco ou nenhum aproveitamento industrial, o que representa não apenas um desafio ambiental, mas também uma oportunidade concreta de transformação (Guimarães; Shiosaki; Mendes, 2021; Pereira et al., 2025). A perspectiva de transformar esses subprodutos em insumos úteis dialoga diretamente com os princípios da bioeconomia e da economia circular, especialmente quando se considera a produção de materiais de alto valor agregado, como biocatalisadores (Kumar Mishra; Singh; Acharya, 2024).

Entre os recursos com esse potencial, destaca-se o licuri (*Syagrus coronata*). Conhecida como “ouro do sertão”, uma palmeira nativa do semiárido brasileiro desempenha papel fundamental na subsistência das comunidades locais, especialmente no estado da Bahia (Guimarães; Shiosaki; Mendes, 2021; Rodrigues; Mendes; Jesus, 2020). Seus frutos são utilizados na alimentação e na medicina tradicional e constituem uma fonte importante de renda para as populações do sertão. Atualmente, cooperativas locais utilizam o licuri na produção de produtos como balas, sorvetes e biscoitos, gerando resíduos subaproveitados, como cascas e tortas (Guimarães; Shiosaki; Mendes, 2021). Esses resíduos apresentam características adequadas à produção de materiais funcionais, como o carvão ativado (Kumar Mishra; Singh; Acharya, 2024).

Derivado de processos termoquímicos como a pirólise, o termo carvão ativado (CA) é utilizado para descrever materiais à base de carbono que possuem uma elevada área superficial, estrutura porosa interna desenvolvida, bem como grande número de grupos funcionais oxigenados, estabilidade química, alta resistência mecânica e boa reciclabilidade (He et al., 2023; Zhang; Zuo, 2024). Além disso, a superfície do carvão ativado pode ser modificada quimicamente, conferindo-lhe características específicas que ampliam sua aplicabilidade. Nesse contexto, a funcionalização do carvão ativado com genipina e nanopartículas de ferro tem despertado interesse por promover estabilidade química e biocompatibilidade, além de conferir propriedades magnéticas úteis para separação e recuperação em processos industriais, e por introduzir grupos funcionais capazes de favorecer a ancoragem e a estabilidade de

enzimas, especialmente lipases. Essas características tornam esses materiais suportes promissores para processos de imobilização enzimática (Doğan et al., 2024; Santos et al., 2022; Wolf et al., 2025).

A imobilização enzimática é uma técnica que ajusta e aprimora as propriedades da enzima, incluindo a atividade catalítica, a seletividade, a especificidade, a estabilidade e a capacidade de reutilização (Bai; Yu; Du, 2025; Zdarta et al., 2018). Os métodos de imobilização consistem no confinamento da enzima em um determinado espaço, com estabilidade e retenção da atividade biológica, destacando como principais vantagens: simplicidade de execução, baixo custo operacional, facilidade de recuperação e reutilização da molécula e boa especificidade para o substrato. Devido a essas vantagens, a imobilização enzimática é uma estratégia utilizada para superar as limitações do uso da enzima livre (Adamian et al., 2021; Sampaio et al., 2022). Dentre as enzimas amplamente estudadas em processos de imobilização devido à sua vasta aplicação biotecnológica, estão as lipases.

Lipases são catalisadores biológicos cuja função natural é catalisar a hidrólise de triacilgliceróis em diacilgliceróis, monoacilgliceróis e ácidos graxos livres. No entanto, também possuem mecanismo de atuação em reações de esterificação, transesterificação e interesterificação quando submetidas a condições não aquosas (Salgado; dos Santos; Vanetti, 2022). Entre as enzimas industriais, as lipases representam aproximadamente 10% do mercado mundial de enzimas, com previsão de crescimento expressivo nas próximas décadas (Grand View Research, 2023). Esse protagonismo se deve à sua aplicação em setores como os de alimentos, biodiesel, detergentes, têxtil, farmacêutico e cosmético (Chandra et al., 2025). Uma das aplicações em crescimento da lipase na indústria de alimentos é a síntese de lipídeos estruturados (LEs) por meio da interesterificação enzimática.

Os lipídeos estruturados (LEs) são obtidos por meio de processos que reorganizam os ácidos graxos do mesmo triacilglicerol (TAG) ou entre diferentes triacilgliceróis, sem alterar a composição global (Temkov; Mureşan, 2021). A interesterificação enzimática é um processo catalisado por lipases, que oferece vantagens como alta seletividade, uso de condições de reação suaves, menos reações paralelas, menos desperdício e facilidade de recuperação do produto em comparação com a interesterificação química (Ai et al., 2023; Sivakanthan; Madhujith, 2020). Os LEs fornecem propriedades funcionais específicas para os óleos e gorduras, tais como pontos de fusão desejáveis, estabilidade oxidativa aprimorada e digestibilidade melhorada, sendo uma solução promissora em relação a métodos como a hidrogenação tradicional, que gera ácidos graxos trans e impactos ambientais e energéticos durante o processo. Estudos indicam que a versatilidade do processo de interesterificação

permite o uso de óleos vegetais de várias fontes para produzir lipídios estruturados, incluindo opções alternativas (Romero; Minari; Collins, 2022; Zhang et al., 2022). Isso se alinha aos princípios de uma economia circular e de sustentabilidade, cada vez mais valorizados nas práticas industriais e acadêmicas.

Este estudo teve como objetivo desenvolver e integrar processos tecnológicos voltados à valorização dos resíduos do licuri (*Syagrus coronata*), por meio da produção de carvão ativado a partir da casca, de sua aplicação como suporte para imobilização de lipases e do aproveitamento da torta do licuri para produção enzimática, culminando na aplicação do biocatalisador obtido na interesterificação do óleo de licuri. A proposta articula produção enzimática, engenharia de materiais e aplicação catalítica em um modelo orientado à geração de valor a partir de resíduos, com ênfase em sustentabilidade e circularidade. Nesse contexto, o trabalho busca alinhar-se aos Objetivos de Desenvolvimento Sustentável (ODS) da Organização das Nações Unidas, especialmente ao ODS 8 (Trabalho Decente e Crescimento Econômico), ao incentivar a agregação de valor a recursos regionais e o fortalecimento de cadeias produtivas locais; ao ODS 9 (Indústria, Inovação e Infraestrutura), por meio do desenvolvimento de bioprocessos inovadores e tecnologias sustentáveis; e ao ODS 12 (Consumo e Produção Responsáveis), ao promover o aproveitamento integral dos resíduos do licuri e a redução de desperdícios ao longo da cadeia produtiva.

2. REVISÃO BIBLIOGRÁFICA

2.1. Valorização de resíduos agroindustriais na bioeconomia

A geração de resíduos agroindustriais é um dos principais desafios ambientais do mundo contemporâneo. O aumento da produção agrícola e da industrialização dos alimentos tem gerado toneladas de subprodutos e resíduos que, na ausência de estratégias de reaproveitamento, são frequentemente acumulados em lixões ou queimados a céu aberto. Esse descarte inadequado impacta diretamente o solo, os corpos hídricos e a qualidade do ar, além de representar uma perda significativa de recursos que poderiam ser reintroduzidos na cadeia produtiva (Adil et al., 2026). Diante dessa situação, cresce a necessidade de encontrar soluções que aliem gestão ambiental eficiente, inovação tecnológica e geração de produtos a partir do reaproveitamento de materiais antes considerados resíduos (Kumar Mishra; Singh; Acharya, 2024; Ukanwa et al., 2019).

A valorização de biomassas lignocelulósicas tem se destacado como uma estratégia chave dentro da bioeconomia e da chamada química verde. Esses resíduos vegetais, compostos majoritariamente por celulose, hemicelulose e lignina, são abundantes, renováveis e apresentam uma estrutura química versátil que permite sua conversão em uma ampla gama de produtos, como biocombustíveis, bioplásticos, enzimas e materiais adsorventes (Alves et al., 2023; Adil et al., 2026). Além disso, a transformação desses subprodutos em insumos tecnológicos contribui para a redução da pegada ambiental dos processos industriais, gerando alternativas de menor impacto e maior valor agregado. O uso dessas biomassas como matéria-prima secundária também promove inclusão social e desenvolvimento local, especialmente em regiões rurais onde a agroindústria é importante, mas os resíduos ainda são pouco aproveitados (Kumar Mishra; Singh; Acharya, 2024).

O licuri (*Syagrus coronata*), uma palmeira nativa do semiárido baiano que oferece não apenas um fruto nutritivo e oleaginoso, mas também uma série de subprodutos subutilizados, pode ser considerado uma fonte de biomassa com potencial de aproveitamento (Guimarães; Shiosaki; Mendes, 2021). Durante a extração do óleo das amêndoas, a casca externa e a torta residual são frequentemente descartadas, podendo ser utilizadas de maneira rudimentar, como combustível doméstico ou ração animal de baixa qualidade. No entanto, estudos mostram que esses subprodutos apresentam composição lignocelulósica rica, o que os torna promissores como matéria-prima alternativa para processos industriais sustentáveis. Essa abundância aliada à carência de cadeias de reaproveitamento organizadas evidencia a necessidade de desenvolver

soluções que transformem os resíduos do licuri em produtos com maior valor agregado (Borges et al., 2023; Peixoto et al., 2022).

O aproveitamento dos subprodutos do processamento do licuri é particularmente interessante, por se tratar de um recurso típico de uma região marcada por vulnerabilidades sociais, climáticas e econômicas. Ao promover o reaproveitamento, é possível não apenas propor soluções tecnológicas inovadoras, mas também fortalecer arranjos produtivos locais, gerar emprego e renda e valorizar o saber tradicional das comunidades extrativistas (Alves et al., 2024). O resíduo da casca, por exemplo, possui alto teor de carbono fixo, enquanto a torta de prensagem é rica em nutrientes e compostos orgânicos que podem ser aproveitados em processos fermentativos. A existência de uma cadeia produtiva já estabelecida para o óleo, ainda que informal em muitos territórios, reforça o potencial de integração desses resíduos em rotas de biorrefinaria e de uso tecnológico (Alves et al., 2024; Gouveia et al., 2020).

A conversão de resíduos lignocelulósicos em materiais de aplicação tecnológica, como o carvão ativado, constitui uma estratégia viável e sustentável. Por meio de processos termoquímicos, como a pirólise controlada, resíduos, como a casca do licuri, podem ser transformados em materiais porosos com propriedades adsorptivas e estruturais de interesse. Essa abordagem promove a valorização de um resíduo abundante e ainda pouco explorado, ao mesmo tempo em que se alinha aos princípios da economia circular, à redução de impactos ambientais e ao desenvolvimento de soluções regionais com potencial de aplicação industrial.

2.2. Produção e funcionalização de carvão ativado

A palavra carvão ativado (CA) é comumente utilizada para descrever materiais à base de carbono que possuem elevada área superficial, estrutura porosa interna desenvolvida, grande número de grupos funcionais oxigenados, estabilidade química, alta resistência mecânica e boa reciclabilidade (Duan et al., 2021; Gayathiri et al., 2022). Essas características conferem aos carvões ativados grande versatilidade, permitindo sua utilização como adsorventes na remoção de poluentes nas fases gasosa e líquida, na purificação de água, bem como em aplicações médicas, industriais e farmacêuticas. Além disso, podem atuar como catalisadores ou como suportes catalíticos em diferentes processos (Borges et al., 2023; Doğan et al., 2024; Kuśmierk; Szala; Świątkowski, 2016). Na Figura 1 é apresentada uma ilustração esquemática do processo de conversão do resíduo agroindustrial do licuri, uma biomassa lignocelulósica, em carvão ativado.

As propriedades químicas do carvão ativado são influenciadas principalmente pelos seus grupos funcionais de superfície e presença de heteroátomos. Mais especificamente, as

transformações químicas e eliminação de compostos voláteis durante a preparação do carvão ativado geram vários grupos de superfície, como grupos hidroxila, carbonila, carboxila e amino, além de anéis aromáticos (Brito et al., 2020). Devido a esses diferentes grupos funcionais, as características químicas da superfície dos carvões podem variar de ácidas a alcalinas, oxidantes, redutoras, hidrofílicas ou hidrofóbicas (Santos et al., 2020). Tais características influenciam diretamente as propriedades adsorptivas e a reatividade do carvão ativado, que serão determinadas pelo método de síntese e ativação desse material.

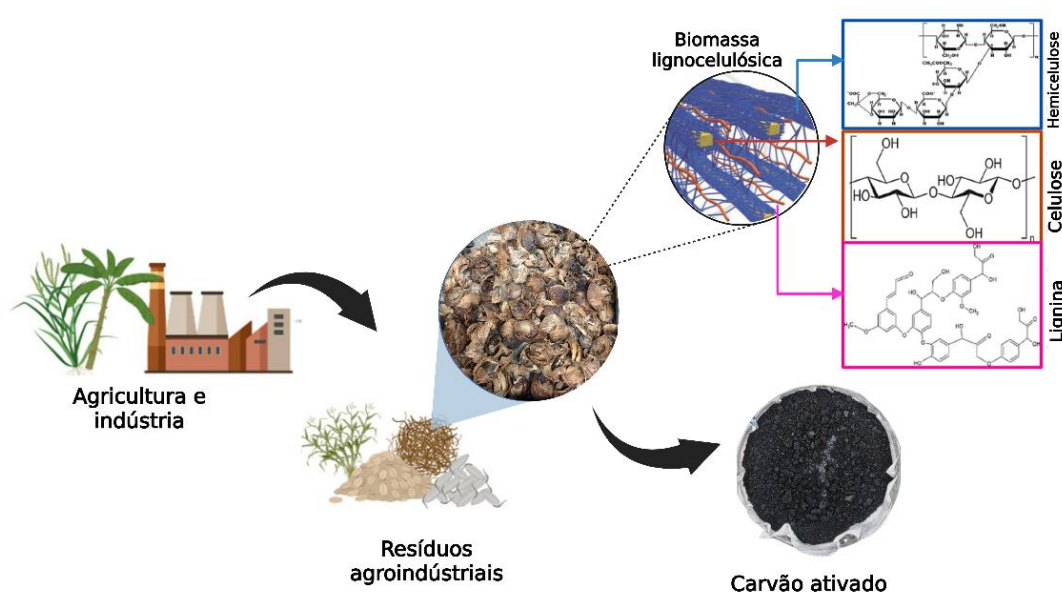


Figura 1- Esquema representativo da conversão de resíduos agroindustriais de licuri (*Syagrus coronata*) em carvão ativado. Imagem elaborada com BioRender.

Os carvões ativados podem ser sintetizados por meio de uma variedade de métodos e técnicas, porém, independentemente do método, há basicamente duas etapas na produção de carvões ativados: ativação e carbonização. Estas etapas podem ocorrer de forma simultânea ou como etapas distintas (Gayathiri et al., 2022), sendo o processo de carbonização indispensável em todas elas. Já o processo de ativação e os agentes utilizados podem variar com base no precursor usado (Kumar Mishra; Singh; Acharya, 2024).

O processo de ativação é uma etapa importante na síntese dos carvões ativados, pois leva ao aumento da porosidade, através do aumento do volume de poros de diâmetro menores e na criação de novos poros no material precursor. A ativação do material pode ser realizada por dois métodos: ativação química e ativação física (Nascimento et al., 2024a). A ativação física

consiste em duas etapas térmicas distintas. Na primeira etapa, o precursor é submetido a altas temperaturas na faixa de 700-900°C, sob atmosfera inerte para evitar a combustão do material carbonáceo. Nesta etapa ocorre a remoção de heteroátomos e voláteis, que resulta em carvões com um alto teor de carbono, mas com baixa porosidade. A segunda etapa do processo de ativação física consiste na remoção seletiva dos átomos de carbono mais reativos por meio de reações de gaseificação controladas que irão gerar a porosidade características dos carvões ativados. Os gases mais comumente utilizados são o vapor de água e gás carbônico (Kumar Mishra; Singh; Acharya, 2024).

A ativação química, mesmo tendo apenas uma etapa térmica, apresenta três diferentes estágios de síntese. O primeiro estágio consiste na impregnação do precursor com o agente ativador. Os agentes de ativação mais comuns são: ácido fosfórico (H_3PO_4), cloreto de zinco (ZnCl_2), hidróxido de potássio (KOH) e hidróxido de sódio (NaOH) (Neme; Gonfa; Masi, 2022). Após a impregnação, ocorre o tratamento térmico em atmosfera inerte em diferentes temperaturas, dependendo do agente ativador escolhido. Nesta etapa ocorre reações de despolimerização, desidratação e condensação, resultando em maiores rendimentos de carbono do que na ativação física, devido à restrição na formação de alcatrões e voláteis. Finalmente, o último estágio envolve a lavagem para remover os agentes ativadores remanescentes e os subprodutos da reação, que obstruem os poros recém-formados, e a secagem (Lewoyehu, 2021). Esse método de ativação oferece várias vantagens em relação a ativação física, que incluem principalmente temperatura de ativação mais baixa (geralmente $< 500^\circ\text{C}$) em comparação com a temperatura de ativação física (500-900°C), etapa de ativação única, maiores rendimentos, melhores características porosas e menores tempos de ativação (Duan et al., 2021).

Independentemente do método de síntese, a capacidade de adsorção do carvão ativado pode ser influenciada por diversos fatores, que vão desde suas características físico-químicas, do adsorbato e condições ambientais. As características do adsorvente que determinam seu desempenho são: área superficial específica, estrutura porosa e grupos funcionais superficiais (He et al., 2023; Lewoyehu, 2021). A área superficial específica refere-se à área superficial total do adsorvente por unidade de massa, esse parâmetro é considerado de grande importância no desempenho de adsorção do carvão ativado, uma vez que, diversos estudos demonstraram que uma boa capacidade de adsorção está relacionada com uma elevada área superficial (Borges et al., 2023; Nascimento et al., 2024a; Pinto Brito et al., 2020).

Após a carbonização e ativação, forma-se uma estrutura porosa no carvão ativado, que pode apresentar diferentes tipos e tamanhos de poros. Foi adotada oficialmente pela União

Internacional de Química Pura e Aplicada (IUPAC) uma classificação convencional para o tamanho de poros de acordo com sua largura média, sendo classificados como: macroporosos (>50 nm), mesoporosos (2–50 nm) e microporosos (< 2 nm) (Duan et al., 2021). Os microporos geralmente conferem aos carvões ativados maior capacidade de adsorção, pois fornecem os principais sítios de adsorção devido à sua elevada área superficial, enquanto os mesoporos e macroporos possibilitam o acesso do adsorbato aos poros de menor tamanho, localizados mais profundamente na estrutura do carvão ativado (Jawad et al., 2022; Nascimento et al., 2024a).

Além das características estruturais inerentes ao processo de ativação, como a área superficial específica e a distribuição de poros, a superfície do carvão ativado pode ser modificada quimicamente para atender a requisitos específicos de aplicação, especialmente na área de biocatálise. Embora o carvão ativado já possua propriedades adsorptivas vantajosas, sua superfície pode necessitar de grupos funcionais específicos que favoreçam a fixação eficiente de biomoléculas, como enzimas (Santos et al., 2022; Speranza, 2019). Nesse contexto, a funcionalização do material torna-se uma etapa essencial para ampliar sua afinidade com grupos químicos de interesse, melhorar a estabilidade da ligação entre o suporte e o biocatalisador e permitir um controle mais preciso das interações envolvidas no processo de imobilização (Peixoto et al., 2022).

2.2.1 Funcionalização de carvão ativado

A funcionalização de suportes sólidos tem sido amplamente utilizada como estratégia para melhorar a imobilização de enzimas, pois permite direcionar a fixação de grupos químicos específicos à superfície do material, favorecendo interações mais seletivas e estáveis entre a enzima e o suporte. No caso do carvão ativado, embora apresente naturalmente elevada área superficial e boa estabilidade térmica, sua superfície pode limitar a eficiência da adsorção. Por isso, a modificação química do material tornou-se uma alternativa para promover tanto a quimissorção (através de ligações covalentes) quanto a fisissorção (por forças de Van der Waals, interações hidrofóbicas e ligações de hidrogênio), contribuindo para o aumento da atividade, seletividade e estabilidade do biocatalisador (Lakshmi et al., 2024).

Entre os reagentes mais utilizados na funcionalização está o glutaraldeído, um agente entrecruzante amplamente empregado devido ao seu baixo custo, facilidade de uso e boa eficiência na estabilização de enzimas. Seu mecanismo envolve a aminação prévia da superfície carbonácea, seguida da formação de bases de Schiff entre os grupos aldeído do glutaraldeído e os grupos amina primários presentes na proteína, resultando em ligações covalentes estáveis. No entanto, apesar da eficácia, seu uso suscita preocupações devido à toxicidade do composto

e à tendência à polimerização em meio aquoso, o que pode comprometer a reprodutibilidade e a segurança do processo, especialmente em aplicações alimentares e farmacêuticas (Santos et al., 2022; Speranza, 2019).

Devido a essas limitações, cresce o interesse por agentes de reticulação alternativos, com destaque para a genipina. A genipina é um agente reticulante natural extraído dos frutos de *Gardenia jasminoides*, amplamente reconhecido por sua baixa toxicidade em comparação a agentes sintéticos convencionais, como o glutaraldeído. Sua estrutura química permite reagir com grupos amino presentes em proteínas e em outros materiais biológicos, formando ligações estáveis que podem aumentar a resistência mecânica e a estabilidade de sistemas imobilizados (Qiu; Wang; Weng, 2020; Wolf et al., 2025). Além disso, a genipina apresenta elevada biocompatibilidade e menor impacto ambiental com toxicidade até 10.000 vezes menor que a do glutaraldeído, características que têm estimulado sua aplicação em áreas como engenharia de tecidos, sistemas de liberação controlada e imobilização de biomoléculas (Adamian et al., 2021; Lakshmi et al., 2024; Liu et al., 2019).

Apesar do potencial, o uso da genipina na funcionalização de carvões ativados ainda é pouco explorado na literatura. Existem poucos estudos que avaliem sua aplicação na imobilização de enzimas em suportes carbonáceos, e a maioria concentra-se em proteases (Santos et al., 2022). Essa lacuna reforça a necessidade de investigar seu desempenho em sistemas com lipases, especialmente diante do crescente interesse por catalisadores enzimáticos mais seguros, recicláveis e ambientalmente adequados (Hong et al., 2021; Liu et al., 2019).

Outro método de funcionalização que vem ganhando destaque é a incorporação de nanopartículas magnéticas aos suportes, especialmente as baseadas em óxidos de ferro, como o Fe_3O_4 . A presença dessas partículas confere ao suporte propriedades magnéticas que facilitam sua recuperação e reutilização, além de promover maior estabilidade em diferentes condições de processo. O óxido de ferro (II, III) pode ser obtido por co-precipitação de íons Fe^{2+} e Fe^{3+} e é reconhecido por sua biocompatibilidade, baixa toxicidade e capacidade de formar interações íon-dipolo com grupos funcionais da enzima. Quando aplicadas à funcionalização de carvões ativados, esse método amplia as possibilidades de aplicação em sistemas de biocatálise heterogênea, tornando-se possível promover a fixação eficiente de enzimas por meio de diferentes interações físico-químicas além de facilitar a separação do biocatalisado por campos magnéticos, reduzindo o custo operacional do processo (Chaudhari et al., 2024; Doğan et al., 2024; Lee et al., 2019; Peixoto et al., 2022).

2.3. Imobilização enzimática

A imobilização enzimática é uma estratégia consolidada no campo da biocatálise e refere-se ao confinamento de enzimas em uma fase distinta da que contém os reagentes e os produtos da reação. Essa abordagem permite superar limitações associadas ao uso de enzimas livres, como a instabilidade sob condições operacionais adversas, a dificuldade de separação do meio reacional e a impossibilidade de reutilização (Liu et al., 2021a). De modo geral, enzimas imobilizadas apresentam maior estabilidade térmica, resistência a variações de pH e melhoram a eficiência catalítica em reações contínuas, promovendo uma reação mais eficiente e economicamente viável para aplicações industriais (Abdalbagemohammedabdalsadeg et al., 2024).

De acordo com a natureza das interações estabelecidas entre a enzima e o suporte, a imobilização enzimática é usualmente dividida em métodos físicos, químicos e físico-químicos. Os métodos físicos envolvem adsorção simples e aprisionamento (entrapment), onde a enzima é retida por forças de Van der Waals ou encapsulada em matrizes poliméricas (Ismail; Baek, 2020). Já os métodos químicos envolvem ligações covalentes entre grupos funcionais da enzima e grupos reativos do suporte, proporcionando maior estabilidade, porém com risco de perda de atividade se o sítio catalítico for afetado. Os métodos físico-químicos combinam ambas as abordagens, como na microencapsulação ou na reticulação cruzada entre enzimas (CLEAs – Cross-Linked Enzyme Aggregates), técnica que não requer suporte sólido (Liu et al., 2021b; Sampaio et al., 2022). Na Figura 2 é apresentada uma representação esquemática dos principais métodos utilizados na imobilização enzimática, destacando as diferentes interações entre enzima e suporte.

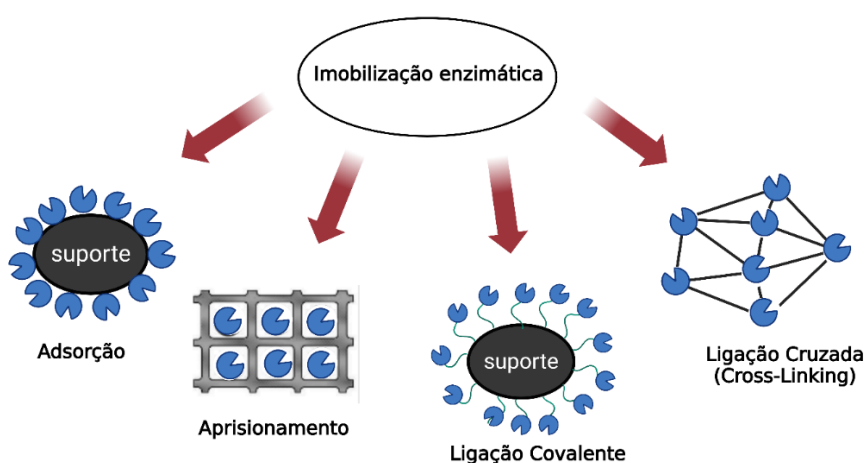


Figura 2- Esquema ilustrativo dos principais métodos de imobilização enzimática.
Imagem elaborada com BioRender

A escolha do método de imobilização e do suporte deve considerar as propriedades químicas da enzima (ponto isoelétrico, hidrofobicidade, estabilidade) e o perfil da aplicação industrial desejada. Materiais como sílica, zeólitas, quitosana, agarose, carvão ativado e nanopartículas magnéticas têm sido utilizados como suportes. Os principais requisitos desejáveis para materiais de suporte utilizados na imobilização enzimática, destacando aspectos estruturais, químicos e operacionais essenciais ao sucesso do processo, são apresentados na Figura 3. Os suportes devem apresentar elevada área superficial, biocompatibilidade, estabilidade química e possibilidade de funcionalização com diferentes agentes de reticulação (Adamian et al., 2021; Nascimento et al., 2024a; Xie; Zhang; Simpson, 2022). A utilização de suportes heterofuncionais, que combinam regiões hidrofóbicas e reativas, tem-se mostrado eficaz para aumentar a eficiência catalítica e permitir coimobilizações (Lee et al., 2019).

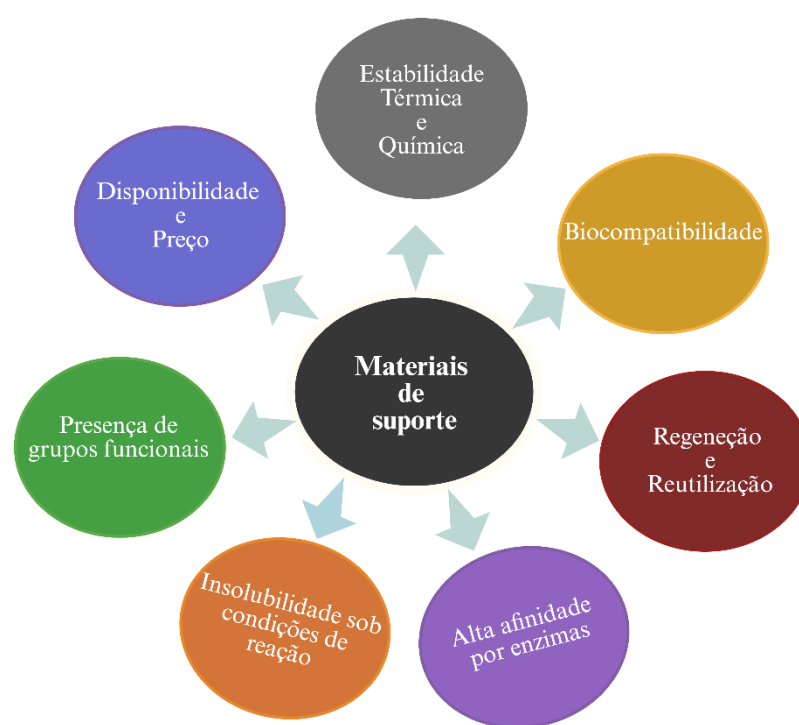


Figura 3- Principais características desejáveis em materiais de suporte para imobilização enzimática. Imagem elaborada com BioRender.

Dentre as diversas enzimas aplicadas na indústria, a lipase tem-se destacado como uma das mais estudadas em processos de imobilização. Sua versatilidade catalítica, aliada à estabilidade em diferentes condições reacionais, torna-a especialmente atrativa para aplicações

nos setores alimentícios, farmacêuticos, cosméticos e energéticos. Além disso, sua capacidade de atuar em múltiplos tipos de reações como hidrólise, esterificação e transesterificação faz com que a lipase seja utilizada como modelo em estudos de desenvolvimento e otimização de biocatalisadores imobilizados (Chandra et al., 2025; Gunathilake; Akanbi; Barrow, 2021; Nascimento et al., 2024b; Pedro et al., 2023; Pinto Brito et al., 2020; Romero; Minari; Collins, 2022).

2.4. Enzimas e sua obtenção por fermentação em estado sólido (FES)

As enzimas são catalisadores biológicos empregados em reações químicas sob condições brandas de temperatura, pressão e pH. Constituídas majoritariamente por proteínas, elas aceleram reações específicas sem serem consumidas no processo, conferindo seletividade e eficiência ao sistema biológico ou industrial no qual atuam (Prabhakar et al., 2025; Xie; Zhang; Simpson, 2022). Na indústria, o uso de enzimas tem crescido exponencialmente, impulsionado pela busca por processos mais sustentáveis e ambientalmente amigáveis, além da necessidade de reduzir custos energéticos e evitar subprodutos tóxicos gerados por catalisadores químicos tradicionais (Guisan et al., 2022).

A aplicação de enzimas abrange diversos setores, incluindo a indústria alimentícia, farmacêutica, têxtil, de detergentes e de biocombustíveis. Seu desempenho superior em termos de especificidade e biocompatibilidade torna as enzimas ferramentas-chave para a inovação tecnológica (Wahab et al., 2020b). De acordo com relatório da Grand View Research (2023), o mercado global de enzimas foi avaliado em aproximadamente USD 13,5 bilhões em 2022, com previsão de crescimento anual superior a 6%, evidenciando o protagonismo dessa área da biotecnologia em diferentes cadeias produtivas (Grand View Research, 2023).

Apesar do potencial, o uso de enzimas em processos industriais enfrenta desafios, como a baixa estabilidade em condições adversas e o alto custo de produção e purificação. Estratégias como a imobilização de enzimas, o uso de sistemas de fermentação em estado sólido (FES) e o desenvolvimento de variantes enzimáticas mais robustas têm sido investigadas para superar essas limitações (Sosa-Martínez et al., 2023; Yafetto, 2022). A FES apresenta-se como uma alternativa promissora para reduzir os custos de produção enzimática, ao utilizar resíduos agroindustriais como substrato, o que alia inovação tecnológica e sustentabilidade.

A tecnologia de fermentação utiliza microrganismos para metabolizar substratos complexos, convertendo-os em compostos mais simples, gerando diversas enzimas e subprodutos ao longo do processo (Chandra et al., 2020). No caso das lipases microbianas, predominantemente extracelulares, sua produção pode ocorrer por fermentação em estado

sólido (FES) ou submersa (FS), sendo o tipo de fermentação um fator determinante no crescimento microbiano e na produção de enzimas em larga escala (Leite et al., 2021).

A principal diferença entre FES e FS é a quantidade de água disponível no meio de cultivo. Na FES, os microrganismos se desenvolvem sobre substratos sólidos de baixa umidade, frequentemente compostos por resíduos agrícolas e industriais simples, baratos e abundantes. Essa abordagem representa uma alternativa biotecnológica eficiente e de baixo custo para a produção de lipases, especialmente por meio de fungos e leveduras (Bharathi; Rajalakshmi, 2019; Leite et al., 2021). Além disso, o uso de subprodutos agroindustriais como substrato favorece a valorização de resíduos, promovendo práticas sustentáveis alinhadas à economia circular e à bioeconomia.

Uma das principais vantagens da FES é a elevada concentração de enzimas produzidas e o uso de substratos de baixo custo. Após a extração do biocatalisador do meio fermentado, o número de etapas de processamento pode ser reduzido em comparação com outros sistemas produtivos, dependendo da aplicação pretendida. (Leite et al., 2021). Lipases produzidas em FES demonstram, em muitos casos, maior atividade, estabilidade térmica e resistência a solventes orgânicos, características essenciais para aplicações em processos como a modificação de óleos e gorduras. Além disso, o perfil hidrofóbico do substrato sólido pode favorecer a expressão de enzimas específicas que atuam em interfaces, como as lipases (Soares et al., 2022).

Uma desvantagem geral da produção industrial de enzimas, incluindo as lipases, é o custo de produção, devido às fontes caras de carbono e nitrogênio, que representam 50% do custo total da produção de enzimas. A exploração de resíduos de matéria-prima de baixo custo torna-se essencial para aumentar a produção de enzimas de origem microbiana (Gaonkar; Furtado, 2021). O uso e valorização destes resíduos são considerados uma abordagem sustentável, uma vez que contribuem para a produção de enzimas de origem microbiana e reduzem os custos de produção e os problemas associados à sua eliminação (Salgado; dos Santos; Vanetti, 2022).

O uso de resíduos agroindustriais como substrato para FES proporciona não apenas benefícios econômicos, mas também ambientais. Substratos como cascas de coco, bagaço de cana, resíduos de cacau e, no presente projeto, a torta de coco licuri, representam fontes abundantes e de baixo valor comercial que, se não aproveitados, podem gerar impactos ambientais importantes (Gaonkar; Furtado, 2021).

Entre os microrganismos empregados na FES, os fungos filamentosos destacam-se pela elevada capacidade de colonizar substratos sólidos e pela secreção eficiente de enzimas extracelulares. Dentre eles, *Penicillium roqueforti* tem despertado interesse biotecnológico

devido à sua capacidade de produzir lipases com potencial de aplicação industrial, embora apresente limitações (Sosa-Martínez et al., 2023; Yafetto, 2022). Esse microrganismo apresenta boa adaptação a resíduos agroindustriais ricos em nutrientes, podendo produzir enzimas com características adequadas para processos de hidrólise e modificação de lipídios, o que o torna um candidato promissor para sistemas de produção enzimática de baixo custo.

2.4.1 Lipases

As lipases (EC 3.1.1.3) são enzimas hidrolíticas que catalisam a clivagem de ligações éster em triacilgliceróis, liberando ácidos graxos livres e glicerol. No entanto, sua versatilidade catalítica vai além da hidrólise: em condições de baixa atividade de água, essas enzimas também podem promover reações de síntese, como esterificações e transesterificações, o que amplia suas possibilidades de aplicação industrial (Nascimento et al., 2021). Essa capacidade de atuar em meios aquosos e orgânicos, aliada à sua alta seletividade, estabilidade e variedade de especificidades de substrato, posiciona os lipases como biocatalisadores estratégicos para o desenvolvimento de processos sustentáveis e inovadores (Pinto Brito et al., 2020).

Devido à sua versatilidade, as lipases são consideradas biocatalisadores de grande importância biotecnológica. Elas representam alternativas sustentáveis aos métodos químicos tradicionais, oferecendo meios mais seguros e eficientes para realizar transformações industriais. Essas enzimas permitem a síntese de compostos naturais ou sintéticos sob condições moderadas de reação e com menor consumo de energia (Guimarães et al., 2023; Salgado; dos Santos; Vanetti, 2022).

A especificidade catalítica das lipases decorre de sua estrutura tridimensional singular, que inclui uma região denominada "tampa" (lid). Essa estrutura sofre uma mudança conformacional ao interagir com interfaces hidrofóbicas, como gotas de óleo ou superfícies hidrofóbicas de suportes de imobilização, ativando o sítio catalítico da enzima, fenômeno conhecido como ativação interfacial (Hwang et al., 2024; Zhang et al., 2021). Essa característica torna as lipases particularmente adequadas para atuar em sistemas heterogêneos óleo-água, como os utilizados na modificação de óleos vegetais.

Entre as enzimas industriais, as lipases representam aproximadamente 10% do mercado mundial de enzimas, com previsão de crescimento expressivo nas próximas décadas (Grand View Research, 2023). Esse protagonismo se deve à sua aplicação em setores como os de alimentos, biodiesel, detergentes, têxtil, farmacêutico e cosmético. No setor alimentício, as lipases são utilizadas para modificar a composição de óleos e gorduras, desenvolver sabores em laticínios, melhorar características de panificação e reduzir o teor de gordura em produtos

cárneos (Salgado; Dos Santos; Vanetti, 2022). A capacidade das lipases de catalisar reações de interesse em condições brandas favorece sua adoção em substituição a processos químicos agressivos. Na indústria de óleos e gorduras, as lipases podem substituir catalisadores químicos empregados em reações de hidrólise, esterificação, transesterificação e interesterificação, reduzindo a necessidade de altas temperaturas, de condições extremas de pH e do uso de reagentes potencialmente poluentes. Além disso, esses biocatalisadores apresentam elevada seletividade, minimizando a formação de subprodutos indesejáveis e contribuindo para processos mais sustentáveis e eficientes (Zhang; Lee; Wang, 2020).

As lipases são classificadas quanto à sua regioespecificidade, ou seja, à capacidade e hidrolisar ácidos graxos em posições específicas da cadeia de glicerol na estrutura dos triglicerídeos. Tais biomoléculas podem ser divididas em específicas para sn-1,3, específicas para sn-2 ou inespecíficas. As lipases específicas sn-1,3 (ex: lipases de *Aspergillus niger*, *Mucor miehei*, *Bacillus thermocatenuatus*, *Candida antarctica* B, *Rhizomucor miehei*, *Thermomyces lanuginosus* e pâncreas do porco) são as mais comuns e hidrolisam ligações éster nas posições sn-1 e sn-3 (Hwang et al., 2024; Rodrigues; Fernandez-Lafuente, 2010). As lipases específicas sn-2 hidrolisam ligações éster na posição sn-2 e as lipases inespecíficas (ex: lipases de *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Candida rugosa*, *Candida cylindracea* e *Chromobacterium viscosum*) hidrolisam aleatoriamente todas as posições dos triglicerídeos. Além disso, algumas lipases apresentam maior preferência por alguns tipos de ácidos graxos, como é o caso das lipases de *Candida rugosa* e *Rhizomucor miehei* que reconhecem preferencialmente substratos contendo ácidos oleico ou elaídico (Gaonkar; Furtado, 2021; Salgado; Dos Santos; Vanetti, 2022). As lipases são encontradas em plantas, animais e microrganismos e variam consideravelmente em suas características. As de origem microbiana têm atraído muita atenção da indústria, devido à sua versatilidade catalítica, especificidade, altos rendimentos, estabilidade superior e maior disponibilidade quando comparado as lipases de origem animal e vegetal (Chandra et al., 2020). Assim, as propriedades das lipases microbianas têm favorecido sua ampla aplicação em processos de modificação de óleos e gorduras, como na produção de lipídios estruturados.

2.5. Produção de lipídeos estruturados

Os óleos e gorduras naturais são compostos principalmente por triacilgliceróis (TAGs). A composição e estrutura molecular dos TAGs determinam as propriedades físico-químicas, funcionais e nutricionais dos lipídeos (Zam, 2020). Ácidos graxos saturados, ácidos graxos monoinsaturados e ácidos graxos poliinsaturados estão ligados às três posições (sn -1, sn -2

e *sn* - 3) da molécula de glicerol e a proporção de cada um desses grupos de ácidos graxos varia com a fonte do lipídio. A interesterificação é um processo de troca de moléculas de ácidos graxos no mesmo TAG ou entre diferentes TAGs e os lipídeos resultantes são denominados lipídios modificados e/ou lipídios estruturados (Temkov; Mureşan, 2021; Zhou et al., 2022).

A interesterificação pode ser realizada quimicamente ou enzimaticamente. A interesterificação enzimática é um processo catalisado por lipases, que oferece vantagens como alta seletividade, uso de condições de reação suaves, menos reações paralelas, menos desperdício e facilidade de recuperação do produto em comparação com a interesterificação química (Da Silva et al., 2022; Sivakanthan; Madhujith, 2020).

As gorduras interesterificadas encontram aplicações na indústria alimentícia, bem como na nutrição clínica, onde a interesterificação é utilizada para produzir alimentos especializados para bebês e para pacientes com distúrbios de má absorção de gordura. As aplicações clínicas incluem o uso de lipídios estruturados na nutrição enteral e parenteral. Além disso, a interesterificação tem sido usada para sintetizar gorduras com menor absorção pelo organismo ao passarem pelo trato gastrointestinal; essa menor absorção reduz a ingestão calórica efetiva. Essas estratégias podem ser utilizadas em produtos destinados ao controle de peso ou dietas com restrição calórica (Ai et al., 2023; Mills; Hall; Berry, 2017; Zhang et al., 2021). Paralelamente às aplicações clínicas, a interesterificação tem ganhado destaque na indústria alimentícia, especialmente na produção de margarinas e gorduras plásticas, tradicionalmente obtidas por hidrogenação parcial (Sivakanthan; Madhujith, 2020).

Margarinas e gorduras são conhecidas como gorduras plásticas devido à sua natureza semissólida. Tradicionalmente, as gorduras plásticas são produzidas pela hidrogenação parcial de óleos vegetais (Sivakanthan; Madhujith, 2020). A hidrogenação parcial leva à redução do grau de insaturação dos ácidos graxos e à produção de níveis significativos de *gorduras trans* como subprodutos, principalmente o ácido elaídico (C18:1 *trans* -9), que são conhecidos por serem bem correlacionados com doenças cardiovasculares (Mills; Hall; Berry, 2017). No entanto, durante a interesterificação, o conteúdo original de ácidos graxos insaturados é mantido e *trans*ácidos graxos não são gerados. Assim, a interesterificação surgiu como uma alternativa promissora à hidrogenação parcial para produzir gorduras plásticas (Zhang; Lee; Wang, 2020).

3. REFERÊNCIAS

- ABDALBAGEMOHAMMEDABDALSADeg, Sanad *et al.* Catalase immobilization: Current knowledge, key insights, applications, and future prospects - A review. *International Journal of Biological Macromolecules*, 2024.
- ADAMIAN, Younes *et al.* Recent Developments in the Immobilization of Laccase on Carbonaceous Supports for Environmental Applications - A Critical Review. *Frontiers in Bioengineering and Biotechnology*, 2021.
- AI, Hongzeng *et al.* Structured lipids produced from palm-olein oil by interesterification: A controllable lipase-catalyzed approach in a solvent-free system. *Food Chemistry*, v. 412, 2023.
- ALDI, Muhammad *et al.* Fermentation-based valorization of agro-industrial cereal wastes and by-products. *Trends in Food Science & Technology*, v. 168, 105464, 2026.
- ALVES, José Luiz Francisco *et al.* Valorization of royal palm tree agroindustrial waste via pyrolysis with a focus on physicochemical properties, kinetic triplet, thermodynamic parameters, and volatile products. *Biomass and Bioenergy*, v. 177, 1 out. 2023.
- ALVES, Simone da Paz Leôncio *et al.* *Syagrus coronata* (Mart.) Becc: importância biológica, nutricional e socioeconômica. *Cuadernos de Educación y Desarrollo*, v. 16, n. 10, p. e6131, 25 out. 2024.
- BAI, Haotian; YU, Dan; DU, Xiaowei. Review of porous microspheres for enzyme immobilization: Strategies, applications, and prospects. *International Journal of Biological Macromolecules Elsevier B.V.*, , 1 mar. 2025.
- BHARATHI, D.; RAJALAKSHMI, G. Microbial lipases: Na overview of screening, production and purification. *Biocatalysis and Agricultural Biotechnology*, v. 22, 2019.
- BORGES, Jéssica Ferreira *et al.* Laccase Immobilization on Activated Carbon from Hydrothermal Carbonization of Corn Cob. *Waste and Biomass Valorization*, v. 15, n. 1, p. 501–520, 1 jan. 2023.
- CHANDRA, Kunal *et al.* Advancements in lipase immobilization: Enhancing enzyme efficiency with nanomaterials for industrial applications. *International Journal of Biological Macromolecules*, v. 311, p. 143754, jun. 2025.
- CHANDRA, Prem *et al.* Microbial lipases and their industrial applications: a comprehensive review. *Microbial Cell Factories*, v. 19, n. 1, p. 169, 26 dez. 2020.
- CHAUDHARI, Dinesh S. *et al.* A review on sustainable iron oxide nanoparticles: syntheses and applications in organic catalysis and environmental remediation. *Green Chemistry Royal Society of Chemistry*, , 22 maio 2024.
- DA SILVA, Mayanny Gomes *et al.* Developed and characterization of nanostructured lipid carriers containing food-grade interesterified lipid phase for food application. *Food Research International*, v. 155, p. 111119, maio 2022.
- DOĞAN, Yusuf *et al.* Activated carbon-coated iron oxide magnetic nanocomposite (IONPs@CtAC) loaded with morin hydrate for drug-delivery applications. *Frontiers in Chemistry*, v. 12, 2024.
- DUAN, Dengle *et al.* Activated carbon from lignocellulosic biomass as catalyst: A review of the applications in fast pyrolysis process. *Journal of Analytical and Applied Pyrolysis Elsevier B.V.*, , 1 set. 2021.

- GAONKAR, S. K.; FURTADO, I. J. Valorization of low-cost agro-wastes residues for the maximum production of protease and lipase haloextremozymes by *Haloferax lucentensis* GUBF-2 MG076078. *Process Biochemistry*, v. 101, p. 72–88, 2021.
- GAYATHIRI, Muniandy *et al.* Activated carbon from biomass waste precursors: Factors affecting 1 production and adsorption mechanism. *[S.l.: S.n.]*.
- GOUVEIA, Alison Batista Vieira Silva *et al.* Alimentos alternativos utilizados para codornas de corte: revisão. *Research, Society and Development*, v. 9, n. 2, p. e172922250, 1 jan. 2020.
- GRAND VIEW RESEARCH. Enzymes Market Size, Share & Trends Analysis Report By Product (Industrial Enzymes, Specialty Enzymes), By Application (Industrial, Specialty), By Source, By Region, And Segment Forecasts, 2023 - 2030. San Francisco: *[S.n.]*.
- GUIMARÃES, José Renato *et al.* A review of lipase immobilization on hydrophobic supports incorporating systematic mapping principles. *Reaction Chemistry & Engineering*, v. 8, n. 11, p. 2689–2702, 2023.
- GUIMARÃES, Julian dos Santos; SHIOSAKI, Ricardo Kenji; MENDES, Marianne Louise Marinho. Licuri (*Syagrus coronata*): Characteristics, importance, potential and perspectives of the small coconut from Brazil. *Desenvolvimento e Meio Ambiente Universidade Federal do Paraná* 1, , 1 dez. 2021.
- GUISAN, Jose M. *et al.* Enzyme immobilization strategies for the design of robust and efficient biocatalysts. *Current Opinion in Green and Sustainable Chemistry Elsevier B.V.*, , 1 jun. 2022.
- GUNATHILAKE, Tharuka; AKANBI, Taiwo O.; BARROW, Colin J. Lipase-produced omega-3 acylglycerols for the fortification and stabilization of extra virgin olive oil using hydroxytyrosyl palmitate. *Future Foods*, v. 4, 1 dez. 2021.
- HE, Hongzhe *et al.* Functional Carbon from Nature: Biomass-Derived Carbon Materials and the Recent Progress of Their Applications. *Advanced Science* John Wiley and Sons Inc, , 2 jun. 2023.
- HONG, Jiyeon *et al.* Immobilization of laccase via cross-linked enzyme aggregates prepared using genipin as a natural cross-linker. *International Journal of Biological Macromolecules*, v. 169, p. 541–550, 1 fev. 2021.
- HWANG, Jihyun *et al.* Immobilized *Candida antarctica* lipase B as an sn-1,3 regiospecific biocatalyst for the interesterification of triacylglycerols with fatty acid ethyl esters. *Food Science and Biotechnology*, v. 33, n. 1, p. 159–170, 30 jan. 2024.
- ISMAIL, Abdallah R.; BAEK, Kwang Hyun. Lipase immobilization with support materials, preparation techniques, and applications: Present and future aspects. *International Journal of Biological Macromolecules Elsevier B.V.*, , 15 nov. 2020.
- JAWAD, Ali H. *et al.* Mesoporous high-surface-area activated carbon from biomass waste via microwave-assisted-H₃PO₄ activation for methylene blue dye adsorption: An optimized process. *Diamond and Related Materials*, v. 128, 1 out. 2022.
- KUMAR MISHRA, Ranjeet; SINGH, Bineeta; ACHARYA, Bishnu. A comprehensive review on activated carbon from pyrolysis of lignocellulosic biomass: An application for energy and the environment. *Carbon Resources Conversion* Ke Ai Publishing Communications Ltd., , 1 dez. 2024.
- KUŚMIEREK, Krzysztof; SZALA, Mateusz; ŚWIATKOWSKI, Andrzej. Adsorption of 2,4-dichlorophenol and 2,4-dichlorophenoxyacetic acid from aqueous solutions on carbonaceous

- materials obtained by combustion synthesis. *Journal of the Taiwan Institute of Chemical Engineers*, v. 63, p. 371–378, 1 jun. 2016.
- LAKSHMI, D. *et al.* Functionalized Nanomaterials, Classification, Properties, and Functionalization Techniques. In: [S.l.: S.n.]. p. 65–92.
- LEE, Ja Hyun *et al.* Biodiesel production by lipases co-immobilized on the functionalized activated carbon. *Bioresource Technology Reports*, v. 7, 1 set. 2019.
- LEITE, Paulina *et al.* Recent advances in production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes. *Current Opinion in Green and Sustainable Chemistry*, v. 27, p. 100407, fev. 2021.
- LEWOYEHU, Mekuanint. Comprehensive review on synthesis and application of activated carbon from agricultural residues for the remediation of venomous pollutants in wastewater. *Journal of Analytical and Applied Pyrolysis Elsevier B.V.*, , 1 out. 2021.
- LIU, Shuai *et al.* Smart chemistry of enzyme immobilization using various support matrices – A review. *International Journal of Biological Macromolecules Elsevier B.V.*, , 1 nov. 2021a.
- LIU, Shuai *et al.* Smart chemistry of enzyme immobilization using various support matrices – A review. *International Journal of Biological Macromolecules Elsevier B.V.*, , 1 nov. 2021b.
- LIU, Yangshuo *et al.* Removal of pharmaceuticals by novel magnetic genipin-crosslinked chitosan/graphene oxide-SO₃H composite. *Carbohydrate Polymers*, v. 220, p. 141–148, set. 2019.
- MILLS, C. E.; HALL, W. L.; BERRY, S. E. E. What are interesterified fats and should we be worried about them in our diet? *Nutrition Bulletin*, v. 42, n. 2, p. 153–158, 8 jun. 2017.
- MIRABELLA, Nadia; CASTELLANI, Valentina; SALA, Serenella. Current options for the valorization of food manufacturing waste: A review. *Journal of Cleaner Production*, 15 fev. 2014.
- NASCIMENTO, Priscilla Amaral *et al.* Partitioning of pequi seed (*Caryocar brasiliense* Camb.) lipase in aqueous two-phase systems composed of PEG/2-propanol + ammonium sulfate + water. *Brazilian Journal of Chemical Engineering*, v. 38, n. 4, p. 957–965, 1 dez. 2021.
- NASCIMENTO, Priscilla Amaral *et al.* Revealing the potential of a novel approach for assessing the effect of pyrolysis conditions on sisal waste-based activated carbon for lipase immobilization and ethyl lactate biosynthesis. *Biomass Conversion and Biorefinery*, 2024a.
- NASCIMENTO, Priscilla Amaral *et al.* Synthesis and microencapsulation of acylglycerols rich in omega-3 PUFAs by glycerolysis using lipase immobilized on activated carbon. *Journal of Environmental Chemical Engineering*, v. 12, n. 6, 1 dez. 2024b.
- NEME, Ibsa; GONFA, Girma; MASI, Chandran. Activated carbon from biomass precursors using phosphoric acid: A review. *Heliyon Elsevier Ltd*, , 1 dez. 2022.
- PEDRO, Kelly C. N. R. *et al.* Adsorption of lipases on porous silica-based materials for esterification in a solvent-free system. *3 Biotech*, v. 13, n. 11, p. 380, 25 nov. 2023.
- PEIXOTO, Bruno Salarini *et al.* Highly Functionalized Microporous Activated Biochar from *Syagrus coronata* Waste: Production, Characterization, and Application in Adsorption Studies. *Water (Switzerland)*, v. 14, n. 21, 1 nov. 2022.

- PEREIRA, Gabriel Sthefano Lourenço *et al.* Evaluation of quality parameters, physicochemical and bioactive properties of licuri oil (*Syagrus coronata*). *Food Research International*, v. 208, p. 116157, maio 2025.
- PINTO BRITO, Mylena Junqueira *et al.* Development of activated carbon from pupunha palm heart sheaths: Effect of synthesis conditions and its application in lipase immobilization. *Journal of Environmental Chemical Engineering*, v. 8, n. 5, 1 out. 2020.
- PRABHAKAR, Theja *et al.* Covalent immobilization: A review from an enzyme perspective. *Chemical Engineering Journal*, v. 503, p. 158054, jan. 2025.
- QIU, Yi Ting; WANG, Be Jen; WENG, Yih Ming. Preparation and characterization of genipin cross-linked and lysozyme incorporated antimicrobial sodium caseinate edible films. *Food Packaging and Shelf Life*, v. 26, 1 dez. 2020.
- RODRIGUES, Luanda Kívia de Oliveira; MENDES, José Ubiragi de Lima; JESUS, Djane Santiago de. Análise físico-química do óleo de licuri (*Syagrus Coronata*) in-natura e transesterificado. *Research, Society and Development*, v. 9, n. 8, 16 jul. 2020.
- RODRIGUES, Rafael C.; FERNANDEZ-LAFUENTE, Roberto. Lipase from *Rhizomucor miehei* as a biocatalyst in fats and oils modification. *Journal of Molecular Catalysis B: Enzymatic*, set. 2010.
- ROMERO, Sandra; MINARI, Roque J.; COLLINS, Sebastián E. Lipase-Catalyzed Interesterification of Fully and Partially Hydrogenated Soybean Oil Blends for Bioparaffin Production. *Industrial & Engineering Chemistry Research*, v. 61, n. 9, p. 3254–3262, 9 mar. 2022.
- SALGADO, Cleonice Aparecida; DOS SANTOS, Clarissa Isabela Aparecida; VANETTI, Maria Cristina Dantas. Microbial lipases: Propitious biocatalysts for the food industry. *Food Bioscience*, v. 45, p. 101509, fev. 2022.
- SALGADO, L. A.; DOS SANTOS, J. C. S.; VANETTI, M. C. D. Applications of microbial lipases in the food industry: An overview. *Food Research International*, v. 152, n. 110895, 2022.
- SAMPAIO, Camila S. *et al.* Lipase immobilization via cross-linked enzyme aggregates: Problems and prospects – A review. *International Journal of Biological Macromolecules Elsevier B.V.*, 31 ago. 2022.
- SANTOS, Mateus P. F. *et al.* Pepsin immobilization: Influence of carbon support functionalization. *International Journal of Biological Macromolecules*, v. 203, p. 67–79, 1 abr. 2022.
- SANTOS, Mateus Pereira F. *et al.* New insight about the relationship between the main characteristics of precursor materials and activated carbon properties using multivariate analysis. *Canadian Journal of Chemical Engineering*, v. 98, n. 7, p. 1501–1511, 1 jul. 2020.
- SIVAKANTHAN, Subajiny; MADHUVITH, Terrence. Current trends in applications of enzymatic interesterification of fats and oils: A review. *LWT Academic Press*, 1 out. 2020.
- SOARES, Glêydison Amarante *et al.* Production of a fermented solid containing lipases from *Penicillium roqueforti* ATCC 10110 and its direct employment in organic medium in ethyl oleate synthesis. *Biotechnology and Applied Biochemistry*, v. 69, n. 3, p. 1284–1299, 9 jun. 2022.
- SOSA-MARTÍNEZ, Jazel Doménica *et al.* Agroindustrial and food processing residues valorization for solid-state fermentation processes: A case for optimizing the co-production of hydrolytic enzymes. *Journal of Environmental Management*, v. 347, 1 dez. 2023.

- SPERANZA, Giorgio. The Role of Functionalization in the Applications of Carbon Materials: An Overview. *C*, v. 5, n. 4, p. 84, 11 dez. 2019.
- TEMKOV, Mishela; MUREŞAN, Vlad. Tailoring the structure of lipids, oleogels and fat replacers by different approaches for solving the trans-fat issue—a review. *Foods MDPI AG*, , 1 jun. 2021.
- UKANWA, Kalu Samuel *et al.* A review of chemicals to produce activated carbon from agricultural waste biomass. *Sustainability (Switzerland)MDPI*, 1 nov. 2019.
- WAHAB, Roswanira Abdul *et al.* On the taught new tricks of enzymes immobilization: An all-inclusive overview. *Reactive and Functional Polymers Elsevier B.V.*, , 1 jul. 2020a.
- WAHAB, Roswanira Abdul *et al.* On the taught new tricks of enzymes immobilization: An all-inclusive overview. *Reactive and Functional Polymers Elsevier B.V.*, , 1 jul. 2020b.
- WOLF, M. H. *et al.* Dielectric properties of chitosan membranes cross-linked with glutaraldehyde or genipin. *Reactive and Functional Polymers*, v. 213, 1 ago. 2025.
- XIE, Jiayu; ZHANG, Yi; SIMPSON, Benjamin. Food enzymes immobilization: novel carriers, techniques and applications. *Current Opinion in Food Science Elsevier Ltd*, , 1 fev. 2022.
- YAFETTO, Levi. Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. *Heliyon Elsevier Ltd*, , 1 mar. 2022.
- ZAM, Wissam. Structured lipids: Synthesis, health effects, and nutraceutical applications. *In: Lipids and Edible Oils. [S.l.]*: Elsevier, 2020. p. 289–327.
- ZDARTA, Jakub *et al.* A general overview of support materials for enzyme immobilization: Characteristics, properties, practical utility. *Catalysts MDPI*, , 24 fev. 2018.
- ZHANG, Li; ZUO, Songlin. The Significance of Lignocellulosic Raw Materials on the Pore Structure of Activated Carbons Prepared by Steam Activation. *Molecules*, v. 29, n. 13, 1 jul. 2024.
- ZHANG, Zhen *et al.* Enzymatic Interesterification of Palm Stearin and Palm Olein Blend Catalyzed by sn-1,3-Specific Lipase: Interesterification Degree, Acyl Migration, and Physical Properties. *Journal of Agricultural and Food Chemistry*, v. 69, n. 32, p. 9056–9066, 18 ago. 2021.
- ZHANG, Zhen *et al.* The effects of interesterification on the physicochemical properties of *Pangasius bocourti* oil and its fractions. *Food Chemistry*, v. 371, 1 mar. 2022.
- ZHANG, Zhen; LEE, Wan Jun; WANG, Yong. Evaluation of enzymatic interesterification in structured triacylglycerols preparation: a concise review and prospect. *Critical Reviews in Food Science and Nutrition Taylor and Francis Inc.*, 2020.
- ZHOU, Jun *et al.* Future of Structured Lipids: Enzymatic Synthesis and Their New Applications in Food Systems. *Foods*, v. 11, n. 16, p. 2400, 10 ago. 2022.

CAPÍTULO 2

OBJETIVOS

OBJETIVO GERAL

Desenvolver uma rota integrada para a valorização dos resíduos do licuri (*Syagrus coronata*) por meio de uma rota tecnológica que integra: (i) produção de lipase por fermentação em estado sólido; (ii) síntese de suporte carbonáceo para imobilização enzimática; e (iii) aplicação do sistema catalítico na modificação das propriedades do óleo de licuri

OBJETIVOS ESPECÍFICOS

- Sintetizar carvões ativados a partir da casca de licuri em diferentes condições de síntese;
- Funcionalizar os carvões ativados com genipina e íons metálicos visando melhorar a interação enzima-suporte;
- Caracterizar os suportes obtidos quanto às propriedades físico-químicas, estruturais e superficiais;
- Produzir lipase por fermentação em estado sólido utilizando a torta de licuri como substrato;
- Otimizar a produção enzimática por meio de planejamento experimental;
- Imobilizar lipases (produzidas por FES e comercial) nos suportes desenvolvidos e avaliar a eficiência de imobilização e recuperação de atividade;
- Aplicar a lipase imobilizada na reação de interesterificação enzimática do óleo de licuri sob diferentes condições reacionais;
- Avaliar as modificações induzidas no óleo por meio de análise reológica, com foco no comportamento de fluxo e viscosidade aparente;
- Investigar, de forma indireta, alterações estruturais nos triacilgliceróis com base no comportamento reológico e térmico observado.

ARTIGO 1

Enzymatic interesterification of regional oils: advances in lipase immobilization and perspectives for structured lipid production - A Short Review

*Manuscrito será submetido à revista Trends in Food Science & Technology (ISSN: 1879-3053)

Fator de impacto: 16.18

Enzymatic interesterification of regional oils: advances in lipase immobilization and perspectives for structured lipid production - A Short Review

Highlights

- Regional oils are underexplored feedstocks for enzymatic interesterification
- Immobilization advances enable industrial-scale interesterification of regional oil
- Immobilization supports remain a key barrier to industrial scale-up.
- Structured lipids provide tailored nutritional and functional properties.
- Agro-industrial residues support circular lipid processing strategies.

ABSTRACT

The demand for healthier and more sustainable lipid-based foods has intensified interest in enzymatic interesterification as an alternative to conventional chemical processes. This review critically examines recent advances in lipase-catalyzed interesterification, with particular emphasis on immobilized enzyme systems and their role in improving process selectivity, operational stability, and industrial viability. Immobilization strategies such as physical adsorption, covalent attachment, and functionalized supports have enabled repeated use of lipases while preserving catalytic efficiency under mild reaction conditions. These advances have expanded the production of structured lipids with tailored nutritional and technological properties, including applications in infant nutrition, functional foods, and lipid-based delivery systems. This review also discusses the integration of regional vegetable oils and agro-industrial by-products as sustainable feedstocks, highlighting opportunities aligned with circular economy principles. Despite clear technological progress, challenges related to support design, regulatory acceptance, and large-scale implementation remain. Future research should prioritize interdisciplinary approaches, pilot-scale validation, and the development of food-grade, low-cost immobilization supports to bridge the gap between laboratory innovation and industrial application.

Keywords: Lipid modification; enzyme immobilization; functional fats; circular bioeconomy; Food processing technologies

1. Introduction

The increasing demand for healthier foods with improved technological performance has led to the development of alternatives to traditional lipid modification processes. In this context, structured lipids (SLs) offer a promising solution, providing specific functional properties such as desirable melting points, enhanced oxidative stability, and improved digestibility (Zhang, Lee, et al., 2020a). Traditionally, methods such as partial hydrogenation and chemical interesterification have been used to achieve these characteristics. However, these methods have faced criticism for the formation of trans fatty acids and their environmental and energy impacts (İnanlar & Altay, 2024; Wallis et al., 2022). This trend has directed scientific and industrial efforts to the search for more selective, efficient, and sustainable processes, leading to the promising alternative of enzymatic interesterification (Ni et al., 2025a).

The industrial application of enzymatic interesterification (EIE) offers significant advantages over conventional chemical methods, particularly in terms of specificity and environmental compatibility (Singh et al., 2022). EIE is increasingly relevant in the food industry for its ability to engineer structured lipids with tailored functional properties, such as controlled melting points and optimized rheological behavior (Danthine et al., 2022; Singh et al., 2022). Applications range from chocolate manufacturing, where specific lipid crystallization is essential, to margarine and fat-based systems, which benefit from improved nutritional profiles and cleaner production processes (free of toxic byproducts, unlike chemical interesterification) (J. H. Baoyindugurong et al., 2024; H. Zhang et al., 2022a). In this regard, the integrating of regional vegetable oils and agro-industrial by-products as substrates expands the range of feedstocks for these processes while aligning with circular economy principles, thereby promoting sustainable resource utilization and local value-chain development (Costa et al., 2022; Ligarda-Samanez et al., 2025).

From a technical standpoint, the success of EIE hinges on multiple factors: lipase selection, with microbial variants like *Rhizomucor miehei*, *Thermomyces lanuginosus*, and *Candida antarctica* being preferred due to their specificity and reaction tolerance, process conditions (temperature, pH, solvent systems), and substrate composition (Cai et al., 2015; Ghidde et al., 2022; Ma et al., 2022). Despite these advantages, widespread adoption remains hindered by practical challenges, including high enzyme costs, operational instability, and limited reusability (Abdelmoez & Mustafa, 2014; Deslauriers, 2017). Enzyme immobilization is recognized as a key strategy to mitigate these limitations, enabling catalyst recovery while

enhancing stability and process efficiency (Ismail & Baek, 2020; Pardo-Tamayo et al., 2023a). Various immobilization techniques, such as adsorption, covalent bonding, entrapment, and encapsulation, have been explored, with the choice of support material strongly influencing enzymatic activity and economic feasibility (Pardo-Tamayo et al., 2023a). Recent studies indicate that immobilization can preserve and, in some cases, enhance catalytic activity while facilitating multi-cycle use. However, the outcome depends strongly on the immobilization strategy, support characteristics, enzyme orientation, and mass transfer effects. When properly designed, immobilized systems can significantly improve operational stability and process economics, supporting industrial-scale applications (Abdel-Mageed, 2025).

Despite these advances, challenges persist in large-scale applications. Standardization of protocols, regulatory compliance, consumer acceptance, and the availability of cost-effective immobilization support that is compatible with food-grade standards remain critical barriers (Mia et al., 2025). Nevertheless, the growing demand for healthier foods and sustainable processing has spurred investments in applied research and public-private collaborations, signaling a promising trajectory for EIE adoption (Campbell et al., 2023; Farhan et al., 2025; Mia et al., 2025; Ringling & Marquart, 2020; Wei et al., 2025).

This review critically examines recent advances in the production of structured lipids via immobilized lipase-catalyzed EIE, with a focus on food industry applications. Key discussion points include biochemical mechanisms and comparative advantages of immobilized systems; practical case studies; technical and regulatory challenges for industrial scale-up; and future directions, such as valorizing regional lipid sources and designing robust, reusable biocatalysts. This analysis aims to inform strategies for transitioning toward greener lipid modification technologies by bridging gaps between sustainability, innovation, and commercial viability.

2. Fundamentals of Enzymatic Interesterification (EIE)

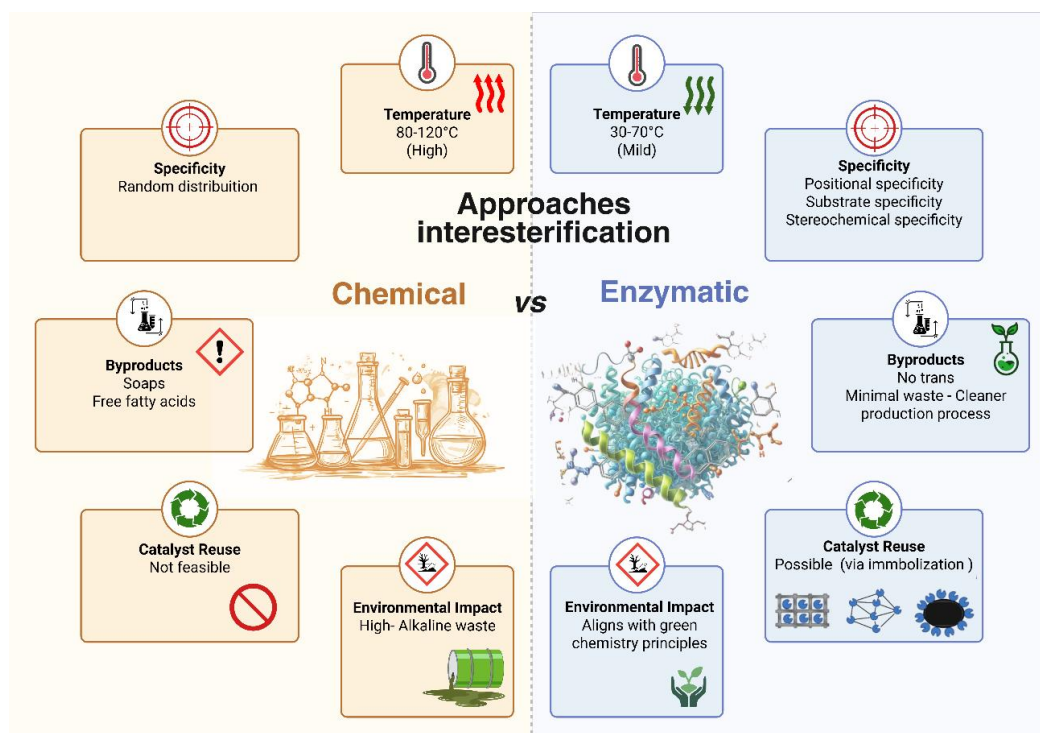
2.1. Principles of reaction and role of lipases

Interesterification is a chemical process that rearranges fatty acids both between and within triacylglycerol (TAG) molecules, aiming to improve their functional and nutritional properties. It modifies the lipid structure while preserving the original fatty acid composition (Singh et al., 2022). This transformation can be achieved through chemical or enzymatic approaches (Temkov & Mureşan, 2021). However, enzymatic interesterification using lipases is increasingly favored over chemical methods due to its higher specificity, milder reaction

conditions, and reduced production of undesirable by-products, such as trans fatty acids (Kanprakobkit et al., 2022; Singh et al., 2022).

The chemical process, typically carried out with alkaline catalysts such as sodium methoxide, is often non-selective, leading to a random redistribution of fatty acids among triacylglycerols (TAGs) (Singh et al., 2022). While this method is effective in terms of yield, it has several drawbacks, including the requirement for high temperatures, the generation of waste, challenges in controlling specificity, and the inability to recycle the catalyst (Kiprono et al., 2022; Singh et al., 2022). In contrast, enzymatic interesterification, which uses lipases, provides high positional and/or substrate selectivity, operates at lower temperatures, and yields purer products with reduced environmental impact (Singh et al., 2022). The main differences between the two processes are summarized in Figure 1. Considering the benefits of enzymatic interesterification, a detailed understanding of the catalytic role and mechanisms of action of lipases becomes increasingly important for advancing this technology (Chen et al., 2022).

Figure 1. Comparison between chemical and enzymatic interesterification of lipids, emphasizing catalyst type, selectivity, operating conditions, industrial applicability, and advantages.



In hydrolysis reactions, lipases (EC 3.1.1.3) specifically target the ester bonds of fatty acids found in triglycerides (Payá-Tormo et al., 2019). Under anhydrous conditions or at low water activity, these enzymes do not catalyze hydrolysis; instead, they promote esterification and transesterification, including interesterification (Zieniuk et al., 2021). A well-known characteristic of lipases is their specificity, which can vary depending on the enzyme source (Hoff & Eisenacher, 2025). This specificity may be expressed as positional specificity, such as a preference for the sn-1 and sn-3 positions on the glycerol backbone, substrate selectivity for certain fatty acids, or regioselectivity in acylation reactions (Mastai, 2015; Singh et al., 2022).

2.2. Mechanisms of EIE: acidolysis, transesterification, and intramolecular interesterification

The main mechanisms involved in enzymatic interesterification (EIE) are acidolysis, transesterification, and intramolecular interesterification (Sivakanthan & Madhujith, 2020). Each of these reactions plays a distinct role in modifying lipid structures, with specific applications depending on the desired functional or nutritional properties of the final product (Temkov & Mureşan, 2021). Acidolysis is an enzymatic reaction that facilitates the exchange of acyl groups between triacylglycerols (TAGs) and free fatty acids (FFAs), driven by the catalytic action of lipases. This process is crucial for synthesizing structured lipids, such as medium- and long-chain triglycerides (MLCTs), which combine fatty acids with varying carbon chain lengths to enhance their nutritional and functional properties (Zhou et al., 2022). Recent research has demonstrated the efficacy of acidolysis in producing MLCTs, particularly emphasizing the preservation of polyunsaturated fatty acids at the sn-2 position when utilizing lipases that selectively target the sn-1,3 positions (Mayookha et al., 2026). This enzymatic specificity plays a key role in the bioavailability and metabolism of lipids in the human body. In addition, acidolysis enables the controlled insertion of essential fatty acids into specific regions of the glycerol molecule, an important aspect in the preparation of lipid substitutes for infant formulas, such as OPO (1,3-dioleoyl-2-palmitoylglycerol) (Ni et al., 2025b). Furthermore, transesterification is another key enzymatic strategy for modifying TAG composition.

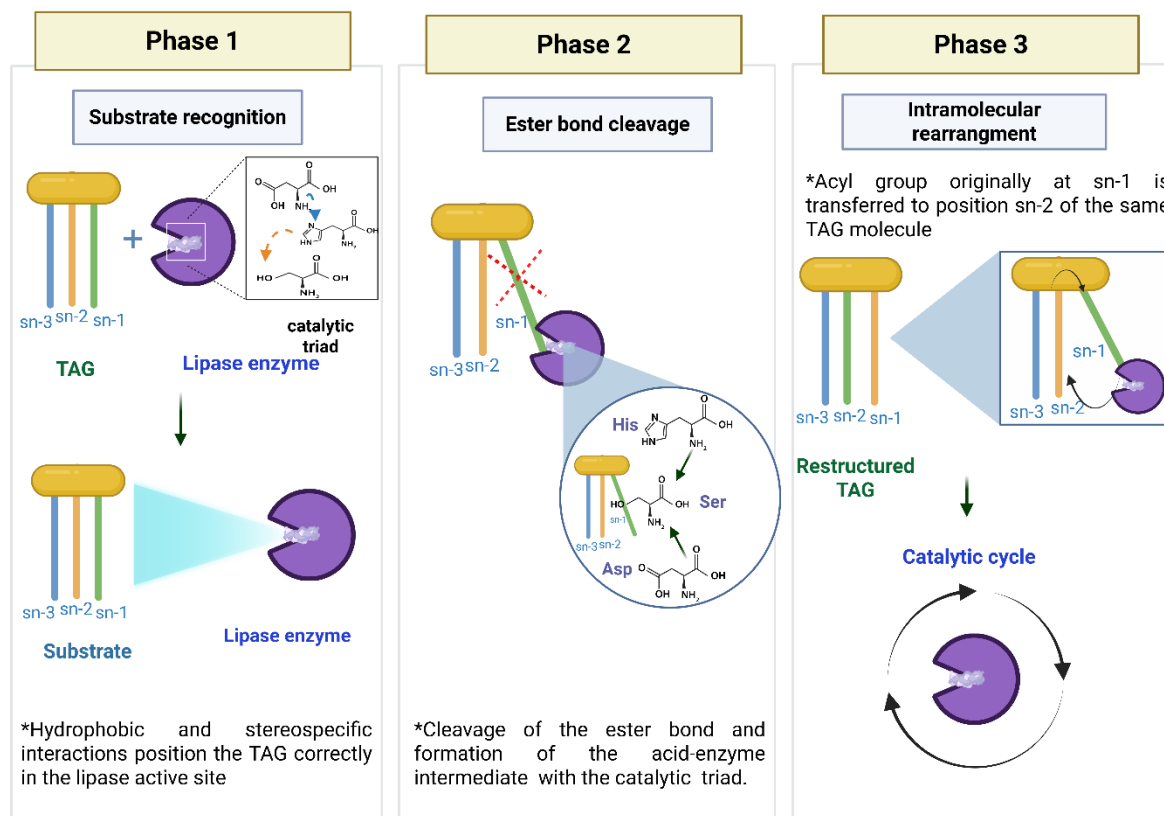
Transesterification is a catalytic process that involves the exchange of acyl groups between esters or between esters and alcohols, facilitated by lipases (Bhardwaj & Gupta, 2017; García-Quinto et al., 2024). This reaction is used to modify lipids, adjusting the composition of triacylglycerols (TAGs) (García-Quinto et al., 2024). This optimization enhances oxidative stability, nutritional profile, and thermal behavior (Singh et al., 2022). An example is the

transesterification of vegetable oils with medium-chain fatty acid esters, which produces structured lipids with potential applications in functional foods (Han et al., 2025; Liu & Akoh, 2024; Melchior et al., 2024). Enzymatic transesterification is used to produce biodiesel as an alternative to traditional chemical catalysts (Amini et al., 2016). The reaction between vegetable oils and low-molecular-weight alcohols (methanol or ethanol), catalyzed by lipases, offers advantages such as smoother reaction conditions and reduced by-product formation (Mahmoud et al., 2022). Moreover, the sn-1,3 positional selectivity of lipases allows precise control over TAG composition, thereby supporting diverse industrial applications (Guan et al., 2024). In parallel, intramolecular interesterification involves the rearrangement of fatty acids within a single TAG molecule, without external acyl donors (Picq et al., 2013).

The mechanism of intramolecular interesterification specifically involves positional exchange of fatty acids within individual TAG molecules, without combining with other lipid molecules (Singh et al., 2022). This controlled internal reorganization enables modulation of physical and nutritional properties, such as melting point, plasticity, and digestibility, which are important characteristics for the development of specialized structured lipids in food and pharmaceutical applications (Alves et al., 2026; Park et al., 2025; Savchina et al., 2025). Intramolecular interesterification mediated by lipases involves a complex enzymatic mechanism that can be divided into three fundamental stages (Singh et al., 2022) (Figure 2).

In the first phase, known as molecular recognition, the triacylglycerol (TAG) molecule is positioned at the enzyme's active site, where hydrophobic and stereospecific interactions facilitate its binding. This initial step is critical for ensuring the positional selectivity characteristic of lipases (Gunn & Neher, 2023; Park & Park, 2022). Subsequently, in the second phase, the active complex forms, which is the enzymatic cleavage of the ester bond at specific glycerol positions (predominantly sn-1 or sn-3), generating an acid-enzyme covalent intermediate. This stage involves the coordinated participation of the catalytic residues of lipase (Serine, Histidine, and Aspartate), which form the so-called "catalytic triad" (Stemler & Scherf, 2024). The last phase of the mechanism is responsible for molecular rearrangement. The acyl group is transferred to another position within the same glycerol molecule, often to the sn-2 position, completing the reesterification cycle. This catalytic cycle can proceed continuously if a suitable substrate is available and favorable reaction conditions are maintained (Batista et al., 2025; Tjørnelund, 2024).

Figure 2. Schematic representation of the intramolecular enzymatic interesterification mechanism catalyzed by lipases.



This sustained catalytic behaviour is closely related to the intrinsic structural features of lipases, particularly their distinctive three-dimensional conformation and the phenomenon of interfacial activation, which governs their selective action (Hoff & Eisenacher, 2025). Unlike traditional enzymes, lipases possess a dynamic structural element known as a protective "lid", a mobile α -helical peptide segment that covers the active site under aqueous conditions. This lid undergoes conformational changes at hydrophobic interfaces, exposing the catalytic center and enabling substrate access (Abdelaziz et al., 2025; Milovanović et al., 2025). This structural element undergoes conformational changes upon exposure to lipid-water interfaces, thereby exposing the catalytic site and triggering enzymatic activity (Guan et al., 2024; Milovanović et al., 2025). This sophisticated structural rearrangement accounts for the pronounced positional specificity exhibited by certain lipases, which preferentially target triglyceride sn-1 and sn-3 positions while typically leaving the sn-2 position intact (Park et al., 2025).

Such high specificity has considerable nutritional implications, especially for infant formula lipids that replicate human milk fat. In breast milk, approximately 70% of palmitic acid is strategically located at the sn-2 position, a configuration that enhances fat absorption and prevents the formation of indigestible calcium soaps in the infant's gut (Hageman et al., 2019). By utilizing these precise enzymatic capabilities, food technologists can design structured lipids with optimized nutritional profiles and bioavailability, thus opening new opportunities in pediatric and clinical nutrition (Alves et al., 2026; Mayookha. et al., 2026; Yao et al., 2026).

The regioselectivity and mild operating conditions of lipase-catalyzed reactions have stimulated growing interest in their application for lipid modification. Based on these characteristics, several studies have explored intramolecular enzymatic interesterification as an effective tool for restructuring vegetable oils and producing structured lipids with tailored physicochemical and nutritional properties (Alves et al., 2026; Liu et al., 2025; Park et al., 2025; Yuan et al., 2025). Table 1 presents studies that analyzed intramolecular interesterification in the modification of vegetable oils.

Table 1. Vegetable oils modified by lipases through enzymatic interesterification.

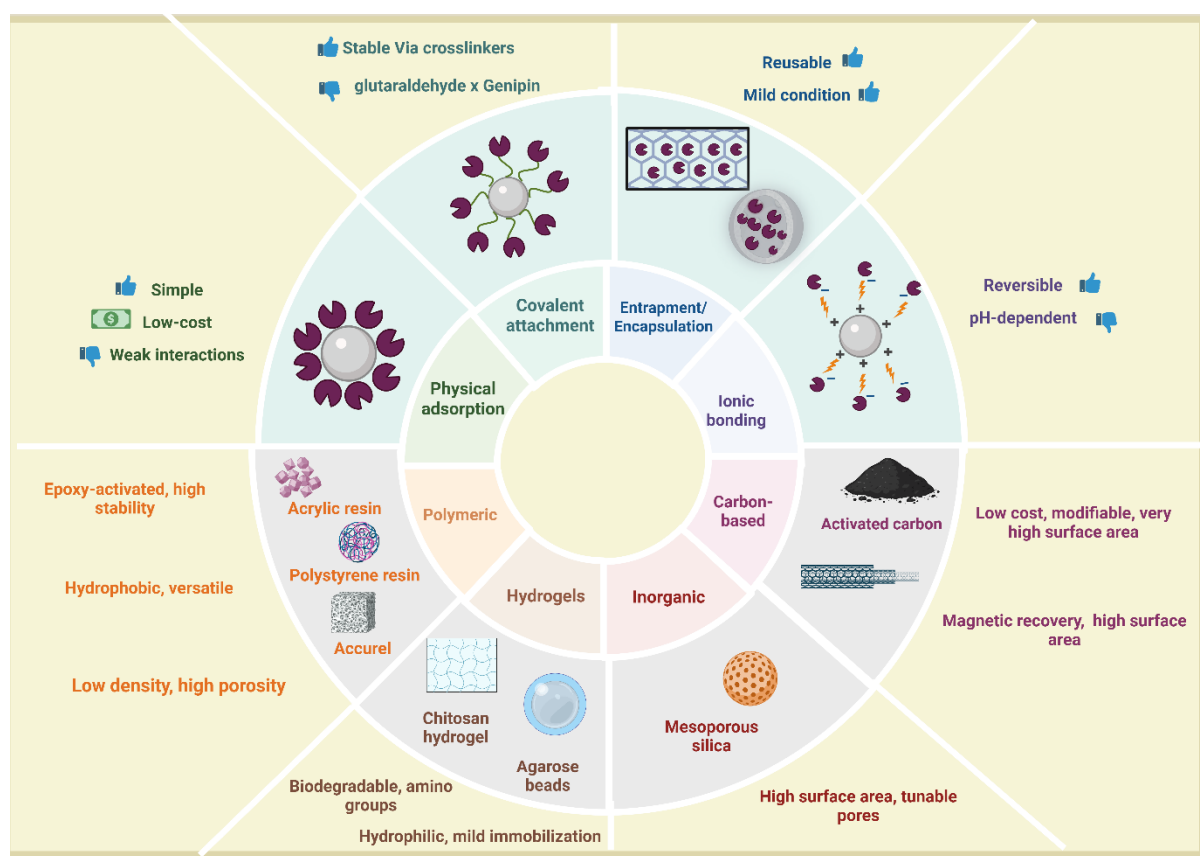
Oil used	Lipase	Main objective of the modification	Reference
Palm stearin+ palm olein+palm kernel oil	<i>Thermomyces lanuginose</i> lipase	Modification of physical properties	(Zhang, Ye, et al., 2021)
Soybean oil + medium-chain TAGs	Lipozyme TL IM (<i>Thermomyces lanuginosus</i>)	Pilot-scale production of functional MLCTs	(Zhang, Zhang, et al., 2020)
Palm olein	sn-1,3-specific lipase	Production of structured lipids with controlled acyl migration	(Ai et al., 2023)
Fully and partially hydrogenated soybean oil	Novozym 435 (<i>Candida antarctica</i>)	Production of bioparaffins with physicochemical properties like mineral waxes	(Romero et al., 2022)
Palm stearin + olein	sn-1,3-specific lipase	Assessment of interesterification degree and acyl migration behavior	(Zhang, Lee, et al., 2021)
Tigernut oil (<i>Cyperus esculentus</i>)	Lipozyme RMIM	Production of structured lipids with functional food applications	(Ijaz & Sun, 2024)
Hydrogenated crambe oil + olive/soybean oil	<i>Thermomyces lanuginosa</i> lipase	Production of behenic acid-rich lipids with anti-obesity potential	(Zuin et al., 2022)

Despite these advances, the industrial application of enzymatic interesterification still faces challenges, particularly regarding enzyme stability at large scale and process efficiency. The immobilization of lipases has proven to be an effective strategy to address these limitations, facilitating enzyme reuse, reducing operational costs, and enhancing industrial feasibility (Abdelaziz et al., 2025; Zhang et al., 2026).

3. Immobilized lipase in interesterification: Strategies and Supports

The immobilization of enzymes, particularly lipases, has emerged as a transformative approach to overcoming the operational limitations of biocatalysis in industrial processes. By anchoring these biological catalysts to solid supports, researchers have significantly improved thermal stability, resistance to substrate and product inhibition, and enzyme reusability, key factors in enhancing process economics (Robescu & Bavaro, 2025; Upadhyay, 2025). This technology simplifies enzyme recovery, enabling continuous reactor operation and precise process control. When combined with the inherent versatility of enzymatic catalysis, immobilization unlocks new possibilities for tailored lipid modification, thus allowing the development of functional foods and specialized formulations with targeted properties (García-Quinto et al., 2025; Guan et al., 2024). Various immobilization strategies (Figure 3) have been developed to implement these benefits effectively, each with distinct advantages and limitations depending on the operational context.

Figure 3. Schematic overview of lipase immobilization strategies and support materials applied in enzymatic interesterification, highlighting their advantages and limitations.



The optimal method depends on the lipase characteristics, support material properties, and intended application. Covalent bonding, while providing considerable stability, may compromise enzyme activity if catalytic residues, amino acid residues located in the active site that directly participate in the catalytic mechanism, are affected (Abdelaziz et al., 2025; Lee et al., 2024; Moentamaria et al., 2024). Physical adsorption offers simplicity and cost-effectiveness but often suffers from enzyme leaching. Recent innovations have explored functionalized supports, including modified silica, epoxy resins, organic-inorganic hybrids, and carbon-based materials like amino-, carboxyl-, or epoxy-group functionalized activated carbon and biochar (Castro et al., 2026; Almeida et al., 2026; Erol et al., 2026; Yameen et al., 2024; Nascimento, Borges, Santos, et al., 2024; Santos et al., 2022).

Activated carbon as a support material has attracted increasing attention, although it remains less explored than traditional commercial supports such as Sepabeads (styrene-divinylbenzene resin), Accurel (polypropylene-based porous polymer), and Eupergit (epoxy-activated acrylic resin) (Asrani et al., 2025; Trisunaryanti et al., 2024). Its high surface area,

porous structure, and potential for surface modification with functional agents are responsible for this growing interest (Qiu et al., 2024; Vera-Baquero et al., 2025). Functionalization with agents such as genipin, glutaraldehyde, or polyethylene imine facilitates the introduction of reactive groups that can covalently bind to the amino acid residues of lipases, such as lysine or cysteine, thereby promoting a more stable immobilization (Almeida et al., 2024; García-Quinto et al., 2024; Moentamaria et al., 2024). While studies using activated carbon in interesterification remain limited, recent findings suggest that this support could emerge as a promising alternative for pilot-scale applications, particularly in contexts that prioritize sustainability and the reuse of agro-industrial waste (Alvez-Tovar et al., 2025; Montfort et al., 2025).

Several studies have already demonstrated the success of interesterification by immobilized lipase on different supports (Table 2). In a study by Baoyindugurong et al. (2024), the use of immobilized lipase for the interesterification of Sunite sheep tail fat with flaxseed oil led to a marked increase in the incorporation of α -linolenic acid at the sn-2 position of TAGs, thereby improving their nutritional profile. Hwang et al. (2023) reported the use of immobilized *Candida antarctica* lipase B as an sn-1,3 regiospecific biocatalyst to produce structured lipids enriched with polyunsaturated fatty acids at specific positions within TAG molecules. These modifications enhanced the functional and metabolic properties of the resulting lipids, reinforcing the value of lipase immobilization strategies for targeted lipid structuring in food and nutrition applications.

As shown in Table 2, a wide range of support materials and immobilization strategies have been explored, highlighting the versatility of immobilized lipase systems across different applications. This diversity enables the use of vegetable oils from various sources to produce structured lipids, including those derived from alternative and underutilized feedstocks (Alves et al., 2026; Caporusso et al., 2025). Such flexibility aligns with circular economy principles, supporting the valorization of renewable resources and promoting more sustainable lipid processing approaches.

Table 2. Enzymatic interesterification using immobilized lipases: support materials, immobilization methods, applications, and effects on triacylglycerol composition.

Lipase Source	Support Material	Immobilization type	Application	Study highlights	Reference
<i>Rhizomucor miehei</i>	Polystyrene resin	Physical adsorption	Fat modification	Improved substrate specificity	(Abdi Hasibuan et al., 2021)

Lipase B from <i>Candida antarctica</i>	Acrylic resin	Physical adsorption	Oil modification	Improved Character of coconut oil	(Kanprakobkit et al., 2023)
<i>Geobacillus zalihae</i>	Agarose beads	Covalente binding	Synthesis of specific TAGs	Hight regioselectivity	(Rahman et al., 2007)
<i>Candida rugosa</i>	Chitosan hydrogel	Physical adsorption	Stabilization of lipids in oxidizing food environments	Improved enzyme stability against lipid oxidation products	(Pirozzi et al., 2023)
<i>Candida lipolytica</i>	Magnetic carbon nanotubes	Physical adsorption	Synthesis of OPO lipids (infant formula fat substitutes)	Higher performance than commercial lipases	(Ni et al., 2025b)
<i>Candida rugosa</i>	Mesoporous silica	Physical adsorption	Synthesis of fatty acid ethyl esters	High catalytic efficiency in solvent-free systems	(Pedro et al., 2023)
<i>Aspergillus niger</i>	Activated carbon	Physical adsorption	Production biodiesel from <i>Phoenix dactylifera</i> L.	Enhanced stability and reusability compared to the free enzyme.	(Karunanithi & Varadappan, 2023)

4. Types of Structured Lipids: Technological and Functional Applications

Lipids, as fundamental biomolecules, play key roles in human physiology beyond energy storage. They are essential structural components of cellular membranes and transport fat-soluble vitamins (A, D, E, and K). Additionally, lipids act as precursors for bioactive molecules, including eicosanoids and steroid hormones (Dakal et al., 2025; Parton & Simons, 2024). From a nutritional standpoint, the type of fatty acids and their positional distribution within triacylglycerols (TAGs) significantly influence digestion, absorption, and metabolic outcomes. Furthermore, the sensory properties of lipids, such as texture, flavor release, and mouthfeel, are important to the palatability of many food products. In industrial applications, lipids are used in food systems as well as in pharmaceuticals, cosmetics, and biofuels, underscoring their versatility across sectors (Kırnapcı & Özbek, 2026; Savchina et al., 2025).

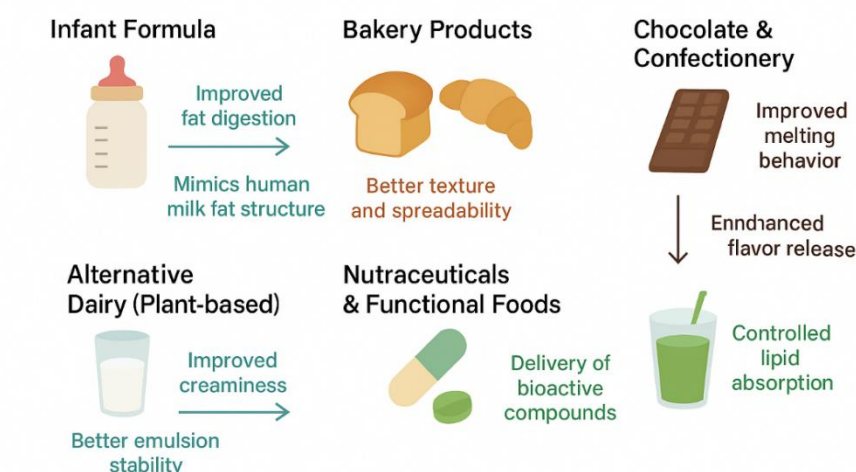
Traditional lipids, particularly those rich in saturated fatty acids or trans isomers, have been consistently associated with increased risk of cardiovascular and metabolic diseases (Medeiros et al., 2021). With this challenge, science has directed efforts toward developing

innovative lipids that combine tunable nutritional and functional properties. In this context, structured lipids (SLs) have gained increasing attention because they are enzymatically modified to incorporate specific fatty acids at predetermined positions on the glycerol backbone, resulting in functionalities that differ from those of conventional triacylglycerols. This molecular design enables the adjustment of characteristics such as melting point, oxidative stability, and digestibility, directly influencing its metabolic behavior (Dovale-Rosabal et al., 2022). As a result, they become ideal for applications in functional foods or products that require specific technological properties (Kupikowska-Stobba et al., 2024; Rosa et al., 2025). In Figure 4, the main applications of structural lipids are shown.

One of the most-researched structured lipids is 1,3-dioleoyl-2-palmitoylglycerol (OPO), a molecule designed to replicate the lipid composition of breast milk. Its unique structure improves calcium absorption in infants, making it a key ingredient in advanced infant formulas (Shen et al., 2021). Recent research shows that the production of this lipid via enzymatic interesterification with specific lipases is highly efficient when reaction conditions are carefully adjusted (Guan et al., 2024; Majumder et al., 2026; Venkatachalam, 2026). The same approach has been adapted to produce triglycerides of the medium-long-medium (MLM) type, which combine two essential benefits: rapid energy supply and balanced lipid metabolism. For these characteristics, these compounds are especially valuable in clinical nutrition and sports supplements (Mayookha. et al., 2026; Qiu et al., 2026).

Figure 4. Overview of the main applications of structured lipids in food and pharmaceutical systems, highlighting their role in improving functionality, nutritional quality, and physicochemical properties.

Applications of Structured Lipids (SLs) in Food Systems



Structured lipids (SLs) provide functional and nutritional advantages in diverse food systems, enabling formulation of healthier and performance-enhanced products.

Applying SLs also extends to the formulation of products with specific nutritional characteristics. In the study conducted by Conti et al. (2020), structured lipids obtained by interesterification of tributyrin with flaxseed oil were evaluated for their chemotherapeutic potential during the initial promotion phase of experimental hepatocarcinogenesis in rats (Conti et al., 2020). Treatment with SLs showed chemopreventive activity comparable to that of tributyrin and even resulted in a lower hepatic concentration of butyric acid. The inhibitory effects of SLs on hepatocarcinogenesis indicated a valuable approach to preventing liver cancer.

SLs have demonstrated potential in pharmaceutical systems, where they serve as lipid-based carriers for lipophilic drugs, improving their solubility and enabling controlled release (Bukke et al., 2024). Additionally, certain SLs show promise in cosmetic formulations by enhancing skin absorption and moisturizing properties. Recent trends indicate that SLs are integrated into nutraceuticals and personalized nutrition, in which lipid molecules are tailored to specific genetic or metabolic profiles (Berciano et al., 2022).

Structured lipids have also demonstrated successful integration into several food matrices. Structured lipids transform the baking industry by providing healthier alternatives while maintaining quality. These innovative compounds effectively replace saturated fats in products such as breads, cakes, and biscuits, leading to a substantial reduction in unhealthy fat content while maintaining the desired texture and shelf stability expected by consumers (Alves et al., 2026; Mohammadi & MB, 2025; Peixoto et al., 2026). The main results of the research indicate that bakery products formulated with structured lipids retain the taste, appearance, and familiar feel of traditional recipes. Sensory assessments confirm consumer acceptance of the

reformulated product relative to the conventional product, and nutritional benefits are achieved without compromising product performance or consumer satisfaction (Ferdaus et al., 2026; Martínez et al., 2025). Beyond baking, structured lipids are increasingly employed in the broader food industry to tailor nutritional profiles and enhance functional attributes across a diverse range of products, reflecting a growing consumer demand for health-conscious food options (Madankar et al., 2025; Zhou et al., 2022).

Furthermore, applying SLs to dairy analogs and spreads contributes to desirable melting behavior and flavor release. Dietetic chocolates and ice creams have benefited from SLs, which replicate milk fat characteristics while reducing saturated fat content (Chaves et al., 2024). These technological capabilities and the ability to modulate nutritional profiles make SLs particularly appealing for developing functional foods and medical nutrition (Ferdaus et al., 2026; Lim & Baik, 2025; Nath et al., 2025).

Structured lipids continue to prove their industrial value through adaptable functional properties. With each innovation in lipase stabilization and reaction engineering, potential applications expand from nutritional formulations to sustainable packaging. This progress addresses the threefold challenge of delivering functionality, promoting health, and ensuring environmental sustainability in the modern food system (Lopes et al., 2023; Zhou et al., 2022).

4. Challenges and Future Perspectives in Structured Lipid Research

Despite the advances observed, there remain relevant gaps in the literature that warrant future investigation. One of the main reasons is the lack of studies on alternative, environmentally sustainable, and economically viable supports for the immobilization of lipases (Ostojčić et al., 2025; Reis et al., 2025; Silva et al., 2025). Activated carbon, although already used in other industrial segments, has still not been explored to support the synthesis of SL (Zhao et al., 2022; Nascimento et al., 2024). This represents an opportunity for innovation, especially in countries with abundant lignocellulosic biomass, such as Brazil. Examples include residues from licuri (*Syagrus coronata*), coconut, babassu, cashew, sugarcane, coffee processing, and fruit agro-industries, which are generated in large quantities and often remain underutilized. Their valorization promotes waste reduction, supports the circular economy, and aligns with sustainable development goals related to responsible production and innovation (Bhardwaj et al., 2025; Vtieri et al., 2025).

Another important point is the lack of comprehensive comparative studies. Although the preliminary results are encouraging, we still need consistent data on how these modified lipids

behave like traditional fats in terms of oxidative stability during storage, the bioavailability of essential fatty acids, and their metabolic effects in living organisms (Alves et al., 2026; Liu et al., 2025). This information is essential to scientifically validate the benefits of structured lipids, support nutritional claims, and obtain regulatory approvals, determinants for consumer market acceptance (Bucchini, 2019; Meynier & Génot, 2017).

Future developments point towards combining knowledge from food science, biochemical engineering, and nanotechnology to develop systems for the controlled release of nutrients, Lipid mixtures with specific functionalities, and customized foods for special metabolic needs. In this context, structured lipids are no longer mere ingredients but powerful tools of nutritional intervention. For all this potential to be realized, adopting an integrated vision that simultaneously considers the technological aspects, economic viability, and environmental impact is essential.

The next frontier in structured lipid research demands interdisciplinary collaboration, pilot-scale testing, and partnerships between academia and industry. Although the challenges are considerable, the potential opportunities are even more promising. With a strategic approach, we can revolutionize the production and consumption of fats, promote sustainable practices, and significantly advance innovation in the global food chain.

6. Conclusion

Enzymatic interesterification (EIE) represents an important advancement in lipid modification, combining precision, sustainability, and nutritional functionality. The immobilization of lipases has enhanced enzyme stability and reusability, addressing key limitations for industrial applications and enabling the production of structured lipids with targeted physical and nutritional properties. From infant formulas to bakery products, structured lipids demonstrate improved performance and health benefits, aligning with global trends toward cleaner labels and reduced saturated fat content. Despite these advances, large-scale adoption still requires overcoming technical challenges, including optimizing immobilization supports, ensuring regulatory compliance, and validating long-term product stability and safety. In this regard, the valorization of agro-industrial residues offers an additional advantage by providing low-cost, sustainable raw materials for both lipid substrates and immobilization support. From a broader perspective, enzymatic interesterification can be considered a mature yet evolving technology for sustainable lipid modification. Continued advances in lipase immobilization, combined with the use of renewable biomass resources, are

expected to play a key role in the development of next-generation functional lipids. Strategic collaboration between academia and industry will be essential to translate these innovations into scalable and regulatory-compliant food solutions.

7. References

- Abdelaziz, A., Abo-Kamar, A. M., Elkotb, E. S., & Al-Madboly, L. A. (2025). Microbial lipases: advances in production, purification, biochemical characterization, and multifaceted applications in industry and medicine. *Microbial Cell Factories*, 24(1), 40. <https://doi.org/10.1186/s12934-025-02664-6>
- Abdel-Mageed, H. M. (2025). Frontiers in nanoparticles redefining enzyme immobilization: a review addressing challenges, innovations, and unlocking sustainable future potentials. *Micro and Nano Systems Letters*, 13(1). <https://doi.org/10.1186/s40486-025-00228-2>
- Abdelmoez, W., & Mustafa, A. (2014). Oleochemical Industry Future through Biotechnology [Review of *Oleochemical Industry Future through Biotechnology*]. *Journal of Oleo Science*, 63(6), 545. Japan Oil Chemists' Society. <https://doi.org/10.5650/jos.ess14022>
- Almeida, Â., Pereira, M. V., Peleja, A. B., Rocha, H. F., Otero, M., Pereira, G., & Calisto, V. (2026). Laccase Immobilization on Carbon-Based Materials Derived from Spent Brewery Grains: Optimization and Stability Evaluation. *Molecules*, 31(4), 738. <https://doi.org/10.3390/molecules31040738>
- Almeida, C. S., Neto, F. S., Sousa, P. da S., Aires, F. I. da S., Filho, J. R. de M., Cavalcante, A. L. G., Sousa, P. G. de, Melo, R. L. F., & Santos, J. C. S. dos. (2024). Enhancing Lipase Immobilization via Physical Adsorption: Advancements in Stability, Reusability, and Industrial Applications for Sustainable Biotechnological Processes. *ACS Omega*, 9(47), 46698. <https://doi.org/10.1021/acsomega.4c07088>
- Alves, V., Furtado, G. de F., Roman, M. A. S., Delboni, L. de M. P., Macedo, J. A., Vianna, C. L. de C., Luccas, V., & Macêdo, G. A. (2026). Palm Oil-Free Structured Lipids: A Novel Structuring Fat for Sandwich Cookie Fillings. *Foods*, 15(1), 178. <https://doi.org/10.3390/foods15010178>
- Alvez-Tovar, B., Scalize, P. S., Angiolillo-Rodríguez, G., Albuquerque, A., Ebang, M. N., & Oliveira, T. F. de. (2025). Agro-Industrial Waste Upcycling into Activated

- Carbons: A Sustainable Approach for Dye Removal and Wastewater Treatment. *Sustainability*, 17(5), 2036. <https://doi.org/10.3390/su17052036>
- Amini, Z., Ilham, Z., Ong, H. C., Mazaheri, H., & Chen, W. (2016). State of the art and prospective of lipase-catalyzed transesterification reaction for biodiesel production. *Energy Conversion and Management*, 141, 339. <https://doi.org/10.1016/j.enconman.2016.09.049>
- Asrani, A., Prasad, B., Gariya, N., Singh, H., Kumar, N., Kumar, K., Bhowmik, A., Khanna, V., Samal, P., John, V., & Kumar, A. (2025). Sustainable advances in activated carbon for environmental and industrial applications. *Environmental Progress & Sustainable Energy*, 45(1). <https://doi.org/10.1002/ep.70176>
- Batista, D., Macêdo, G. A., Luccas, V., & Macedo, J. A. (2025). Enzymatic Interesterification: An Innovative Strategy for Lower-Calorie Lipid Production From Refined Peanut Oil. *Journal of Food Science*, 90(10). <https://doi.org/10.1111/1750-3841.70603>
- Berciano, S., Figueiredo, J. P., Brisbois, T. D., Alford, S., Koecher, K., Eckhouse, S., Ciati, R., Kussmann, M., Ordovás, J. M., Stebbins, K., & Blumberg, J. B. (2022). Precision nutrition: Maintaining scientific integrity while realizing market potential [Review of *Precision nutrition: Maintaining scientific integrity while realizing market potential*]. *Frontiers in Nutrition*, 9. Frontiers Media. <https://doi.org/10.3389/fnut.2022.979665>
- Bhardwaj, A. K., Thakur, B., Tripathi, S. K., Turabayev, M. E., Aljulaib, A. A., Kharlamova, T., & Kulinich, S. A. (2025). Recent Advances in Valorizing Agricultural Waste: A Sustainable Approach. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-025-03419-5>
- Bhardwaj, K. K., & Gupta, R. (2017). Synthesis of Chirally Pure Enantiomers by Lipase. *Journal of Oleo Science*, 66(10), 1073. <https://doi.org/10.5650/jos.ess17114>
- Bucchini, L. (2019). Nutrition and health claims in Europe: oils & fats related claims, regulatory and labeling challenges. *OCL*, 26, 48. <https://doi.org/10.1051/ocl/2019041>
- Bukke, S. P. N., Venkatesh, C., Rajanna, S. B., Saraswathi, T. S., Kusuma, P. K., Goruntla, N., Balasuramanyam, N., & Munishamireddy, S. (2024). Solid lipid nanocarriers for drug delivery: design innovations and characterization strategies—a comprehensive review. *Discover Applied Sciences*, 6(6). <https://doi.org/10.1007/s42452-024-05897-z>

- Campbell, B., Meadu, V., Loboguerrero, A. M., Nowak, A., Rosenstock, T. S., Shilomboleni, H., Zougmore, R. B., Läderach, P., Born, L., Shelton, S., Broeze, J., Pereira, L., Cosgrove, B., Huyer, S., Koerner, J., Thornton, P. K., Campbell, B., Aggarwal, P., Keating, B. A., & Zeppenfeldt, L. (2023). Transforming Food Systems Under Climate Change through Innovation. In *Cambridge University Press eBooks*. Cambridge University Press. <https://doi.org/10.1017/9781009227216>
- Caporusso, A., Radice, M., Biundo, A., Gorgoglione, R., Agrimi, G., & Pisano, I. (2025). Waste cooking oils as a sustainable feedstock for bio-based application: A systematic review. *Journal of Biotechnology*, 400, 48. <https://doi.org/10.1016/j.jbiotec.2025.02.003>
- Castro, É. C. de, Fernandes, T. S. M., Aires, F. I. da S., Sousa, P. G. de, Silva, L. E. C. e, Santos, K. M. dos, Filho, J. R. de M., Araújo, S. da C., & Santos, J. C. S. dos. (2026). Engineering functional support materials for Enzyme immobilization: Technologies, performance and industrial relevance. *Next Chemical Engineering*, 2, 100028. <https://doi.org/10.1016/j.nxcen.2026.100028>
- Chen, G., Khan, I. M., He, W., Li, Y., Jin, P., Campanella, O. H., Zhang, H., Huo, Y., Chen, Y., Yang, H., & Miao, M. (2022). Rebuilding the lid region from conformational and dynamic features to engineering applications of lipase in foods: Current status and future prospects. *Comprehensive Reviews in Food Science and Food Safety*, 21(3), 2688. <https://doi.org/10.1111/1541-4337.12965>
- Conti, A. de, Tryndyak, V., Heidor, R., Jimenez, L., Moreno, F., Beland, F. A., Rusyn, I., & Pogribny, I. P. (2020). Butyrate-containing structured lipids inhibit RAC1 and epithelial-to-mesenchymal transition markers: a chemopreventive mechanism against hepatocarcinogenesis. *The Journal of Nutritional Biochemistry*, 86, 108496. <https://doi.org/10.1016/j.jnutbio.2020.108496>
- Costa, A. R., Salgado, J. M., Lopes, M., & Belo, I. (2022). Valorization of by-products from vegetable oil industries: Enzymes production by *Yarrowia lipolytica* through solid state fermentation. *Frontiers in Sustainable Food Systems*, 6. <https://doi.org/10.3389/fsufs.2022.1006467>
- Dakal, T. C., Xiao, F., Bhusal, C. K., Sabapathy, P. C., Segal, R., Chen, J., & Bai, X. (2025). Lipids dysregulation in diseases: core concepts, targets and treatment

- strategies. *Lipids in Health and Disease*, 24(1), 61. <https://doi.org/10.1186/s12944-024-02425-1>
- Danthine, S., Closset, S., Maes, J., Mascrez, S., Blecker, C., Purcaro, G., & Gibon, V. (2022). Enzymatic interesterification to produce zero-trans and dialkylketones-free fats from rapeseed oil. *OCL*, 29, 36. <https://doi.org/10.1051/ocl/2022029>
- Deslauriers, M. G. (2017). Considerations for implementation of novel enzyme-based processes. *Research Portal Denmark*, 203. <https://local.forskningsportal.dk/local/dki-cgi/ws/cris-link?src=dtu&id=dtu-1beb4d95-3b44-4451-8d85-b343f1b6497f&ti=Considerations%20for%20implementation%20of%20novel%20enzyme-based%20processes>
- Dovale-Rosabal, G., Espinosa, A., Rodríguez, A., Barriga, A., Palomino-Calderón, A., Romero, N., Troncoso, R., & Aubourg, S. P. (2022). Effect of Structured Phenolic Lipids with EPA/DHA and Gallic Acid against Metabolic-Associated Fatty Liver Disease (MAFLD) in Mice. *Molecules*, 27(22), 7702. <https://doi.org/10.3390/molecules27227702>
- Erol, K., Veyisoğlu, A., Tatar, D., Kocabas, B. B., Alacabey, İ., & Gökmeşe, E. (2026). Enhanced kinetic performance and stability of catalase immobilized on epoxy-functionalized kaolinite. *Scientific Reports*, 16(1). <https://doi.org/10.1038/s41598-026-38910-z>
- Farhan, M., Hasani, I. W., Khafaga, D. S. R., Ragab, W. M., Kazi, R. N. A., Aatif, M., Muteeb, G., & Fahim, Y. A. (2025). Enzymes as Catalysts in Industrial Biocatalysis: Advances in Engineering, Applications, and Sustainable Integration. *Catalysts*, 15(9), 891. <https://doi.org/10.3390/catal15090891>
- Ferdaus, J., Mahmud, N., Matamoros, J. R., Aragon-Alegro, L. C., & Silva, R. (2026). Structured Plant-Based Oil as Healthier Alternatives to Conventional Fats in Chocolate Cream Fillings. *Journal of the American Oil Chemists Society*, 103(4), 245. <https://doi.org/10.1002/aocs.70045>
- García-Quinto, E., Guisán, J. M., & Fernández-Lorente, G. (2024). Enzymatic synthesis of structured DHA phospholipids in a solvent-free medium with potential effects in the treatment of neurodegenerative diseases. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-4821561/v1>

- García-Quinto, E., Guisán, J. M., & Fernández-Lorente, G. (2025). Enzymatic Synthesis of Bioactive Structured DHA Phospholipids via Stable Immobilized Phospholipase-Catalyzed Esterification in a Solvent-Free Medium. *Processes*, 13(2), 442. <https://doi.org/10.3390/pr13020442>
- Guan, A., Hou, Y., Yang, R., & Qin, J. (2024). Enzyme engineering for functional lipids synthesis: recent advance and perspective. *Bioresources and Bioprocessing*, 11(1). <https://doi.org/10.1186/s40643-023-00723-7>
- Gunn, K. H., & Neher, S. B. (2023). Structure of dimeric lipoprotein lipase reveals a pore adjacent to the active site. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-38243-9>
- Hageman, J. H. J., Keijer, J., Dalsgaard, T. K., Zeper, L. W., Carrière, F., Feitsma, A. L., & Nieuwenhuizen, A. G. (2019). Free fatty acid release from vegetable and bovine milk fat-based infant formulas and human milk during two-phase in vitro digestion. *Food & Function*, 10(4), 2102. <https://doi.org/10.1039/c8fo01940a>
- Han, Y., Fang, H., Nie, X., Zhang, X., Lu, Z., Nian, B., Hu, Y., & Cheng, W. (2025). Potential applications of lipases rational design in the synthesis of medium- and long-chain triglycerides: Current advances and perspectives. *Food Chemistry*, 493, 146049. <https://doi.org/10.1016/j.foodchem.2025.146049>
- Hoff, K. L., & Eisenacher, M. (2025). Process Intensification Strategies for Esterification: Kinetic Modeling, Reactor Design, and Sustainable Applications. *International Journal of Molecular Sciences*, 26(15), 7214. <https://doi.org/10.3390/ijms26157214>
- İnanlar, B., & Altay, F. (2024). The Method That Makes Oils and Fats Healthier: Interesterification. *Black Sea Journal of Engineering and Science*, 7(5), 1081. <https://doi.org/10.34248/bsengineering.1310721>
- İnel, M., & Ozyilmaz, E. (2026). Immobilised lipase applications in health. *International Journal of Pharmaceutics*, 693, 126686. <https://doi.org/10.1016/j.ijpharm.2026.126686>
- Kanprakobkit, W., Kielarova, S. W., Wichai, U., Bunyaphatsara, N., & Kielar, F. (2022). Incrementing MCT Character of Coconut Oil Using Enzyme Catalyzed Interesterification. *Journal of Oleo Science*, 72(1), 87. <https://doi.org/10.5650/jos.ess22269>

- Kiprono, J., Rutto, H., & Seodigeng, T. (2022). Production of Biodiesel Using Phosphate Rock as a Heterogeneous Catalyst. An Optimized Process Using Surface Response Methodology. *Environmental and Climate Technologies*, 26(1), 822.
<https://doi.org/10.2478/rtuct-2022-0062>
- Kırnapcı, M., & Özbek, Z. A. (2026). Emerging trends and recent innovations in oleogel-based fat substitutes for sustainable foods. *European Food Research and Technology*, 252(2). <https://doi.org/10.1007/s00217-025-04962-0>
- Kupikowska-Stobba, B., Niu, H., Kłodová, I., Agregán, R., Lorenzo, J. M., & Kasprzak, M. (2024). Controlled lipid digestion in the development of functional and personalized foods for a tailored delivery of dietary fats. *Food Chemistry*, 466, 142151.
<https://doi.org/10.1016/j.foodchem.2024.142151>
- Lee, H., Yang, E., Kwon, H. K., Kim, B. K., Lee, T., Yoo, H. Y., & Park, C. (2024). Innovative approach for semi-continuous production of puerarin palmitate via transesterification with novel immobilized lipase. *Chemical and Biological Technologies in Agriculture*, 11(1). <https://doi.org/10.1186/s40538-024-00550-8>
- Ligarda-Samanez, C. A., Huamán-Carrión, M. L., Ponce, W. C. C., Cruz, G. D. la, Huamaní, D. F. C., Cabel-Moscoso, D. J., García-Espinoza, A. J., León, R. S., Durán, Y. A., Muñoz-Saenz, J. C., Muñoz-Melgarejo, M., & Jilaja-Carita, E. E. (2025). Technological Innovations and Circular Economy in the Valorization of Agri-Food By-Products: Advances, Challenges and Perspectives. *Foods*, 14(11), 1950.
<https://doi.org/10.3390/foods14111950>
- Lim, H., & Baik, M. (2025). Emulsifiers for the plant-based milk alternatives: a review. *Food Science and Biotechnology*, 34(2), 515. <https://doi.org/10.1007/s10068-024-01814-7>
- Liu, X., & Akoh, C. C. (2024). Enzymatic Synthesis and Characterization of MLM-type Structured Lipid Using Grapeseed Oil and Capric Acid. *Journal of Oleo Science*, 73(7), 977. <https://doi.org/10.5650/jos.ess23212>
- Liu, Y., Gao, P., Yang, Y., Liu, C., Zhong, W., Yin, J., & Reaney, M. J. T. (2025). Enzymatically Interesterified Triadica sebifera Oil: A Novel Shortening for Enhanced Nutritional Quality and Sustainability. *Foods*, 14(4), 590.
<https://doi.org/10.3390/foods14040590>

- Madankar, C. S., Bhagat, A. A., Nasser, E. K., Altemimi, A. B., Calabrò, F., & Mahna, N. (2025). Structured Lipids: Synthesis, Genetic Engineering, and Applications. *Journal of the American Oil Chemists Society*, 102(9), 1325.
<https://doi.org/10.1002/aocs.12970>
- Mahmoud, E. A., Zaki, E. G., Ismail, D. A., Gaffer, A., & Mazrouaa, A. M. (2022). Green valorization of waste date seed oil for biodiesel production. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-1749903/v1>
- Majumder, D., Saha, T. N., Bhattacharya, D., Singh, S., Rajeev, M., Nag, M., Lahiri, D., & Pandit, S. (2026). Comparative Analysis of Translational Innovations in Sn-2 and Sn-1,3-Specific Lipase-Mediated Regioselective Catalysis for Enhanced Diacylglycerol Production. *Journal of Agricultural and Food Chemistry*, 74(1), 15.
<https://doi.org/10.1021/acs.jafc.5c05671>
- Martínez, E., González, J. E. P., Álvarez-Ortí, M., Martínez-Navarro, M. E., & Rabadán, A. (2025). Sensory and Lipid Profile Optimization of Functional Brownies Through Cold-Pressed Nut Oil Substitution for Butter. *Applied Sciences*, 15(1), 454.
<https://doi.org/10.3390/app15010454>
- Mastai, Y. (2015). Advanced Topics in Crystallization. In *InTech eBooks*.
<https://doi.org/10.5772/58651>
- Medeiros, L. B., Alves, S. P., Bessa, R. J. B., Soares, J. K. B., Costa, C. N. M., Aquino, J. de S., Guerra, G. C. B., Araújo, D. F. S., Toscano, L. T., Silva, A. S., Alves, A. F., Lacerda, M. M. P. D., Araújo, W. J., Medeiros, A. N. de, Oliveira, C. J. B. de, & Queiroga, R. de C. R. do E. (2021). Metabolic parameters and gut microbiota composition of rats fed high-fat diets based on ruminant fat compared with hydrogenated vegetable fat. *Research Square (Research Square)*.
<https://doi.org/10.21203/rs.3.rs-267429/v1>
- Melchior, S., Plazzotta, S., Miao, S., Manzocco, L., Nicoli, M. C., & Calligaris, S. (2024). Design of Fat Alternatives Using Saturated Monoglycerides. *Food Engineering Reviews*, 16(4), 635. <https://doi.org/10.1007/s12393-024-09379-1>
- Meynier, A., & Génot, C. (2017). Molecular and structural organization of lipids in foods: their fate during digestion and impact in nutrition. *OCL*, 24(2).
<https://doi.org/10.1051/ocl/2017006>

- Mia, M., Ahmed, Md. M., & Zzaman, W. (2025). Valorization of food waste into functional ingredients supports a sustainable strategy for the food industry. *Discover Food*, 5(1). <https://doi.org/10.1007/s44187-025-00584-3>
- Milovanović, J., Banjanac, K., Nikolić, J., Nikodinović-Runić, J., & Prlainović, N. (2025). The Organic-Functionalized Silica Nanoparticles as Lipase Carriers for Biocatalytic Application: Future Perspective in Biodegradation. *Catalysts*, 15(1), 54. <https://doi.org/10.3390/catal15010054>
- Moentamaria, D., Irfan, Z., Chumaidi, A., Widjaja, A., Widjaja, T., Muharja, M., & Darmayanti, R. F. (2024). Development of a Novel Support Modification for Efficient Lipase Immobilization: Preparation, Characterization, and Application for Bio-flavor Production. *BULLETIN OF CHEMICAL REACTION ENGINEERING AND CATALYSIS*, 19(2), 215. <https://doi.org/10.9767/bcrec.20130>
- Mohammadi, Z., & MB, A. (2025). A Review of Plant Based Bakery Products. *International Journal For Multidisciplinary Research*, 7(4). <https://doi.org/10.36948/ijfmr.2025.v07i04.49839>
- Montfort, G. R.-C., Azamar-Barrios, J. A., Quintana, P., Rejón, V., González-Gómez, W. S., & Madera-Santana, T. J. (2025). Production and Physicochemical Characterization of Activated Carbon from the Mesocarp of the Coconut (*Cocos nucifera* L.) Variety Alto del Pacifico. *Chemistry*, 7(3), 88. <https://doi.org/10.3390/chemistry7030088>
- Nascimento, P. A., Borges, J. F., Alves, A. N., Holland, B., Bonomo, R. C. F., Mathesh, M., Yang, W., & Veloso, C. M. (2024). Synthesis and microencapsulation of acylglycerols rich in omega-3 PUFAs by glycerolysis using lipase immobilized on activated carbon. *Journal of Environmental Chemical Engineering*, 12(6). <https://doi.org/10.1016/j.jece.2024.114617>
- Nascimento, P. A., Borges, J. F., Santos, M. P. F., Alves, A. N., Santos, L. S., da Costa Ilhéu Fontan, R., Bonomo, R. C. F., Yang, W., & Veloso, C. M. (2024). Revealing the potential of a novel approach for assessing the effect of pyrolysis conditions on sisal waste-based activated carbon for lipase immobilization and ethyl lactate biosynthesis. *Biomass Conversion and Biorefinery*. <https://doi.org/10.1007/s13399-024-06318-2>

- Nath, M., Ramkumar, S., Das, A., & Das, M. (2025). An overview on application of fat-replacers in different dairy products and its health implications. *Discover Food*, 5(1). <https://doi.org/10.1007/s44187-025-00332-7>
- Nguyen, L. V., & Shahidi, F. (2025). Structured lipids from virgin coconut oil and omega-3 fatty acids: Process optimization. *Journal of the American Oil Chemists Society*, 102(5), 957. <https://doi.org/10.1002/aocs.12942>
- Ostojčić, M., Brekalo, M., Stjepanović, M., Rajs, B. B., Velić, N., Šarić, S., Djerdj, I., Budžaki, S., & Strelec, I. (2025). Cellulose Carriers from Spent Coffee Grounds for Lipase Immobilization and Evaluation of Biocatalyst Performance. *Sustainability*, 17(21), 9633. <https://doi.org/10.3390/su17219633>
- Moyookha, V. P., Bhumika, K. P., Raksha U., Nanishankar, V. H., Soujanya, K. V., Suresh, G. (2026). Lipase-catalysed acidolysis for enrichment of medium-chain triacylglycerols in virgin coconut oil and its functional properties. *Food & Function*. <https://doi.org/10.1039/d5fo04699h>
- Park, J., Lee, J., Kim, J.-H., & Chang, P. (2025). Chemoenzymatic synthesis and identification of medium- and long-chain triacylglycerol congeners. *Food Science and Biotechnology*. <https://doi.org/10.1007/s10068-025-02006-7>
- Park, J., & Park, K. (2022). Lipase and Its Unique Selectivity: A Mini-Review. *Journal of Chemistry*, 2022, 1. <https://doi.org/10.1155/2022/7609019>
- Parton, R. G., & Simons, K. (2024). The Biology of Lipids. *Cold Spring Harbor Perspectives in Biology*, 16(8). <https://doi.org/10.1101/cshperspect.a041713>
- Payá-Tormo, L., Rodríguez-Salarichs, J., Prieto, A., Martínez, M. J., & Barriuso, J. (2019). Improvement of the Activity of a Fungal Versatile-Lipase Toward Triglycerides: An in silico Mechanistic Description. *Frontiers in Bioengineering and Biotechnology*, 7. <https://doi.org/10.3389/fbioe.2019.00071>
- Peixoto, V. O. D. S., Brito, G. B., Conte-Júnior, C. A., Marinho, T. O., Nele, M., Torres, A. G., & Castelo-Branco, V. N. (2026). Use of solid fat-tailored oleogels loaded with α -tocopherol as fat replacers to improve the nutritional profile of cookies. *Frontiers in Soft Matter*, 5. <https://doi.org/10.3389/frsfm.2025.1741918>

- Picq, M., Bernoud-Hubac, N., & Lagarde, M. (2013). Synthesis and Biological Interest of Structured Docosaehaenoic Acid-Containing Triacylglycerols and Phospholipids. *HAL (Le Centre Pour La Communication Scientifique Directe)*.
<https://hal.inrae.fr/hal-02649095>
- Qiu, L., Mokhtarinori, N., Liu, H., Jiang, D., Yang, Z., & Dai, S. (2024). The Carbon Challenge: Design, Synthesis, and Chemisorption Behavior of Solid Sorbents in Direct Air Capture of Carbon Dioxide. *Materials Today Energy*, 47, 101740.
<https://doi.org/10.1016/j.mtener.2024.101740>
- Qiu, Y.-Z., Yang, M.-T., Liu, Z., Chen, X., Chen, J.-D., Huang, S., Lan, Q.-Y., Hu, Y., Zhou, X. R., Wei, W., Wang, X., Xingwang, Y., & Zhu, H.-L. (2026). Impact of medium- and long-chain triacylglycerols and sn -2 palmitate on temperament development in term infants: potential role of gut Bifidobacterium. *Food & Function*, 17(5), 2399. <https://doi.org/10.1039/d5fo03451e>
- Reis, W. de S. M., Preto, A. O., Santana, G. de O., Tessaro, Í., Ferreira, A. L. G., Pereira, E. B., & Carvalho, A. K. F. de. (2025). Sustainable Alternative Media for the Production of Lipolytic Cells and Fatty Acid Concentrates: Integration of the Enzyme and Food Industries. *Foods*, 14(6), 990. <https://doi.org/10.3390/foods14060990>
- Ringling, K., & Marquart, L. (2020). Intersection of Diet, Health, and Environment: Land Grant Universities' Role in Creating Platforms for Sustainable Food Systems. *Frontiers in Sustainable Food Systems*, 4. <https://doi.org/10.3389/fsufs.2020.00070>
- Robescu, M. S., & Bavaro, T. (2025). A Comprehensive Guide to Enzyme Immobilization: All You Need to Know. *Molecules*, 30(4), 939.
<https://doi.org/10.3390/molecules30040939>
- Rosa, J. D., Marin, D. R., Silva, A. C. F., Souza, E. H. de, Rech, E., Nepomuceno, A. L., & Mertz-Henning, L. M. (2025). Biotechnological advances in enhancing soybean oil quality for human health and food industry applications. *Frontiers in Food Science and Technology*, 5. <https://doi.org/10.3389/frfst.2025.1658589>
- Santos, M. P. F., Porfírio, M. C. P., Junior, E. C. S., Bonomo, R. C. F., & Veloso, C. M. (2022). Pepsin immobilization: Influence of carbon support functionalization. *International Journal of Biological Macromolecules*, 203, 67–79.
<https://doi.org/10.1016/j.ijbiomac.2022.01.135>

- Savchina, E., Grosso, A. L., Massoner, P., Morozova, K., Scampicchio, M., & Ferrentino, G. (2025). From liquid to solid: Exploring techniques, applications, and challenges of structured oils as fat replacements in food formulations. *Comprehensive Reviews in Food Science and Food Safety*, 24(2). <https://doi.org/10.1111/1541-4337.70163>
- Shen, L., Huang, W., Xu, X., Wang, L., Wang, Q., Li, S., & Yuan, X. (2021). Stool Saponified Fatty Acid, Behavior, Growth, and Stool Characteristics in Infants Fed a High-OPO Formula: A Randomized, Double-Blind Clinical Trial. *Frontiers in Pediatrics*, 9. <https://doi.org/10.3389/fped.2021.712201>
- Silva, A., Gonçalves, L. R. B., & Silva, I. J. da. (2025). Innovations in packed-bed reactors utilizing immobilized lipase catalysts: A comprehensive technical and scientific review. *Molecular Catalysis*, 573, 114814. <https://doi.org/10.1016/j.mcat.2024.114814>
- Singh, P. K., Chopra, R., Garg, M., Dhiman, A., & Dhyani, A. (2022). Enzymatic Interesterification of Vegetable Oil: A Review on Physicochemical and Functional Properties, and Its Health Effects [Review of *Enzymatic Interesterification of Vegetable Oil: A Review on Physicochemical and Functional Properties, and Its Health Effects*]. *Journal of Oleo Science*, 71(12), 1697. Japan Oil Chemists' Society. <https://doi.org/10.5650/jos.ess22118>
- Stemler, C. D., & Scherf, K. A. (2024). Substrate specificities of baking lipases for use in fine bakery goods. *Lebensmittelchemie*, 78. <https://doi.org/10.1002/lemi.202452243>
- Tjørnelund, H. D. (2024). Computational screening tool for lipase performance in organic media. *Research Portal Denmark*, 158. <https://local.forskningsportal.dk/local/dki-cgi/ws/cris-link?src=dtu&id=dtu-e95d411e-fcfe-40ab-a1bd-162600c41d4b&ti=Computational%20screening%20tool%20for%20lipase%20performance%20in%20organic%20media>
- Trisunaryanti, W., Triyono, T., Wijaya, K., Kartini, I., Purwono, S., Rodiansono, R., Mara, A., & Dewi, I. A. (2024). Hydrodeoxygenation of Refined Palm Kernel Oil Into Bioavtur Using Spray-Dry Impregnated Activated Carbon Supported-Mo Catalysts. *Evergreen*, 11(2), 652. <https://doi.org/10.5109/7183322>

- Upadhyay, M. K. (2025). Biocatalysts vs. Metal Catalysts: A meta-analysis of green chemistry efficiency, sustainability, and industrial applications. *International Journal of Chemical Studies*, 13(5), 16. <https://doi.org/10.22271/chemi.2025.v13.i5a.12583>
- Vatieri, C., Cirillo, T., & Esposito, F. (2025). Waste to worth: bioplastic synthesis from lignocellulosic food waste in the age of the circular bioeconomy. *Frontiers in Sustainable Food Systems*, 9. <https://doi.org/10.3389/fsufs.2025.1698348>
- Venkatachalam, R. N. (2026). Microbial production of human milk fat substitutes: a review. *Discover Biotechnology*, 3(1). <https://doi.org/10.1007/s44340-026-00054-1>
- Vera-Baquero, F. L., Morante-Zarcero, S., Pérez-Quintanilla, D., & Sierra, I. (2025). Exploring Adsorption Performance of Functionalized Mesoporous Silicas with a Different Pore Structure as Strong Cation-Exchange Sorbents for Solid-Phase Extraction of Atropine and Scopolamine. *Applied Sciences*, 15(2), 646. <https://doi.org/10.3390/app15020646>
- Wallis, J. G., Bengtsson, J. D., & Browse, J. (2022). Molecular Approaches Reduce Saturates and Eliminate trans Fats in Food Oils. *Frontiers in Plant Science*, 13, 908608. <https://doi.org/10.3389/fpls.2022.908608>
- Wei, S., Kumar, A., Hailu, G. G., Choi, S., Lee, O., Chelliah, R., Oh, D., & LIU, S. (2025). Exploring the Effects of Novel Food Processing Methods on Food Proteins: A Review. *Food Science & Nutrition*, 13(6). <https://doi.org/10.1002/fsn3.70373>
- Yameen, M. Z., Naqvi, S. R., Juchelková, D., & Khan, M. N. A. (2024). Harnessing the power of functionalized biochar: progress, challenges, and future perspectives in energy, water treatment, and environmental sustainability. *Biochar*, 6(1). <https://doi.org/10.1007/s42773-024-00316-3>
- Yang, K., Wang, Y., Zheng, W., Zhu, Y., & Duan, Z. (2026). Physical and chemical properties of commercial medium- and long-carbon-chain triglyceride products: triglyceride composition, volatile substances, thermodynamic properties. *Journal of the Science of Food and Agriculture*, 106(6), 3564. <https://doi.org/10.1002/jsfa.70449>
- Yao, Y.-Y., Yuan, Y., Xiong, K., Mao, S., Jiang, J., Chen, Y., Li, X., Liu, H., Liu, L.-C., Cai, B., & Song, S. (2026). Current Progress and Future Directions of Enzyme Technology in Food Nutrition: A Comprehensive Review of Processing, Nutrition, and Functional Innovation. *Foods*, 15(2), 402. <https://doi.org/10.3390/foods15020402>

- Yuan, T., Guo, L., Gao, X., Song, G., Wang, D., Li, L., & Gong, J. (2025). Enzymatic synthesis and characterization of structured lipids: Medium- and long-chain triacylglycerols enriched with lauric acid and diverse long-chain fatty acids. *Food Bioscience*, 64, 105956. <https://doi.org/10.1016/j.fbio.2025.105956>
- Zhang, Z., Zhang, S., Lee, W. J., Lai, O. M., Tan, C. P., & Wang, Y. (2020). Production of Structured Triacylglycerol via Enzymatic Interesterification of Medium-Chain Triacylglycerol and Soybean Oil Using a Pilot-Scale Solvent-Free Packed Bed Reactor. *Journal of the American Oil Chemists' Society*, 97(3), 271–280. <https://doi.org/10.1002/aocs.12319>
- Zhang, Z., Lee, W. J., & Wang, Y. (2020b). Evaluation of enzymatic interesterification in structured triacylglycerols preparation: a concise review and prospect. In *Critical Reviews in Food Science and Nutrition* (pp. 1–15). Taylor and Francis Inc. <https://doi.org/10.1080/10408398.2020.1793725>
- Zhang, Z., Ye, J., Lee, W. J., Akoh, C. C., Li, A., & Wang, Y. (2021). Modification of palm-based oil blend via interesterification: Physicochemical properties, crystallization behaviors and oxidative stabilities. *Food Chemistry*, 347. <https://doi.org/10.1016/j.foodchem.2021.129070>
- Zhang, Z., Lee, W. J., Xie, X., Ye, J., Tan, C. P., Lai, O. M., Li, A., & Wang, Y. (2021). Enzymatic Interesterification of Palm Stearin and Palm Olein Blend Catalyzed by sn-1,3-Specific Lipase: Interesterification Degree, Acyl Migration, and Physical Properties. *Journal of Agricultural and Food Chemistry*, 69(32), 9056–9066. <https://doi.org/10.1021/acs.jafc.0c06297>
- Zhang, Z., Xie, X., Lee, W. J., Zhao, G., Li, C., & Wang, Y. (2022). The effects of interesterification on the physicochemical properties of *Pangasius bocourti* oil and its fractions. *Food Chemistry*, 371. <https://doi.org/10.1016/j.foodchem.2021.131177>
- Zhang, L., & Zuo, S. (2024). The Significance of Lignocellulosic Raw Materials on the Pore Structure of Activated Carbons Prepared by Steam Activation. *Molecules*, 29(13). <https://doi.org/10.3390/molecules29133197>

- Zhang, H., Jiang, H., Yu, F., Zhang, H., Wu, Y., Shi, M., & Wang, C. (2026). Advances in lipase immobilization and applications in biofabrication. *Reaction Chemistry & Engineering*. <https://doi.org/10.1039/d5re00493d>
- Zhou, J., Lee, Y., Mao, Y., Wang, Y., & Zhang, Z. (2022). Future of Structured Lipids: Enzymatic Synthesis and Their New Applications in Food Systems. *Foods*, 11(16), 2400. <https://doi.org/10.3390/foods11162400>
- Zieniuk, B., Białecka-Florjańczyk, E., Wierzchowska, K., & Fabiszewska, A. (2021). Recent advances in the enzymatic synthesis of lipophilic antioxidant and antimicrobial compounds [Review of *Recent advances in the enzymatic synthesis of lipophilic antioxidant and antimicrobial compounds*]. *World Journal of Microbiology and Biotechnology*, 38(1). Springer Science+Business Media. <https://doi.org/10.1007/s11274-021-03200-5>

Valorization of Licuri Coconut Shell Waste into Functional Activated Carbon for Enzyme
Immobilization and Biocatalysis

*Manuscrito será submetido à revista Industrial crops and products (ISSN: 0926-6690)

Fator de impacto: 6.2

Valorization of Licuri Coconut Shell Waste into Functional Activated Carbon for Enzyme Immobilization and Biocatalysis

Highlights

- *Syagrus coronata* shell waste as a renewable precursor for high-performance activated carbon.
- RCCD optimization maximized biocatalytic performance under mild conditions.
- Fe³⁺ functionalization introduced specific sites but induced conformational constraints.
- Genipin crosslinking improved enzyme orientation and conformational flexibility.
- Licuri-derived residues show high potential for sustainable biocatalyst development.

ABSTRACT

Syagrus coronata shells, an abundant residue from licuri processing, were investigated as a lignocellulosic precursor to produce activated carbon tailored for enzyme immobilization. The material exhibited a high cellulose (35.78%) and hemicellulose (26.09%) content, low ash content (2.22%), and favorable composition for porous carbon development. Activated carbon synthesis was optimized using Response Surface Methodology, yielding optimal conditions at 107.3 min and an impregnation ratio of 2.53 g·g⁻¹. Under these conditions, high immobilization capacity (~493 mg·g⁻¹) and enzyme activity (~72 U) were achieved. The resulting carbon presented a high surface area (1082 m²·g⁻¹) and mesoporous structure (Dp ≈ 4.66 nm), suitable for lipase immobilization. Surface functionalization slightly reduced surface area (981-904 m²·g⁻¹) but preserved pore accessibility. Despite this reduction, catalytic activity was mainly determined by surface chemistry rather than textural properties. Genipin-functionalized carbon exhibited the highest activity recovery (~65%), greater than activated carbon and iron-functionalized samples (~50%), due to improved enzyme orientation and conformational flexibility. In contrast, Fe³⁺ incorporation increased surface polarity and induced conformational constraints, reducing activity despite introducing specific binding sites. Thermal analysis confirmed enhanced stability for genipin-modified carbon and reduced stability for iron-functionalized samples due to catalytic decomposition effects. These results demonstrate that controlled surface modification is a key role for optimizing enzyme-support interactions and highlight licuri processing residues as a high-value, sustainable feedstock for advanced biocatalytic materials.

Keywords: *syagrus coronata*; genipin functionalization; iron-assisted modification; lipase stabilization; circular bioeconomy.

1. Introduction

The licuri palm (*Syagrus coronata*) is a native species of the Caatinga biome in Brazil's semi-arid region and has attracted increasing attention as a renewable lignocellulosic resource. It is widely exploited for oil extraction, food products, and cosmetics in northeastern Brazil, contributing to the socioeconomic development of local communities (Leal et al., 2025). Reported fruit yields range from approximately 2000 to 4000 kg/ha/year, depending on environmental conditions and management practices, indicating its productive potential in semi-arid systems (Brito et al., 2020; Nascimento et al., 2024; Santos et al., 2020). Furthermore, market data indicate that whole fruits are sold at approximately US\$ 2.18/kg, whereas raw kernels can reach around US\$ 5.45/kg (Leal et al., 2025).

The increasing processing of licuri fruits generates considerable quantities of residues, particularly shells, which represent an abundant yet underexploited lignocellulosic resource. Despite their availability, these residues are still largely directed to low value uses such as fuel or handicrafts. However, their structural and chemical features, especially their high carbon content and lignocellulosic composition, make them suitable precursors to produce porous activated carbons with tailored surface properties (Peixoto et al., 2022). As a sustainable approach to valorizing this residue, the conversion of licuri shell waste into functional carbon materials represents a promising pathway for developing high-value applications, including use as supports for enzyme immobilization (Sousa et al., 2024).

Activated carbon (AC) is one of the most widely used carbonaceous materials due to its high surface area, porous structure, and tunable surface chemistry, characteristics that make it attractive for adsorption, catalysis, and enzyme immobilization (Adamian et al., 2021; Alagöz et al., 2020). In recent years, increasing attention has been directed toward the production of AC from renewable lignocellulosic residues, driven by the need to reduce environmental impacts and promote circular economy strategies. Agricultural by-products rich in carbon and low in commercial value have emerged as promising feedstocks for the development of sustainable activated carbons (Vajitha et al., 2023).

Among these residues, licuri coconut shell (*Syagrus coronata*) represents an attractive yet underexplored biomass source. Generated in large quantities during fruit processing in the Brazilian semi-arid region, this residue exhibits characteristics favorable for conversion into activated carbon, including high lignocellulosic content, structural rigidity, and carbon-rich composition. The transformation of licuri shell into activated carbon offers an opportunity to

simultaneously address waste management challenges and generate high-value materials for biotechnological applications.

In addition to textural properties, surface chemistry is a key propriety that determine the performance of activated carbons in advanced applications. Surface functionalization strategies have been widely employed to introduce targeted chemical groups, enabling controlled interactions at the carbon interface. Functionalization with organic crosslinkers such as genipin (Sousa et al., 2024) or with metal species, including iron ions (Li et al., 2022), has proven effective in tailoring surface polarity, reactivity and binding capacity. These modifications are particularly important for applications requiring stable interactions between carbon materials and biological or catalytic species (Doğan et al., 2024; Hong et al., 2021).

Enzyme immobilization represents a promising application of functionalized porous carbons, as it directly benefits from tailored surface chemistry, high surface area, and accessible pore structure. Moreover, the use of activated carbon as a support for enzyme immobilization represents a value-added strategy for biomass-derived materials, enabling the conversion of low-cost residues into functional biocatalytic systems with enhanced stability, reusability, and operational performance (Wahab et al., 2020). Previous studies have demonstrated that functionalized activated carbons can successfully host enzymes such as laccases, proteases, and lipases, highlighting the importance of surface properties in stabilizing bio-carbon interfaces (Adamian et al., 2021; Nascimento et al., 2024; Santos et al., 2022). Among the different classes of enzymes, lipases (EC 3.1.1.3) are frequently employed industrially due to their catalytic versatility in hydrolysis and transesterification reactions and their ability to operate under non-aqueous conditions (Nascimento et al., 2024; Zhang et al., 2022).

This study investigates the production and surface functionalization of activated carbon derived from licuri coconut shells, aiming to develop functional supports for enzyme immobilization and provide insights into the design of biomass-derived carbon materials for biocatalytic applications. To optimize the synthesis conditions of the activated carbon, an innovative approach was employed in which, instead of using conventional parameters such as textural properties, yield, or other physicochemical characteristics, enzymatic activity and lipase immobilization capacity were used as the primary response variables. This strategy enables the direct selection of materials with suitable performance for enzyme immobilization, while reducing analytical complexity and overall process costs. Lipase (EC 3.1.1.3) was selected because of its broad industrial application, catalytic versatility in hydrolysis and transesterification reactions, and its ability to operate under non-aqueous conditions (Cea et al.,

2019). The effects of genipin and iron-based functionalization were evaluated using comprehensive characterization.

2. Materials and methods

2.1. Materials and reagents

Licuri coconut (*Syagrus coronata*) shells were used as precursor material for the synthesis of activated carbon (AC). The shells were acquired in Capim Grosso, Bahia, Brazil (11°22'51" S, 40°00'46" W), through cooperative manufacturing of products based on the Licuri coconut. Lipase from *Candida rugosa* type VII (CAS number:9001-62-1) was purchased from Sigma-Aldrich Brazil. Phosphoric acid (85% P.A-ACS; CAS number: 7664-38-2) was obtained from Synth (Brazil). For the surface modification, genipin extract was prepared through the alcohol extraction of the unripe genipap (*Genipa americana* L.) as detailed by Santos et al., 2022).

2.2.Synthesis of activated carbons

2.2.1. Preparation and characterization of the precursor material

Licuri coconut shells were oven-dried at 105 °C for 24 h, ground using a knife mill, and sieved using a 20-mesh sieve, resulting in more uniform particles. The biomass was characterized for ash and moisture contents in accordance with AOAC guidelines (AOAC, 1995). Lignocellulosic components, including lignin, cellulose, and hemicellulose, were determined using the neutral detergent fiber (NDF) and acid detergent fiber (ADF) methodologies described by Soest et al., 1991. Functional groups were identified by Fourier Transform Infrared Spectroscopy (FTIR). The sample was analyzed directly using the attenuated total reflectance (ATR) technique in the 4000-600 cm⁻¹ infrared region with an Agilent Cary 630 FTIR spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA).

2.2.2. Activated carbon synthesis

Activated carbons were synthesized by the chemical activation method using phosphoric acid (H₃PO₄) following Santos et al., 2020. The precursor materials were impregnated with different concentrations of the activating agent and dried in an oven at 105 °C for 24 h. The carbonization step was performed in a muffle furnace under a nitrogen gas flow (50 mL/min) at a constant temperature of 550 °C, with varying times and impregnation ratios, according to the design shown in Table 1. The resulting materials were washed with hot water between 60 - 80 °C until they reached a pH of 7.0. The samples were then dried at 105 °C for 24 h and packaged in airtight containers.

2.2.3. Optimization of activated carbon synthesis

A rotatable central composite design (RCCD) was applied to optimize the synthesis parameters. Response surface methodology (RSM) was used to evaluate the effects of two independent variables: carbonization time (minutes) and the impregnation ratio (mass of activating agent to mass of precursor). The response variables analyzed were enzymatic activity (U) and immobilization capacity (mg/g^{-1}). A total of 12 experimental runs were conducted, including four factorial points, four axial points, and one central point, each performed in quadruplicate (Table 1). The results were fitted to a second-order polynomial model (Eq. 1).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 \quad (1)$$

Where β_0 to β_5 are regression coefficients estimated by least squares. The adequacy of the model was assessed using significance ($p < 0.05$), lack of fit ($p > 0.05$), and the coefficient of determination (R^2). All statistical analyses were performed using SAS Studio software.

2.3. Functionalization of the activated carbon

Activated carbon obtained under the optimized synthesis conditions was selected for surface modification to evaluate the effect of functional groups on lipase immobilization.

2.3.1. Modification with genipin

The surface modification with genipin was performed as follows. First, 10 g of activated carbon (AC) were mixed with an amine solution (2.5% v/v) containing 0.85 mL of ethylenediamine (99%; CAS: 107-15-3) and 32.5 mL of acetone (CAS: 67-64-1). The mixture was stirred manually for 10 min, and the AC was left to react for 24 h. In the second step, the amino-functionalized carbon was immersed in 33.5 mL of genipin extract and 33.5 mL of acetone (CAS: 67-64-1), stirred at 150 rpm for 30 min, and dried at 60 °C for 24 h. After solvent evaporation, the modified material was washed with distilled water to remove unbound compounds and dried again at 60 °C for 6 h (Santos et al., 2022). The final genipin-functionalized material (AC-GF) was sieved through a 40 mesh and stored in airtight containers.

2.3.2. Modification with iron salts and chelating

Surface functionalization with iron salts was carried out by co-precipitating Fe^{2+} and Fe^{3+} ions on the carbon surface, following (Mohan et al., 2011). Before metal deposition, the AC was treated with iminodiacetic acid (IDA) to enhance metal binding. Five grams of dried AC were mixed with 20 mL of $1 \text{ mol} \cdot \text{L}^{-1} \text{Na}_2\text{CO}_3$ under stirring for 1 h, then with 20 mL of 0.5

mol·L⁻¹ IDA (CAS: 142-73-4), adjusting the pH to 11 with 1 mol·L⁻¹ NaOH. The mixture was incubated at 40 °C for 14 h under shaking (20 rpm). The carbon was then washed with 1% acetic acid until the pH reached neutral.

After chelating insertion, the activated carbon was mixed with 250 mL of ultrapure water and stirred in a magnetic stirrer. The iron salts solution was prepared by mixing 200 mL of a solution containing 0.067 mol of trivalent iron (FeCl₃·6H₂O; CAS: 10025-77-1), 200 mL of a solution with 0.033 mol of bivalent iron (FeSO₄·7H₂O; CAS: 7782-63-0), and 2.5 mL of 37% v/v HCl. The ferrous sulfate and ferric chloride solutions were added to activated carbon and stirred vigorously for 15 min at 70 °C. After this period, 500 mL of a 4 mol·L⁻¹ sodium hydroxide (NaOH) solution was added to the initial solution to precipitate the particles, and the mixture was stirred magnetically for 2 h. The precipitate was collected in Falcon tubes, washed 10 times with 40 mL of ultrapure water, and centrifuged at 4000 rpm for 5 min. After washing, the magnetic activated carbon (AC-MF) was dried in an oven at 60 °C for 24 h.

2.4. Supports characterization

All supports were characterized for surface and structural properties. Nitrogen adsorption-desorption isotherms were used to determine the specific surface area and pore structure. Additional analyses included determination of the point of zero charge (pH_{PCZ}); Fourier Transform Infrared Absorption Spectrophotometry (FTIR); X-ray Diffraction (XRD); Scanning Electron Microscopy (SEM); and Energy-Dispersive X-ray (EDX).

Textural properties of the activated carbons were evaluated by nitrogen adsorption-desorption isotherms at 77 K. Prior to the analysis, the samples were degassed under vacuum to remove moisture and adsorbed gases. The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) method (Lippens, 1964), while the micropore area and volume were estimated by the t-plot method. Pore size distribution and mesopore volume were determined using the Barrett-Joyner-Halenda (BJH) model applied to the adsorption and desorption branches of the isotherms (Barrett et al., 1951)

The zero charge point of the supports was determined according to the methodology proposed by Kuśmierek et al. (2016). The functional groups were analyzed directly using the attenuated total reflectance (ATR) technique on an Agilent Cary 630 FTIR spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). The spectra were collected in the 4000–600 cm⁻¹ infrared region with a resolution of 4 cm⁻¹ and 64 scans. X-ray diffractograms were acquired using diffractometers (D2 PHASER, Bruker, Germany) equipped with a Cu K α monochromatic radiation source (λ = 1.54184 Å). The generator was set to 30 kV and 10 mA.

The crystallinity of the support was assessed at room temperature, with 2θ ranging from 10° to 90° and a scanning speed of $4^\circ\text{C}/\text{min}$.

The support morphology was examined using scanning electron microscopy (SEM). The samples were securely mounted on appropriate stubs and then coated with a thin layer of gold for enhanced conductivity. Afterward, the samples were analyzed with a QUANTA 250 SEM (FEI COMPANY, Waltham, MA, USA). Additionally, the samples' elemental composition was determined through an energy-dispersive X-ray fluorescence Spectrometer (EDX). This analysis was performed on sintered pellets utilizing an EDX-7200/800HS (SHIMADZU, EUA).

2.5. Lipase immobilization on carbon supports

2.5.1. Immobilization on activated carbons from experimental design

Lipase immobilization was employed as a probe technique to evaluate the surface properties and functionalization efficiency of the activated carbons. The materials produced according to the RCCD were used as supports for lipase immobilization via physical adsorption, following the method described by Nascimento et al., 2024. *Candida rugosa* lipase type VII (Sigma-Aldrich; CAS number: 9001-62-1) solution with a concentration of $10\text{ mg}\cdot\text{mL}^{-1}$, prepared in $0.1\text{ mol}\cdot\text{L}^{-1}$ sodium acetate buffer at pH 5.0, was added to tubes containing 0.1 g of each support material. The tubes were maintained under constant agitation (20 rpm) in an orbital shaker in a BOD incubator chamber for 2 h at 30°C . After incubation, the samples were centrifuged at 3500 rpm for 5 min, and the supernatant was collected for protein quantification using the Bradford method (Bradford, 1976). Successive washing steps were performed to remove weakly adsorbed enzyme molecules until no detectable protein was observed in the supernatant, indicating adsorption equilibrium. The immobilized systems were stored at 4°C for further analyses.

The immobilization capacity of each carbon was determined as the difference between the initial and final protein concentrations using Eq. (2).

$$C_{Im} = \frac{V(C_{in}-C)}{m_s} \quad (2)$$

where: C_{Im} is the immobilization capacity ($\text{mg}\cdot\text{g}^{-1}$); V is the volume of solution (mL); C_{in} is the initial concentration of the solution ($\text{mg}\cdot\text{L}^{-1}$); C is the concentration of solution at equilibrium ($\text{mg}\cdot\text{L}^{-1}$); m_s is the mass of the support (g).

2.5.2. Immobilization on functionalized supports under optimal conditions

The supports produced under the optimized conditions and subsequently functionalized with genipin (AC-GP) or iron salts (AC-FC) were also used for lipase immobilization under the same conditions as described in Section 2.5.2. The resulting enzymatic derivatives were stored at 4°C for subsequent analyses.

2.5.3. Lipase activity assay

The catalytic activity of the immobilized systems was evaluated to assess whether surface modification preserved the functional integrity of the adsorbed enzyme. Hydrolysis was conducted using the olive oil emulsion method described by Brito et al. (2020) substrate was prepared by mixing 5 mL of olive oil with 3.75 g of 3% gum arabic under stirring until a homogeneous solution was achieved. Next, 5 mL of sodium phosphate buffer (0.1 mol·L⁻¹, pH 8.0) and the derivative or 1 mL of lipase solution were added to 125 mL erlenmeyer flasks. The samples were incubated in a shaker bath at 37 °C for 10 min, stirring at 150 rpm. The reaction was halted by adding 10 mL of 92.5% ethanol to the flasks. The liberated fatty acids were then titrated with 30 mM NaOH until reaching a pH of 11. Enzyme activity was calculated according to Eq. 3, where one unit of activity is defined as the amount of enzyme that releases 1 μmol of fatty acid per minute under the specified assay conditions.

$$U \left(\frac{\mu\text{mol}}{\text{min}} \right) = \frac{(V_a - V_b) \cdot M \cdot 10^3}{t} \quad (3)$$

where V_a is the NaOH volume used in the sample titration (mL), V_b is the NaOH volume used in the control titration (mL), M is the molar concentration of NaOH solution, and t is the reaction time (min).

2.6. Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation. The results were analyzed using analysis of variance (ANOVA), and significant differences between means were evaluated using Tukey's test ($p < 0.05$). Statistical analyses were performed using SAS Studio software, and graphs were prepared using OriginPro 2018.

3. Results and discussion

3.1 Lignocellulosic Composition and Ash Content of Licuri Shells

The lignocellulosic composition of the licuri coconut shell is an important property that influences the quality and performance of the activated carbon. The shell presents a high content of cellulose ($35.78 \pm 1.09\%$) and hemicellulose ($26.09 \pm 0.83\%$), with a moderate lignin content ($14.80 \pm 1.55\%$). These components directly affect the thermal degradation behavior and the development of porosity during carbonization (Mishra et al., 2024). Cellulose and

hemicellulose decompose at lower temperatures, generating volatile compounds and promoting the formation of initial pore structures. This thermal decomposition process creates a porous matrix by releasing gases such as CO, CO₂, and light hydrocarbons. In contrast, lignin decomposes over a wider temperature range and contributes to the formation of more thermally stable aromatic structures, which are essential for maintaining the integrity of the carbon matrix (Nascimento et al., 2024; Lu & Gu, 2022; Zhang et al., 2022; Byrne et al., 2017). The low ash content (2.22±0.24%) indicates minimal inorganic waste, which is desirable for activated carbon synthesis (Mishra et al., 2024). The combination of high holocellulose content (cellulose + hemicellulose) and sufficient lignin fraction favors the development of a hierarchical porous structure, where the volatilization of polysaccharides creates pores, while lignin-derived carbon stabilizes the structure, preventing collapse during activation. This balance is particularly advantageous for producing activated carbons with high surface area and well-developed porosity (Neme et al., 2022).

3.2 Optimization of Activated Carbon Synthesis

The synthesis of activated carbon (AC) was optimized using a Rotatable Central Composite Design (RCCD) within Response Surface Methodology (RSM), enabling evaluation of the combined effects of process variables on the responses via a second-order polynomial model. The optimization strategy was selected based on biocatalytic performance rather than on textural properties alone. Thus, the main objective was to enhance lipase immobilization capacity and catalytic activity, recognizing that high surface area alone does not necessarily ensure effective enzyme-support interactions. The independent variables investigated were carbonization time and impregnation ratio (the ratio of the activating agent to the precursor mass). Table 1 summarizes the experimental conditions and the responses obtained.

Statistical optimization utilizing RCCD is widely acknowledged for its effectiveness in identifying optimal process conditions with fewer experiments. For example, Borges et al. (2023) successfully employed RCCD to establish the ideal synthesis parameters for hydrochar derived from corn cob. Their findings indicated that both carbonization temperature and time significantly impact the formation of oxygen functional groups (OFGs), which play an important role in enzyme immobilization. Another study (Nascimento et al., 2024) found that specific conditions produced activated carbon with enhanced structural properties, including increased porosity and improved enzyme-support interactions.

Table 1. Immobilization capacity and enzyme activity (U) of lipase activated carbons synthesized under the conditions defined by the Rotatable Central Composite Design (RCCD).

Assay	(X ₁) ^a x ₁ ^b	(X ₂) ^a x ₂ ^b	Immobilization capacity (mg.g ⁻¹)	Enzyme activity (U)
1	(-1) 52.00	(-1) 0.90	468.89±4.32	9.10±1.65
2	(-1) 52.00	(+1) 2.60	486.48±2.72	31.50±1.56
3	(+1) 158.00	(+1) 2.60	487.52±1.28	39.50±5.82
4	(+1) 158.00	(-1) 0.90	472.32±2.66	18.10±1.88
5	(-1.41) 30.00	(0) 1.75	473.14±3.94	13.10±2.62
6	(+1.41)180.00	(0) 1.75	475.14±2.22	13.40±1.65
7	(0) 105.00	(-1.41) 0.50	467.47±1.31	20.60±0.62
8	(0) 105.00	(+1.41) 3.00	486.74±5.65	72.30±9.83
9	(0) 105.00	(0) 1.75	493.13±0.86	72.50±6.33
10	(0) 105.00	(0) 1.75	492.56±1.09	58.00±3.30
11	(0) 105.00	(0) 1.75	490.91±0.76	56.30±5.27
12	(0) 105.00	(0) 1.75	493.86±0.54	57.30±2.16

^a Coded values.

^b Uncoded values of: x₁ = time (min) and x₂ = impregnation ratio (%w/w).

In this study, the polynomial model and variables obtained from the RCCD data were evaluated using analysis of variance (ANOVA) and tested for significance with the *F*-test at the 5% significance level. The results demonstrated a strong correlation between the response variables, namely immobilization capacity and enzyme activity, as evidenced by the statistical significance of the models ($p < 0.0001$) and high coefficients of determination (R^2), with R^2 values of 0.92 and 0.90, respectively. These values indicate a strong agreement between the experimental data and the fitted quadratic models. The estimated parameters of the regression model are presented in Table 2.

Table 2. Parameters of the quadratic model were estimated for enzyme activity and immobilization capacity. X₁ time (min) and X₂ impregnation ratio (%w/w).

Variables	Immobilization capacity		Enzyme activity	
	Parameter estimate	p- Value	Parameter estimate	p-Value
Intercept	492.615	<0.0001	61.025	<0.0001
X ₁	0.963	<0.0001	2.178	<0.0001
X ₂	7.505	<0.0001	14.614	<0.0001
X ₁ ²	-8.385	<0.0001	-25.213	<0.0001
X ₂ ²	-6.978	<0.0001	-8.612	<0.0001

The statistical analysis showed a significant quadratic effect of carbonization time and impregnation ratio on the response variables of enzymatic activity and immobilization capacity. The regression equation for the uncoded variables, as described in Eqs. 4 and 5, was also determined for use in response surface methodology (RSM) (Figs. 1 and 2), validating the models and identifying the optimal region for AC production.

$$IC = 413.66 + 0.66t + 40.56r - 0.003t^2 - 9.13r^2 \quad (4)$$

$$U = -107.18 + 1.96t + 55.25r - 0.01t^2 - 10.90r^2 \quad (5)$$

Where IC ($\text{mg} \cdot \text{g}^{-1}$) is immobilization capacity; U (U) is enzyme activity; t (min) is carbonization time, and r (w/w%) is the impregnation ratio

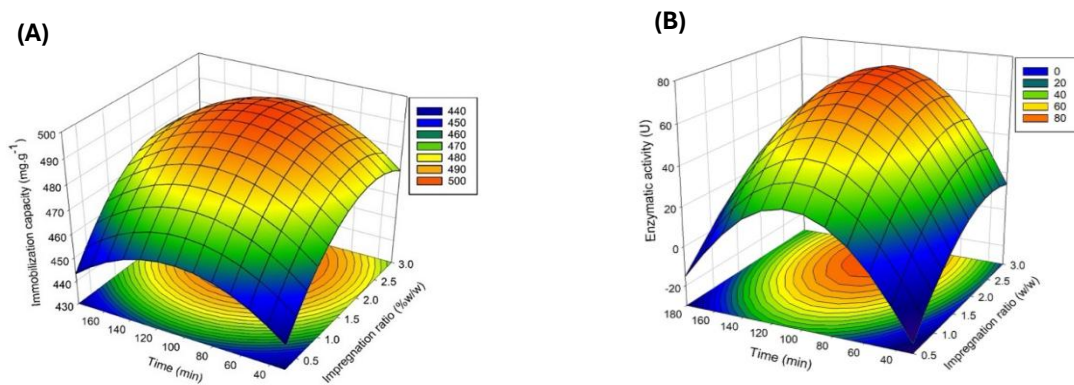


Figure 1. Response surface with optimization of activated carbon production for better immobilization condition (A) and (B) response surface with optimization of activated carbon production for better recovery condition of enzyme activity.

Based on the decoded model equations and response surface analysis, the optimal conditions for AC synthesis were identified as 107.3 min and an impregnation ratio of 2.53 $\text{g} \cdot \text{g}^{-1}$, maximizing immobilization capacity (IC) and enzyme activity (U). Under these conditions, the material exhibited its highest biocatalytic performance, highlighting the importance of statistically based process optimization. Similarly, activation times close to 2 h have been widely reported as optimal for achieving high surface area without compromising carbon yield (Bamerdhah et al., 2025; Neme et al., 2022). For example, Nazem et al. (2020) reported optimal conditions for phosphoric acid activation of agricultural residues using impregnation ratios between 1.5 and 3.0 $\text{g} \cdot \text{g}^{-1}$ and activation times of 60–120 min, resulting in high surface areas ($>1000 \text{ m}^2/\text{g}$) and well-developed mesoporosity. Additionally, the obtained

pore diameter is particularly suitable for lipase immobilization, as *Candida rugosa* has molecular dimensions of 3-5 nm. Mesopores in this range facilitate enzyme diffusion and anchoring while minimizing steric hindrance that may limit catalytic activity (Li et al., 2025; Ramlee et al., 2022).

3.3 Textural and surface properties of optimized and functional carbons

The activated carbon employed in the immobilization assays was produced under the optimal conditions determined by response surface methodology. Unlike studies that optimize activated carbon based primarily on textural properties, the present work adopted enzymatic activity as the response variable. This approach was based on the understanding that catalytic performance depends not only on surface area but also on enzyme-support interactions, preservation of enzyme conformation, and accessibility of active sites (Lim & Jo, 2025; Robescu & Bavaro, 2025). The textural properties of the activated carbon and its functionalized derivatives are summarized in Table 3. The non-functionalized activated carbon (AC) exhibited a high surface area, with a predominance of mesopores and an average pore diameter of 4.66 nm, indicating a well-developed porous network suitable for enzyme immobilization. The non-functionalized activated carbon (AC) exhibited a high surface area, with a predominance of mesopores and a pore diameter of 4.66 nm, indicating a well-developed porous network suitable for enzyme immobilization (Caldas et al., 2017). This structural characteristic is directly related to the lignocellulosic composition of the precursor. The high content of cellulose and hemicellulose promotes the formation of mesopores via thermal decomposition, while the presence of lignin enhances structural stability during activation, thereby preserving the pore architecture (Feng et al., 2024; Toor et al., 2026; Zhang & Zuo, 2024).

Table 3. Textural properties of activated carbons

Sample	Surface area (m ² .g ⁻¹)	Mesoporos volume (cm ³ .g ⁻¹)	Microporos volume (cm ³ .g ⁻¹)	Pore diameter D _p (nm) ^a
AC	1082	1.260	0.104	4.659
AC-GF	981	1.100	0.074	4.487
AC-MF	904	0.805	0.078	3.563

^a Maximum pore size distribution

Compared to other lignocellulosic residues such as rice husk or sugarcane bagasse, which often present higher ash content or lower structural stability, licuri coconut shell offers a

favorable balance between porosity development and carbon framework stability, making it a promising precursor for enzyme immobilization applications (Barakat et al., 2024; Dhashnamoorthy et al., 2026; Ortega-Ortiz et al., 2026). This pore diameter range is suitable for lipase immobilization, as *Candida rugosa* has a molecular dimension of approximately 2-5 nm (Menezes et al., 2021). Mesopores in this range facilitate enzyme diffusion and anchoring while minimizing steric hindrance that may limit catalytic activity (Pota et al., 2021).

Surface functionalization led to a slight reduction in surface area and pore volume, with the iron-functionalized material (AC-MF) showing a slightly greater reduction than the genipin-functionalized (AC-GF). This behavior is attributed to partial pore filling and surface coverage by functional groups and metallic species, a phenomenon widely reported for chemically modified activated carbons (Aziz et al., 2026; Zhang & Zuo, 2024). Despite the reduction in surface area, the average pore diameters remained within the mesoporous range of 3-5 nm, preserving size compatibility with lipase molecules. These results reinforce that a slight reduction in surface area does not necessarily impair catalytic performance when pore accessibility and surface chemistry are suitably tailored.

3.4 Effect of surface functionalization on carbon surface chemistry

The yield of activated carbon is a key indicator of the efficiency of biomass-to-carbon conversion and is strongly influenced by both precursor composition and activation conditions. Under optimized chemical activation conditions, the activated carbon derived from licuri coconut shells achieved an approximately 78% yield. This relatively high yield can be attributed to the combined effect of the activation conditions and the intrinsic composition of the biomass, particularly the balance between holocellulose and lignin fractions. While lignin contributes to the formation of thermally stable carbon structures, the presence of cellulose and hemicellulose also helps maintain structural integrity during controlled activation processes. Reported yields for lignocellulosic precursors typically range from 26.1% to 85%, depending on precursor composition and activation strategy (Neme et al., 2022). In this context, the yield obtained in this study is comparable to, or higher than, values reported for other lignin-rich materials chemically activated with phosphoric acid, reinforcing the suitability of licuri shells as an efficient and sustainable precursor for activated carbon production (Neme et al., 2022).

Mishra et al. (2024) highlighted the importance of achieving high yields in the conversion of lignocellulosic biomass into activated carbon (AC) for environmental and energy-related applications. Januszewicz et al. (2023) reported that coconut shells yield around 75% when chemically activated with phosphoric acid at concentrations of 40-50 wt%, in agreement with

the results obtained in the present work. Phosphoric acid is known to promote dehydration and crosslinking reactions within lignocellulosic matrices, particularly in lignin-rich precursors, resulting in high carbon yields and well-developed porous structures (Ivanichok et al., 2023; Zhang et al., 2022).

The point of zero charge (pHpzc) is an important parameter for carbon materials, indicating the pH at which the surface has no net electrical charge. This parameter significantly influences how the carbon interacts with biomolecules, such as enzymes, during immobilization processes (Silva et al., 2021). The pHpzc of the activated carbon (AC) was 5.32 (Figure 2), and after functionalization with genipin, the value remained practically unchanged (5.30), indicating that the treatment did not significantly alter the surface acidity of the material. In comparison, the iron-functionalized activated carbon (AC-FC) exhibited a more neutral pHpzc value (6.37), which can be attributed to the functionalization steps carried out under basic conditions, mainly during the precipitation with NaOH. Since lipase immobilization was carried out in acetate buffer at pH 5.0, close to both the pHpzc of AC and the isoelectric point ($pI \approx 4.6-5.0$) of *Candida rugosa* lipase, electrostatic interactions are expected to be minimized for AC and AC-GP, favoring adsorption driven by hydrophobic interactions. In contrast, the more positively charged surface of AC-FC under these conditions may enhance electrostatic contributions, altering enzyme-support interactions (Muronetz et al., 2022; Vallejo-García et al., 2024).

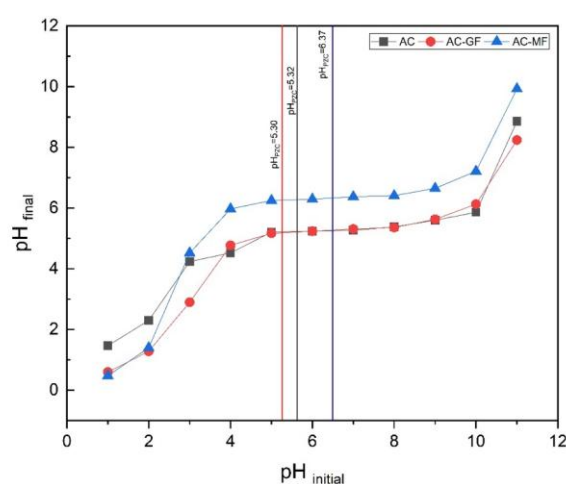


Figure 2. Point of zero charge of activated carbon (AC), functionalized with genipin (AC-GF), and activated carbon functionalized with iron particles and chelator agent (AC-MF).

Similar behavior was reported by Alagöz et al. (2020) for enzyme immobilization on supports functionalized with inorganic particles, highlighting how surface modifications can tailor support-enzyme interactions for specific biotechnological applications. Furthermore, understanding and controlling the pHpzc plays an important role in improving the performance of activated carbons, particularly in biocatalysis (Gonçalves et al., 2021) and adsorption processes (Feitoza et al., 2022; Pimentel et al., 2023). Adjusting the surface charge can enhance enzyme-support interactions, improving immobilization efficiency and catalytic activity (Muronetz et al., 2022; Zhao, 2023).

The FTIR spectra of the supports are shown in Figure 3. The observed bands indicate functional groups and chemical modifications resulting from activation and functionalization.

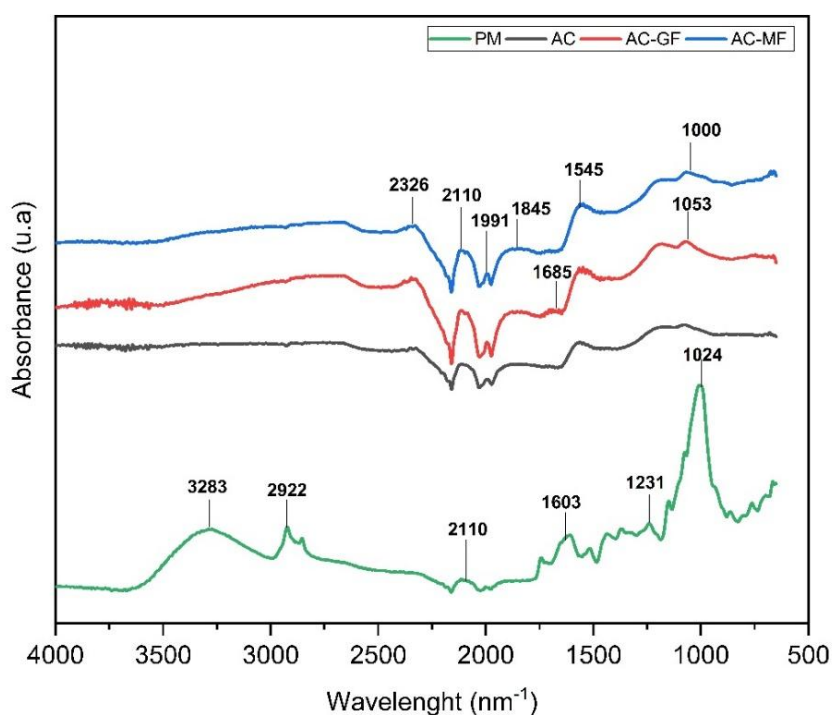


Figure 3. FTIR of the shell of the licuri coconut, activated carbon (AC), activated carbon functionalized with genipin (AC-GF), and activated carbon functionalized with iron ion particles and chelator (AC-MF).

In the analysis of the precursor material (PM), a broad band around 3283 cm^{-1} was observed, corresponding to the axial vibration of -OH groups. These groups are commonly found in lignocellulosic materials due to the presence of cellulose, hemicellulose, and lignin

(Mtibe et al., 2022). Additionally, the band at 2920 cm^{-1} is attributed to C–H stretching of methyl and methylene groups and shows a slight separation into two components, a spectral characteristic frequently observed in plant-derived organic structures (Salim et al., 2021; Taheri-Shakib et al., 2024). The bands at 1603 , 1231 , and 1024 cm^{-1} are assigned to vibrations of carbonyl groups (C=O), aromatic C=C structures, and glycosidic bonds (C–O–C) present in cellulose and hemicellulose, confirming the lignocellulosic nature of the residue (Bichang'a et al., 2024; Olabisi et al., 2023).

After activation, the activated carbon (AC) exhibited a decrease in the intensity of the bands associated with -OH groups (3340 cm^{-1}) and C-H stretching (2920 cm^{-1}). This behavior indicates thermal degradation and the release of volatile components, particularly cellulose and hemicellulose, which decompose during pyrolysis. These changes demonstrate the effectiveness of the thermal treatment in promoting the development of a more aromatic and less functionalized carbonaceous matrix. The FTIR spectra showed bands associated with oxygen-containing functional groups, characteristic of lignocellulosic precursors, that were partially preserved after carbonization. Bands observed in the range of 1845 – 1545 cm^{-1} are assigned to C=O and C=C stretching vibrations commonly found in activated carbons. The increased intensity of these bands after activation suggests an enrichment of oxygenated surface functionalities rather than their exclusive formation during chemical treatment.

In the spectrum of activated carbon functionalized with genipin (AC-GF), a discrete band is observed around 1685 cm^{-1} , indicating the formation of conjugated bonds such as C=N or C=O, resulting from reactions between genipin and amino groups on the carbon surface. A band at 1545 cm^{-1} present across all materials analyzed can be attributed to the C=C stretching vibration of aromatic rings, commonly observed in carbonaceous materials such as activated carbon and biochar, as well as in partially carbonized lignocellulosic structures. Furthermore, the band at 1053 cm^{-1} in AC-GF is more clearly defined and is associated with C-O stretching, typically found in ethers and other oxygenated groups. This enhancement may be associated with the crosslinking action of genipin and the introduction of new oxygenated groups. These results suggest modifications to the carbonaceous surface that align with existing literature on the functionalizing lignocellulosic precursors using genipin (Santos et al., 2022).

Minor spectral changes were observed for the metalized activated carbon (AC-MF) compared to the non-functionalized material. Specifically, a slight shift and increased definition of the band at 1545 cm^{-1} may be associated with interactions between iron species and functional groups introduced by the chelating agent used during metallization. (Pryshchepa

et al., 2022). Although no new distinct vibrational modes were observed, the overall increase in band definition in the region of 1800–1000 cm^{-1} , compared with AC, may reflect changes in surface chemistry due to the functionalization process. These results suggest that incorporating Fe^{3+} ions and chelating agents altered the chemical environment of the carbon surface, potentially increasing its capacity to interact with biomolecules. Nevertheless, additional characterization techniques would be necessary to confirm the specific nature of these interactions (Boukhvalov et al., 2022).

SEM micrographs (Figure 4) show clear morphological differences among the evaluated supports. In Figure 4A, the activated carbon exhibited a heterogeneous surface with well-developed pores, characteristic of chemically activated lignocellulosic materials (Ali et al., 2025; Attaurrazaq et al., 2025). After functionalization with genipin (AC-GF), a slight change in surface texture was observed, accompanied by the appearance of superficial structures consistent with the modification detected by FTIR analysis. Although SEM does not provide direct chemical identification, these morphological alterations suggest that the functionalization procedure affected the surface organization of the carbon (Qiu et al., 2020; Santos et al., 2022). For AC-MF (Figure 4C), dense spherical aggregates were observed on the support surface, indicating morphological features compatible with the presence of iron-containing phases, which were later corroborated by EDX and XRD analyses (Chaudhari et al., 2024). These structures may act as active sites for lipase anchoring, as reported in previous studies (Chaudhari et al., 2024; Suo et al., 2019). These modifications may influence enzyme-support interactions by creating new anchoring sites and altering local surface properties. Overall, the SEM observations demonstrate that both functionalization strategies modified the carbon surface morphology, potentially contributing to the distinct immobilization behaviors observed for the evaluated supports.

Energy-dispersive X-ray spectroscopy (EDX) was used to investigate the elemental composition of the supports (Figure 5). EDX is a semi-quantitative technique that detects inorganic elements with atomic numbers greater than 3, including metals and non-metals such as phosphorus and sulfur (Agrawal et al., 2024; Garrido-García et al., 2024). Due to their detection limits, elements such as hydrogen are often underrepresented or undetected (Adeniyi et al., 2021; Agrawal et al., 2024). Therefore, while EDX spectra provide insights into the surface's inorganic composition, they do not comprehensively reflect the presence of light organic elements, which is particularly relevant for carbon-based materials (Faber et al., 2021; Kwon et al., 2023).

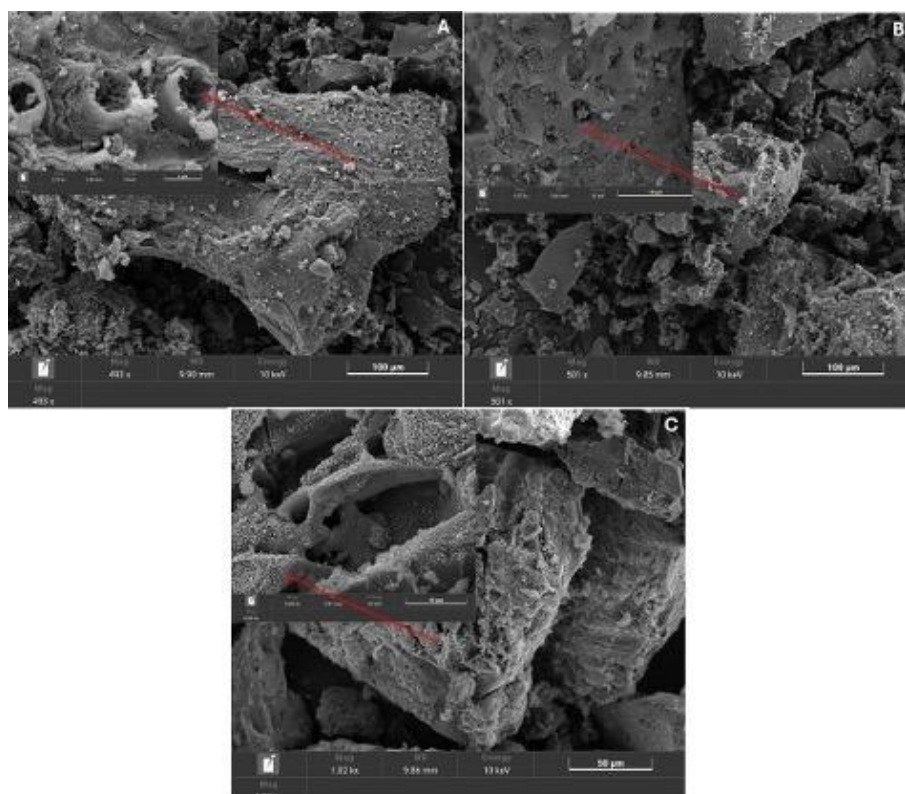


Figure 4. SEM images of AC (493x;9.79kx) (A), AC-GF (501x;9.07kx) (B) and AC-MF (1.02kx; 6.89kx) (C).

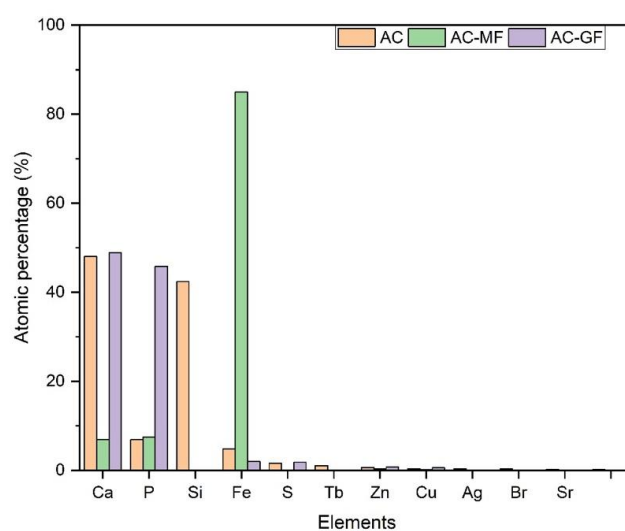


Figure 5. Elemental composition of activated carbon unfunctionalized (AC); functionalized with genipin (AC-GF) and functionalized with iron and chelating agent (AC-MF) by EDX analysis.

The activated carbon (AC) exhibited high levels of calcium (48.02%) and silicon (42.38%). These elements are likely derived from the intrinsic mineral content of the licuri biomass (Peixoto et al., 2022).

The sample functionalized with genipin (AC-GF) maintained a similar profile to the AC regarding calcium content, suggesting that the functionalization did not remove these minerals. Low concentrations of zinc (0.77%) and copper (0.58%) were detected. Although these elements were not intentionally introduced during the process, they may originate from trace contaminants or from impurities present in the reagents used (ElNasri et al., 2022).

In contrast, the carbon functionalized with iron in the presence of a chelating agent (AC-MF) showed a sharp reduction in calcium and phosphorus levels and a pronounced increase in iron content (84.96%), indicating effective metallization of the carbon matrix. This behavior is in accordance with the use of chelating agents during functionalization, which enhances the binding of metal ions to the support surface, leading to increased metal incorporation as detected by Energy-Dispersive Spectroscopy (EDS).

The EDX results demonstrate that functionalization strategies distinctly influence the inorganic surface composition of activated carbon. While genipin leads to mild surface modification without altering the native mineral profile, the iron-chelating system promotes high incorporation of metal species. Additionally, detecting elements such as strontium, zinc, and copper across all samples may reflect residual components intrinsic to the original biomass or incidental sources introduced during processing (Şen et al., 2023).

The structural characterization of the activated and functionalized carbon was conducted using X-ray diffraction (XRD) to verify its amorphous nature and detect any crystalline structures formed during functionalization. Figure 6 shows the diffractograms obtained for activated carbon (AC) and activated carbon functionalized with iron and a chelating agent (AC-MF). The AC-GF sample was not analyzed by XRD because genipin functionalization primarily introduces organic surface modifications rather than significantly altering the carbon material's bulk crystalline structure.

All analyzed materials exhibited a diffractometric pattern typical of amorphous carbon structures, characterized by a broad diffuse halo in the regions of 2θ 20°-30° and 40°-50°. This pattern indicates the presence of disordered graphitic domains, a behavior commonly observed in activated carbons derived from lignocellulosic biomasses (Antil et al., 2025). No distinct crystalline peaks were observed in the analyzed range, confirming the predominantly amorphous nature of both materials. The absence of sharp reflections in the region of 2θ 20°-

30 ° further indicates that no significant crystalline phases, such as silica or metal oxides, were formed or retained at detectable levels in the samples (Habte et al., 2025).

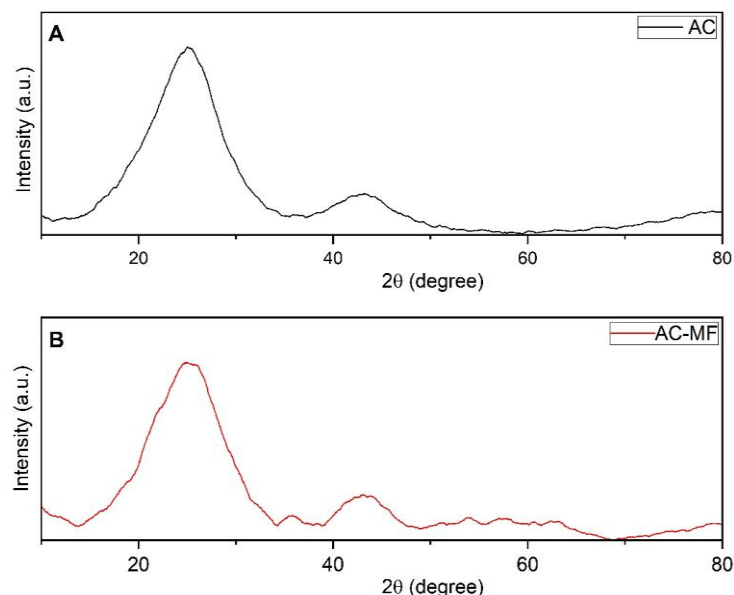


Figure 6. Diffractogram of activated carbon (AC) (A) and activated carbons modified with iron ion particles and chelator (AC-MF) (B).

The iron-functionalized sample (AC-MF) exhibited changes in the diffractometric profile, with the appearance of more defined features at approximately 2θ values of 35°, 50°, and 62°, suggesting a tendency toward the formation of crystalline domains. These signals can be attributed to iron oxide phases such as hematite (Fe_2O_3) and magnetite (Fe_3O_4), as reported in the literature (Fasfous et al., 2021). This behavior is consistent with the partial preservation of the activated carbon's amorphous structure after metallization, thereby confirming the successful incorporation of iron species via the chelating-assisted functionalization route.

XRD analysis was not performed for the genipin-functionalized sample (AC-GF) because the expected structural modifications are predominantly organic, poorly crystalline, and unlikely to produce distinct diffraction signals detectable by conventional X-ray diffraction. Therefore, complementary techniques such as FTIR were considered more appropriate for evaluating the effects of genipin functionalization.

The thermal analysis conducted using thermogravimetry (TG), derivative thermogravimetry (DTG), and differential thermal analysis (DTA), was used to investigate the thermal stability and decomposition behavior of the activated carbon. Figure 7 presents the

thermal curves, which provide important insights into physicochemical changes during heating (Islam et al., 2025; Sari et al., 2025).

All materials exhibited an initial mass loss between 50 and 150 °C, associated with the loss of physically adsorbed water and low-molecular-weight volatile compounds, characteristic of carbonaceous materials derived from lignocellulosic biomass (Sklepova et al., 2022). The main degradation stage observed between 300 and 600 °C in AC and AC-GF corresponds to the decomposition of the carbon matrix and the breakdown of oxygenated surface functional groups, such as carboxylic acids and phenolics (Olivier et al., 2022). For the AC-MF sample, the thermogravimetric profile shows two main mass-loss events occurring within distinct temperature ranges. The first event, observed slightly above 300 °C, is associated with the decomposition of less stable carbon structures and surface functional groups. The second event, occurring around 400 °C, exhibited lower intensity, suggesting the presence of more thermally stable structures that require more energy for degradation. These ions may accelerate carbon bond cleavage, particularly in the presence of a chelating agent such as IDA, which enhances metal dispersion and interaction with the matrix (Hua et al., 2023).

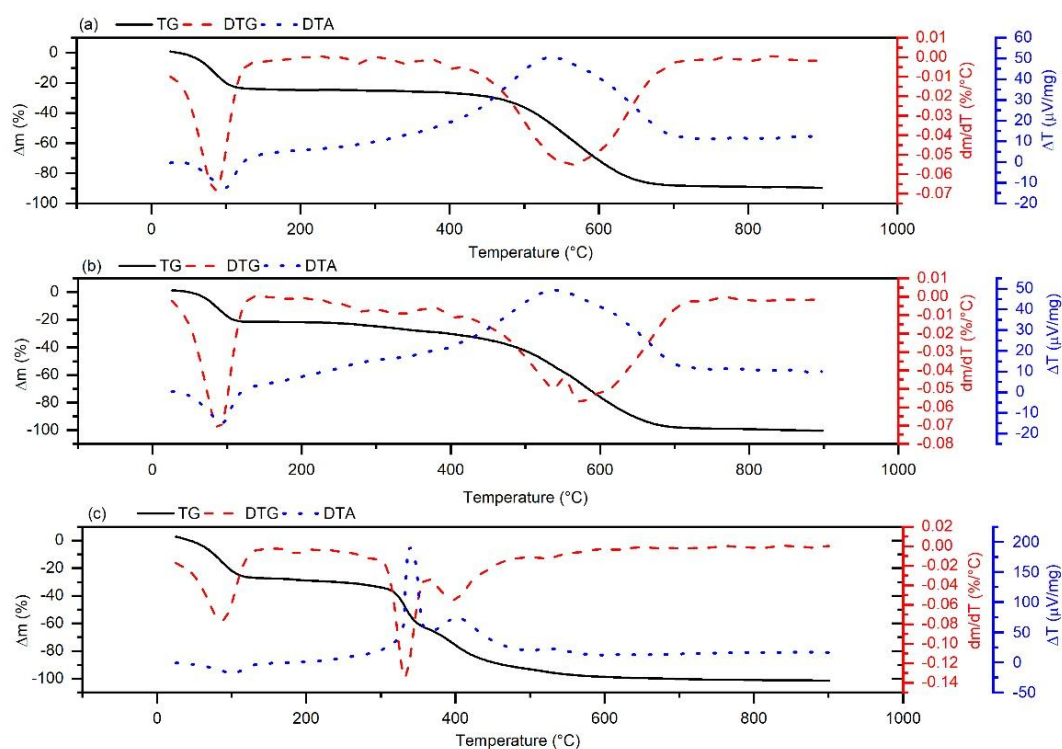


Figure 7. Thermogravimetric (TG), differential thermal (DTA), and derivative thermogravimetric (DTG) curves of unfunctionalized activated carbon (AC) (a), genipin-functionalized carbon (AC-GF) (b), and iron/chelating agent-functionalized carbon (AC-MF) (c).

Compared to the non-functionalized carbon, AC-MF exhibited a shift of the degradation events toward slightly lower temperatures, indicating a reduction in thermal stability. This behavior may be related to the presence of iron species, which can influence heat transfer and promote localized catalytic effects on carbon bond cleavage. However, the presence of two distinct degradation steps suggests heterogeneous interactions between iron ions and the carbon matrix, resulting in regions with different thermal resistances rather than a uniform catalytic degradation effect (Sumathirathne & Euler, 2021).

On the other hand, the genipin-functionalized carbon (AC-GF) demonstrated greater thermal resistance, with a slower decomposition rate during the main degradation stage (350–500 °C). This improved stability may be attributed to crosslinking reactions between genipin and surface functional groups, resulting in a more thermally stable carbon network (Makar et al., 2023). Similar results have been reported for thermally modified biopolymers crosslinked with genipin (Kwiatos et al., 2024).

The DTA analysis corroborated the TG and DTG results, indicating thermal events consistent with the observed mass-loss behavior. The DTA curves (Figure 7C) exhibited endothermic events between approximately 50 and 160 °C, corresponding to moisture removal, with AC-MF showing the most pronounced peak in this region, indicating greater water retention. At temperatures above 300 °C, exothermic events became evident for all samples and were associated with carbon matrix decomposition. In particular, the lower intensity of the exothermic peak observed for AC-MF between 350 and 600 °C suggests that the presence of iron-containing phases influenced the degradation process, reducing the energy required for thermal decomposition while maintaining a similar decomposition temperature range (Li et al., 2025; Schmitt et al., 2024). The occurrence of two exothermic events in both DTG and DTA profiles reinforces the existence of carbon domains with different thermal stabilities, which may be attributed to variations in structural organization and to distinct interactions between iron ions and surface functional groups. In contrast, the genipin-functionalized carbon (AC-GF) exhibited a slower decomposition rate in the main degradation region (350–500 °C), indicating improved thermal stability (Makar et al., 2023). This behavior can be attributed to crosslinking reactions promoted by genipin, which lead to a more rigid and thermally resistant carbon network. Similar effects have been reported for genipin-modified materials, in which increased structural integrity leads to enhanced resistance to thermal degradation (Sousa et al., 2024; Wahba, 2024).

3.5 Immobilization of lipase and activity assay

Enzyme immobilization is a well-established strategy for improving catalyst stability, reusability, and operational control in industrial biocatalytic (Wahab et al., 2020). Figure 8 presents the immobilization efficiency (IE) and relative activity recovery (RA) of *Candida rugosa* lipase immobilized on activated carbon (AC) and on surface-modified carbons (AC-MF and AC-GP). Although all supports exhibited high immobilization efficiencies (>85%), marked differences in activity recovery were observed, indicating that catalytic performance was influenced not only by enzyme loading but also by the physicochemical characteristics of the support (Wahab et al., 2020).

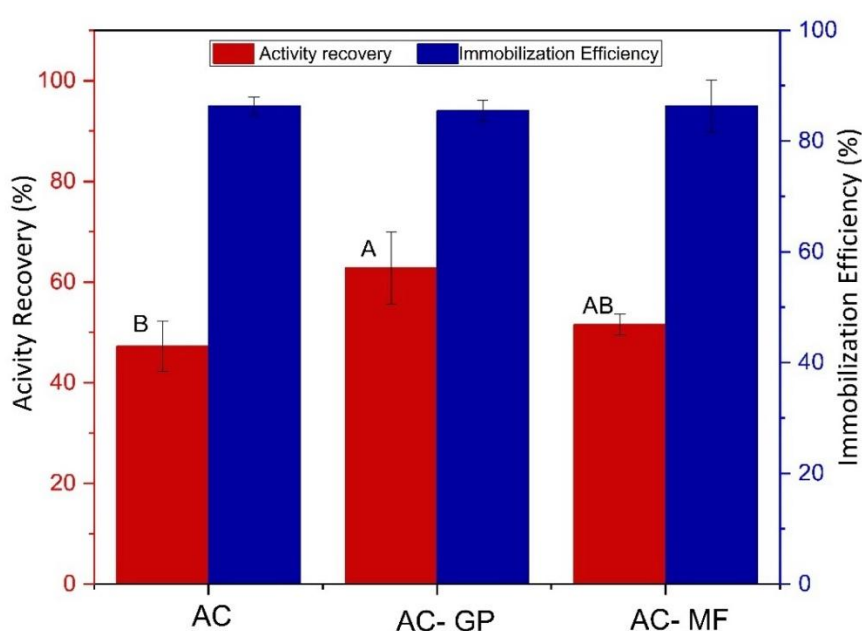


Figure 8. Immobilization efficiency (IE) and relative activity recovery (RA) of *Candida rugosa* lipase immobilized on different supports: activated carbon (AC), genipin-functionalized activated carbon (AC-GP), and iron-chelated activated carbon with iminodiacetic acid (AC-MF). Different uppercase letters indicate statistically significant differences ($p < 0.05$) in RA according to Tukey's test.

The non-functionalized activated carbon (AC) exhibited the lowest relative activity recovery among the evaluated materials. Despite exhibiting the highest BET surface area ($1082 \text{ m}^2 \cdot \text{g}^{-1}$) and well-developed mesoporosity, immobilization on AC was governed predominantly by nonspecific physical adsorption (Brito et al., 2020). Under the immobilization conditions (pH 8.0), electrostatic interactions between the enzyme and the carbon surface were likely

limited, favoring weakly oriented adsorption. Consequently, although a high amount of enzyme was retained on the support, the absence of specific anchoring interactions may have contributed to partial loss of catalytic activity. These results indicate that high surface area alone is insufficient to guarantee superior biocatalytic performance. A similar trend was observed by Cea et al. (2019) those who reported approximately 50% relative activity after immobilizing *Candida rugosa* lipase on biochar derived from agricultural residue. Immobilization resulted in an enzyme activity of around 50% (Cea et al., 2019). In the other study carried out by Oliveira-Ribeiro et al. Oliveira-Ribeiro et al., 2019 when immobilizing lipase in biochar produced from *Manihot esculenta*, an immobilization efficiency of 61.2% and a relative activity around 50% was obtained.

The iron-functionalized carbon (AC-MF) showed an intermediate activity recovery (~50%), despite having a lower BET surface area ($904 \text{ m}^2\cdot\text{g}^{-1}$) and pore volume than AC. Functionalization with Fe^{3+} ions chelated by iminodiacetic acid (IDA) introduced specific interaction sites that promote stronger enzyme-support interactions. The presence of iron-containing species was confirmed by EDX and supported by XRD analysis, while the shift in pH_{pzc} toward neutrality (6.37) indicated significant modification of the surface properties. Although these changes may have improved enzyme anchoring, stronger interactions and increased surface polarity may also have imposed conformational restrictions on the enzyme, potentially limiting the structural flexibility required for optimal catalytic performance, including lid opening and substrate accessibility (Fang et al., 2023).

Among the evaluated supports, genipin-functionalized carbon (AC-GP) exhibited the highest relative activity recovery (~65%). Although its BET surface area ($981 \text{ m}^2\cdot\text{g}^{-1}$) was lower than that of AC, the superior catalytic performance demonstrates that surface chemistry played a more decisive role than textural properties alone. FTIR analysis confirmed the introduction of new surface functionalities after genipin treatment, while pH_{pzc} measurements indicated modifications in the surface chemical environment. Genipin acts as a natural crosslinking agent, establishing covalent interactions between the enzyme's amino groups and functional groups on the support (Wahba, 2024). Such interactions likely promoted a more favorable enzyme orientation and preserved conformational flexibility, creating a microenvironment more compatible with lipase (Wolf et al., 2025).

These results indicate that immobilization performance is determined by both textural and chemical properties (Kuo et al., 2021). Although activated carbon (AC) exhibited the highest surface area, its lower activity recovery suggests suboptimal enzyme-support interactions. The pH_{pzc} values, surface functional groups identified by FTIR, and

morphological changes following functionalization indicate that AC-MF and AC-GP create a more favorable microenvironment for enzyme immobilization compared to non-functionalized carbon. The superior performance of AC-GP underscores the significance of tailoring surface chemistry to maintain catalytic function, demonstrating that controlled functionalization may be more important than maximizing surface area alone. Distinct functionalization strategies influenced enzyme activity differently: iron modification improved enzyme anchoring but may impose conformational constraints, whereas genipin functionalization provided a more balanced interface between enzyme retention and catalytic preservation.

4. Conclusion

Syagrus coronata shells, an abundant residue from licuri processing, are a lignocellulosic material with high potential for producing activated carbon, which, through controlled activation and surface functionalization, can be tailored for use as a support for the immobilization of industrially important enzymes. Optimization via Response Surface Methodology focused on biocatalytic activity rather than exclusively maximizing textural properties, resulting in carbons with high immobilization efficiency and varied catalytic behaviors. BET analysis confirmed that all supports exhibited high surface areas; however, enzymatic activity was largely determined by surface chemistry and enzyme-support interactions rather than by surface area. Among the evaluated materials, genipin-functionalized carbon provided the most favorable interface, combining suitable porosity with a chemically compatible microenvironment that maintained lipase activity. These results highlight the importance of controlled surface modification in carbon-based biocatalysts and demonstrate the potential of licuri processing residues as sustainable precursors for high-value materials. The immobilized lipase systems developed in this study may find applications in enzymatic interesterification, structured lipid production, biodiesel synthesis, esterification, flavor ester production, and other industrial biotransformation processes that require stable, reusable biocatalysts.

5. References

- Adamian, Y., Lonappan, L., Alokpa, K., Agathos, S. N., & Cabana, H. (2021). Recent Developments in the Immobilization of Laccase on Carbonaceous Supports for Environmental Applications - A Critical Review. *Frontiers in Bioengineering and Biotechnology*, 9, 778239. <https://doi.org/10.3389/fbioe.2021.778239>
- Adeniyi, A. G., Abdulkareem, S. A., Ighalo, J. O., Amosa, M. K., Popoola, A., Ogunniyi, S., & Abdulkareem, M. T. (2021). Usage of Biomass-based Carbon Materials as Lubricant

- Additive: Effects on Rheological and Tribological Properties. *Letters in Applied NanoBioScience*, 10(4), 2861. <https://doi.org/10.33263/lianbs104.28612868>
- Agrawal, M. K., Prasad, V., Nijhawan, G., Jalal, S. S., Rajalakshmi, B., & Dwivedi, S. P. (2024). A Comprehensive Review of Electron Microscopy in Materials Science: Technological Advances and Applications. *E3S Web of Conferences*, 505, 1029. EDP Sciences. <https://doi.org/10.1051/e3sconf/202450501029>
- Alagöz, D., Toprak, A., Yıldırım, D., Tükel, S. S., & Fernández-Lafuente, R. (2020). Modified silicates and carbon nanotubes for immobilization of lipase from *Rhizomucor miehei*: Effect of support and immobilization technique on the catalytic performance of the immobilized biocatalysts. *Enzyme and Microbial Technology*, 144, 109739. <https://doi.org/10.1016/j.enzmictec.2020.109739>
- Ali, M. B., Hazzat, M. E., Flayou, M., Moussadik, A., Belekbi, S., Sifou, A., Dahhou, M., Kacimi, M., Benzaouak, A., & Hamidi, A. E. (2025). Advanced kinetic and thermodynamic analysis of sewage sludge combustion: A study of non-isothermal reaction mechanisms and energy recovery. *FirePhysChem*, 5(6), 545. <https://doi.org/10.1016/j.fpc.2025.04.002>
- Antil, B., Olhan, S., & Wal, R. L. V. (2025). Production of Graphitic Carbon from Renewable Lignocellulosic Biomass Source. *Minerals*, 15(3), 262. <https://doi.org/10.3390/min15030262>
- Attaurrazaq, B., Harjanto, S., Ulu, R. M., Setiawan, A., & Kawigraha, A. (2025). Carbothermic reduction of ilmenite using non-carbonized biomass from palm kernel shell. *Case Studies in Chemical and Environmental Engineering*, 11, 101119. <https://doi.org/10.1016/j.cscee.2025.101119>
- Aziz, R. A., Tahiruddin, N. S. M., Ali, R., & Taib, N. I. (2026). Fruit waste to wealth: Adsorption isotherm, kinetics, and thermodynamic studies of methylene blue removal using activated carbon derived from jackfruit peel (*Artocarpus heterophyllus*). *Next Materials*, 11, 101901. <https://doi.org/10.1016/j.nxmte.2026.101901>
- Bamerdhah, S. S., Kumar, N. S., Al-Ghurabi, E. H., Boumaza, M., & Asif, M. (2025). Optimized synthesis of activated carbon from date palm seeds for efficient crude oil adsorption in wastewater treatment. *Scientific Reports*, 15(1). <https://doi.org/10.1038/s41598-025-16831-7>

- Barakat, N. A. M., Mahmoud, M. S., & Moustafa, H. M. (2024). Comparing specific capacitance in rice husk-derived activated carbon through phosphoric acid and potassium hydroxide activation order variations. *Scientific Reports*, 14(1), 1460. <https://doi.org/10.1038/s41598-023-49675-0>
- Barrett, E. P., Joyner, L. G., & Halenda, P. P. (1951). The Determination of Pore Volume and Area Distributions in Porous Substances. I. Computations from Nitrogen Isotherms. *Journal of the American Chemical Society*, 73(1), 373. <https://doi.org/10.1021/ja01145a126>
- Bichang'a, D. O., Oladele, I. O., Alabi, O. O., Aramide, F. O., Oluseye, O., Borisade, S. G., Githinji, D. N., & Ojemaye, M. O. (2024). Coconut Shell Agro-waste-derived Cellulosic Powder as a Potential Reinforcement for Sustainable Polymeric Composite Application. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-4374947/v1>
- Borges, J. F., Nascimento, P. A., Alves, A. N., Santos, M. P. F., Brito, M. J. P., Bonomo, R. C. F., Santos, L. S., & Veloso, C. M. (2023). Laccase Immobilization on Activated Carbon from Hydrothermal Carbonization of Corn Cob. *Waste and Biomass Valorization*, 15(1), 501. <https://doi.org/10.1007/s12649-023-02160-1>
- Boukhvalov, D. W., Zhidkov, I. S., Kurmaev, E. Z., Fazio, E., Cholakh, S. O., & D'Urso, L. (2022). Atomic and electronic structures of stable linear carbon chains on Ag-nanoparticles. *arXiv (Cornell University)*. <https://doi.org/10.48550/arxiv.1711.07119>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Brito, M. J. P., Santos, M. P. F., Júnior, E. C. de S., Santos, L. S., Bonomo, R. C. F., Fontan, R. da C. I., & Veloso, C. M. (2020). Development of activated carbon from pupunha palm heart sheaths: Effect of synthesis conditions and its application in lipase immobilization. *Journal of Environmental Chemical Engineering*, 8(5), 104391. <https://doi.org/10.1016/j.jece.2020.104391>
- Byrne, N., Silva, R. D., Ma, Y., Sixta, H., & Hummel, M. (2017). Enhanced stabilization of cellulose-lignin hybrid filaments for carbon fiber production. *Cellulose*, 25(1), 723. <https://doi.org/10.1007/s10570-017-1579-0>

- Caldas, E. M., Novatzky, D., Deon, M., Menezes, E. W. de, Hertz, P. F., Costa, T. M. H., Arenas, L. T., & Benvenuti, E. V. (2017). Pore size effect in the amount of immobilized enzyme for manufacturing carbon ceramic biosensor. *Microporous and Mesoporous Materials*, 247, 95. <https://doi.org/10.1016/j.micromeso.2017.03.051>
- Cea, M., González, M. E., Abarzúa, M., & Navia, R. (2019). Enzymatic esterification of oleic acid by *Candida rugosa* lipase immobilized onto biochar. *Journal of Environmental Management*, 242, 171. <https://doi.org/10.1016/j.jenvman.2019.04.013>
- Chaudhari, D. S., Upadhyay, R. P., Shinde, G. Y., Gawande, M. B., Filip, J., Varma, R. S., & Zbořil, R. (2024). A review on sustainable iron oxide nanoparticles: syntheses and applications in organic catalysis and environmental remediation. *Green Chemistry*, 26(13), 7579. <https://doi.org/10.1039/d4gc01870b>
- Choi, B.-H., Worsham, E. K., Knighton, T., Reyes, M. A. H. de, Larsen, L., Bryan, H., Molina, E. A. R., & Boardman, R. D. (2023). Nuclear-Driven Chemical Conversion Pathways. *OSTI OAI (U.S. Department of Energy Office of Scientific and Technical Information)*. <https://www.osti.gov/biblio/2377404>
- Dhashnamoorthy, N., Kumar, R. K., & Radhamani, A. V. (2026). Waste-to-energy storage: Bio-Waste derived activated carbon from wood apple shell for symmetric electrochemical supercapacitors. *Results in Surfaces and Interfaces*, 23, 100746. <https://doi.org/10.1016/j.rsurfi.2026.100746>
- Doğan, Y., Öziç, C., Ertaş, E., Baran, A., Rosić, G., Selaković, D., & Eftekhari, A. (2024). Activated carbon-coated iron oxide magnetic nanocomposite (IONPs@CtAC) loaded with morin hydrate for drug-delivery applications. *Frontiers in Chemistry*, 12, 1477724. <https://doi.org/10.3389/fchem.2024.1477724>
- ElNasri, N. A. aziz, Wadidi, N. A., Idris, A. A. A., Woldemicheal, S. K., & Haj, S. I. A. E. (2022). Properties of natural adsorbent prepared from two local Sudanese agricultural wastes mango seeds and date's stones and their uses in removal of contamination from fluid nutrient. *Bulletin of the National Research Centre/Bulletin of the National Research Center*, 46(1). <https://doi.org/10.1186/s42269-022-00768-2>
- Faber, J., Brodzik, K., & Nycz, M. (2021). Understanding technical cleanliness: importance, assessment, maintenance. *Silniki Spalinowe/Combustion Engines*, 186(3), 41. <https://doi.org/10.19206/ce-140531>

- Fang, Y., Liu, F., Shi, Y., Yang, T., Xin, Y., Gu, Z., Shi, G., & Zhang, L. (2023). N-terminal lid swapping contributes to the substrate specificity and activity of thermophilic lipase TrLipE. *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1193955>
- Fasfous, I. I., El-Sheikh, A. H., Awwad, A., Al-Degs, Y. S., Fayyumi, E., & Dawoud, J. N. (2021). Factorial Investigation of Cobalt Retention by Ti and Fe Oxides-Modified Carbon Nanotubes: Multivariate Against Univariate Analysis. *Frontiers in Chemistry*, 9. <https://doi.org/10.3389/fchem.2021.690420>
- Feitoza, U. dos S., Thue, P. S., Lima, É. C., Reis, G. S. dos, Rabiee, N., Alencar, W. S., Mello, B. L., Dehmani, Y., Rinklebe, J., & Dias, S. L. P. (2022). Use of Biochar Prepared from the Açaí Seed as Adsorbent for the Uptake of Catechol from Synthetic Effluents. *Molecules*, 27(21), 7570. <https://doi.org/10.3390/molecules27217570>
- Feng, S., Ouyang, Q., Huang, J., Zhang, X., Ma, Z., Liang, K., & Huang, Q. (2024). Conversion of Lignin into Porous Carbons for High-Performance Supercapacitors via Spray Drying and KOH Activation: Structure-Properties Relationship and Reaction Mechanism. *Journal of Renewable Materials*, 12(7), 1207. <https://doi.org/10.32604/jrm.2024.052579>
- Garrido-García, L. F., Pérez-Martínez, A. L., Reyes-Gasga, J., Aguilar-Del-Valle, M. del P., Wong, Y. H., & Rodríguez-Gómez, A. (2024). A Simple Methodology to Gain Insights into the Physical and Compositional Features of Ternary and Quaternary Compounds Based on the Weight Percentages of Their Constituent Elements: A Proof of Principle Using Conventional EDX Characterizations. *Ceramics*, 7(3), 1275. <https://doi.org/10.3390/ceramics7030085>
- Gonçalves, G. R., Gandolfi, O. R., Bonomo, R. C. F., Fontan, R. da C. I., & Veloso, C. M. (2021). Synthesis of activated carbon from hydrothermally carbonized tamarind seeds for lipase immobilization: characterization and application in aroma ester synthesis. *Journal of Chemical Technology & Biotechnology*, 96(12), 3316. <https://doi.org/10.1002/jctb.6884>
- Habte, G. A., Bullo, T. A., & Ahmed, Y. (2025). Statistical optimization characterizations and Eco- friendly synthesis of silica from sugarcane bagasse. *Scientific Reports*, 15(1). <https://doi.org/10.1038/s41598-025-89366-6>

- Hoang, T., Liu, Y., & Le, M. T. (2024). Synthesis and Characterization of Biochars and Activated Carbons Derived from Various Biomasses. *Sustainability*, 16(13), 5495. <https://doi.org/10.3390/su16135495>
- Hong, J. Y., Jung, D., Park, S., Oh, Y., Oh, K. K., & Lee, S. H. (2021). Immobilization of laccase via cross-linked enzyme aggregates prepared using genipin as a natural cross-linker. *International Journal of Biological Macromolecules*, 169, 541. <https://doi.org/10.1016/j.ijbiomac.2020.12.136>
- Hua, Y., Ahmadi, Y., & Kim, K. (2023). Thermocatalytic Degradation of Gaseous Formaldehyde Using Transition Metal-Based Catalysts. *Advanced Science*, 10(21). <https://doi.org/10.1002/advs.202300079>
- Islam, Md. T., Hossen, Md. F., Kudrat-E-Zahan, Md., Asraf, Md. A., Zakaria, C. M., Hayatullah, & Rana, Md. S. (2025). Effect of temperature and time on purity, morphology and phase transformations of silica from rice husk. *Chemistry of Inorganic Materials*, 5, 100092. <https://doi.org/10.1016/j.cinorg.2025.100092>
- Ismail, A. R., & Baek, K.-H. (2020). Lipase immobilization with support materials, preparation techniques, and applications: Present and future aspects. *International Journal of Biological Macromolecules*, 163, 1624. <https://doi.org/10.1016/j.ijbiomac.2020.09.021>
- Ivanichok, N. Ya., Kolkovskiy, P. I., Soltys, A. M., Boychuk, V., Mandzyuk, V., Yablon, L. S., & Rachiy, B. I. (2023). The effect of orthophosphoric acid on energy-intensive parameters of porous carbon electrode materials. *Physics and Chemistry of Solid State*, 24(1), 34. <https://doi.org/10.15330/pcss.24.1.34-45>
- Karume, I., Bbumba, S., Tewolde, S., Mukasa, I. Z. T., & Ntale, M. (2023). Impact of carbonization conditions and adsorbate nature on the performance of activated carbon in water treatment. *BMC Chemistry*, 17(1). BioMed Central. <https://doi.org/10.1186/s13065-023-01091-1>
- Kuo, P., Lin, Z.-X., Wu, T., Hsu, C., Lin, H., & Wu, T.-S. (2021). Effects of morphology and pore size of mesoporous silicas on the efficiency of an immobilized enzyme. *RSC Advances*, 11(17), 10010. <https://doi.org/10.1039/d1ra01358k>

- Kwiatos, N., Atila, D., Puchalski, M., Kumaravel, V., & Steinbüchel, A. (2024). Cyanophycin modifications for applications in tissue scaffolding. *Applied Microbiology and Biotechnology*, 108(1). <https://doi.org/10.1007/s00253-024-13088-4>
- Kwon, Y., Kim, J.-K., Kim, Y., Je, A. R., Guim, H., & Kim, J.-G. (2023). Effective investigation of murine femoral bone development utilizing correlative light and electron microscopy (CLEM). *Journal of Analytical Science & Technology*, 14(1). <https://doi.org/10.1186/s40543-023-00402-4>
- Leal, G. F., Cruz, M. de S., Santos, J. C. C., Sousa, H. M. S., Martins, G. A. de S., & Queiroz, F. (2025). Syagrus coronata: A bibliometric and critical review on nutritional composition, bioactive compounds, and future applications. *Trends in Food Science & Technology*, 166, 105384. <https://doi.org/10.1016/j.tifs.2025.105384>
- Li, H., Yu, Y., Li, P., Guo, X., Zhang, Y., Wang, J., & Zhang, Y. (2025). Biochar-supported Fe/Mg co-doped MoS₂ synergistically enhances PMS activation for the degradation of tetracycline hydrochloride: A study on the Fe²⁺/Fe³⁺ cycling driving mechanism and catalytic stability. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-7582872/v1>
- Li, L., Cao, S., Li, Z., Huang, J., Jiang, Y., Deng, C., & Liu, Z. (2022). Adsorption and desorption of the flavonoids on activated carbon impregnated with different metal ions. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-2293257/v1>
- Li, Y., Wu, G., Liu, Z., Dai, L., Liu, D., & Du, W. (2025). Enzymatic synthesis of phosphatidyl-EPA/DHA using Candida antarctica lipase B immobilized on mesoporous MIL-88 A. *Bioresources and Bioprocessing*, 12(1), 123. <https://doi.org/10.1186/s40643-025-00959-5>
- Lippens, B. C. (1964). Studies on pore systems in catalysts I. The adsorption of nitrogen; apparatus and calculation. *Journal of Catalysis*, 3(1), 32. [https://doi.org/10.1016/0021-9517\(64\)90089-2](https://doi.org/10.1016/0021-9517(64)90089-2)
- Liu, P. (2021). Study of the interaction between hydrogen and ultradispersed metals for heterogeneous catalysis. *HAL (Le Centre Pour La Communication Scientifique Directe)*. <https://hal.science/tel-03681371>

- Lu, X., & Gu, X. (2022). A review on lignin pyrolysis: pyrolytic behavior, mechanism, and relevant upgrading for improving process efficiency. *Biotechnology for Biofuels and Bioproducts*, 15(1), 106. <https://doi.org/10.1186/s13068-022-02203-0>
- Makar, L. E., Nady, N., Shawky, N., & Kandil, S. (2023). Genipin versus Ferric Chloride cross-linked unmodified Gum Arabic/Chitosan/nano-Hydroxyapatite nanocomposite hydrogels as potential scaffolds for bone regeneration. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-41413-w>
- Menezes, L. H. S. de, Santo, E. L. do E., Santos, M. M. O. dos, Tavares, I. M. de C., Méndes, A. A., Franco, M., & Oliveira, J. R. de. (2021). Candida rugosa lipase immobilized on hydrophobic support Accurel MP 1000 in the synthesis of emollient esters. *Biotechnology Letters*, 44(1), 89. <https://doi.org/10.1007/s10529-021-03196-w>
- Mergbi, M., Galloni, M. G., Aboagye, D., Elimian, E., Su, P., Ikram, B. M., Nabgan, W., Bedia, J., Amor, H. B., Contreras, S., Medina, F., & Djellabi, R. (2023). Valorization of lignocellulosic biomass into sustainable materials for adsorption and photocatalytic applications in water and air remediation. *Environmental Science and Pollution Research*, 30(30), 74544. <https://doi.org/10.1007/s11356-023-27484-2>
- Mirzaei, S., Ahmadpour, A., Shao, Z., & Arami-Niya, A. (2022). Rational design of carbon-based materials for purification and storage of energy carrier gases of methane and hydrogen. *Journal of Energy Storage*, 56, 105967. <https://doi.org/10.1016/j.est.2022.105967>
- Mishra, R. K., Singh, B., & Acharya, B. (2024). A comprehensive review on activated carbon from pyrolysis of lignocellulosic biomass: An application for energy and the environment. *Carbon Resources Conversion*, 7(4), 100228. <https://doi.org/10.1016/j.crcon.2024.100228>
- Mohan, D., Sarswat, A., Singh, V. K., Alexandre-Franco, M., & Pittman, C. U. (2011). Development of magnetic activated carbon from almond shells for trinitrophenol removal from water. *Chemical Engineering Journal*, 172, 1111. <https://doi.org/10.1016/j.cej.2011.06.054>
- Mtibe, A., John, M. J., Andrew, J., & Mokhena, T. C. (2022). Sequential extraction of carbohydrates and lignin from agricultural waste and their structural characterization. *Biomaterials and Polymers Horizon*, 1(2). <https://doi.org/10.37819/bph.001.02.0216>

- Mubarak, M. F., Ahmed, A. M., & Gabr, S. S. (2021). Nanoporous Carbon Materials toward Phenolic Compounds Adsorption. In *IntechOpen eBooks*. IntechOpen. <https://doi.org/10.5772/intechopen.96380>
- Muronetz, V. I., Pozdyshev, D. V., & Semenyuk, P. I. (2022). Polyelectrolytes for Enzyme Immobilization and the Regulation of Their Properties. *Polymers*, 14(19), 4204. <https://doi.org/10.3390/polym14194204>
- Nascimento, P. A., Borges, J. F., Alves, A. N., Holland, B. J., Bonomo, R. C. F., Mathesh, M., Yang, W., & Veloso, C. M. (2024). Synthesis and microencapsulation of acylglycerols rich in omega-3 PUFAs by glycerolysis using lipase immobilized on activated carbon. *Journal of Environmental Chemical Engineering*, 12(6), 114617. <https://doi.org/10.1016/j.jece.2024.114617>
- Nascimento, P. A., Borges-Vilches, J., Santos, M. P. F., Alves, A. N., Santos, L. S., Fontan, R. da C. I., Bonomo, R. C. F., Yang, W., & Veloso, C. M. (2024). Revealing the potential of a novel approach for assessing the effect of pyrolysis conditions on sisal waste-based activated carbon for lipase immobilization and ethyl lactate biosynthesis. *Biomass Conversion and Biorefinery*, 15(11), 16987. <https://doi.org/10.1007/s13399-024-06318-2>
- Nazem, M. A., Zare, M. H., & Shirazian, S. (2020). Preparation and optimization of activated nano-carbon production using physical activation by water steam from agricultural wastes. *RSC Advances*, 10(3), 1463. <https://doi.org/10.1039/c9ra07409k>
- Neme, I., Gonfa, G., & Masi, C. (2022). Activated carbon from biomass precursors using phosphoric acid: A review. *Heliyon*, 8(12). <https://doi.org/10.1016/j.heliyon.2022.e11940>
- Olabisi, A. S., Balogun, A. O., Oni, T. O., Fakinle, B. S., Sotoudehnia, F., McDonald, A. G., & Ikubanni, P. P. (2023). Physicochemical Characterization of Woody Lignocellulosic Biomass and Charcoal for Bio-energy Heat Generation. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-46054-7>
- Oliveira-Ribeiro, L. M., Meili, L., Silva-Belo-Gois, G. N., Rosas-Garcia-Almeida, R. M., & Duarte, J. L. da S. (2019). Immobilization of lipase in biochar obtained from *Manihot esculenta* Crantz. *Revista ION*, 32(2), 7. <https://doi.org/10.18273/revion.v32n2-2019001>

- Olivier, J. A. S., Canepa, J. R. L., García, D. S., Hernández, R., Zappi, M. E., & Monteros, A. E. E. de los. (2022). Activated Carbon: A Review of Residual Precursors, Synthesis Processes, Characterization Techniques, and Applications in the Improvement of Biogas. *Environmental Engineering Research*, 28(3), 220100. Korean Society of Environmental Engineering. <https://doi.org/10.4491/eer.2022.100>
- Ortega-Ortiz, H., Bernardo, M., Matos, I., Fonseca, I., Mota, J. P. B., Jandosov, J., & Ribeiro, R. P. P. L. (2026). Rice husk-based activated carbon honeycomb monolith for biogas upgrading and CO₂ capture from flue gases. *Adsorption*, 32(3). <https://doi.org/10.1007/s10450-026-00687-y>
- Peixoto, B. S., Mota, L. S. de O., Oliveira, P. C. O. de, Veloso, M. C. da C., Romeiro, G. A., & Moraes, M. C. de. (2022). Highly Functionalized Microporous Activated Biochar from *Syagrus coronata* Waste: Production, Characterization, and Application in Adsorption Studies. *Water*, 14(21), 3525. <https://doi.org/10.3390/w14213525>
- Pimentel, C. H., Freire, M. S., Gómez-Díaz, D., & González-Álvarez, J. (2023). Preparation of activated carbon from pine (*Pinus radiata*) sawdust by chemical activation with zinc chloride for wood dye adsorption. *Biomass Conversion and Biorefinery*, 13(18), 16537. <https://doi.org/10.1007/s13399-023-04138-4>
- Pota, G., Bifulco, A., Parida, D., Zhao, S., Rentsch, D., Amendola, E., Califano, V., & Costantini, A. (2021). Tailoring the hydrophobicity of wrinkled silica nanoparticles and of the adsorption medium as a strategy for immobilizing lipase: An efficient catalyst for biofuel production. *Microporous and Mesoporous Materials*, 328, 111504. <https://doi.org/10.1016/j.micromeso.2021.111504>
- Pryshchepa, O., Rafińska, K., Gołębiowski, A., Sugajski, M., Sagandykova, G., Madajski, P., Buszewski, B., & Pomastowski, P. (2022). Synthesis and physicochemical characterization of bovine lactoferrin supersaturated complex with iron (III) ions. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-15814-2>
- Qiu, Y., Wang, B.-J., & Weng, Y. (2020). Preparation and characterization of genipin cross-linked and lysozyme incorporated antimicrobial sodium caseinate edible films. *Food Packaging and Shelf Life*, 26, 100601. <https://doi.org/10.1016/j.fpsl.2020.100601>
- Ramlee, N. N., Illias, R. M., Rahman, R. A., Toemen, S., Selvasembian, R., Ahmad, R. A., Manas, N. H. A., & Azelee, N. I. W. (2022). Biochemical and Physical Characterization

- of Immobilized *Candida rugosa* Lipase on Metal Oxide Hybrid Support. *Catalysts*, 12(8), 854. <https://doi.org/10.3390/catal12080854>
- Salim, R., Asik, J., & Sarjadi, M. S. (2021). Chemical functional groups of extractives, cellulose and lignin extracted from native *Leucaena leucocephala* bark. *Wood Science and Technology*, 55(2), 295. <https://doi.org/10.1007/s00226-020-01258-2>
- Santos, M. P. F., Porfírio, M. C. P., Júnior, E. C. de S., Bonomo, R. C. F., & Veloso, C. M. (2022). Pepsin immobilization: Influence of carbon support functionalization. *International Journal of Biological Macromolecules*, 203, 67. <https://doi.org/10.1016/j.ijbiomac.2022.01.135>
- Santos, M. P. F., Silva, J. F., Fontan, R. da C. I., Bonomo, R. C. F., Santos, L. S., & Veloso, C. M. (2020). New insight about the relationship between the main characteristics of precursor materials and activated carbon properties using multivariate analysis. *The Canadian Journal of Chemical Engineering*, 98(7), 1501. <https://doi.org/10.1002/cjce.23721>
- Sari, N. H., Suteja, S., Hidayatullah, S., Al-Farizi, F. H., & Lokantara, I. P. (2025). Performance evaluation of hybrid sisalana Agave fiber and carbon powder in polyester composites: A study on mechanical, thermal, and microstructural characteristics. *Case Studies in Chemical and Environmental Engineering*, 11, 101215. <https://doi.org/10.1016/j.cscee.2025.101215>
- Schmitt, D., Janka, O., Leiner, R., Kickelbick, G., & Gallei, M. (2024). Preparation of preceramic ferrocene-modified microparticles for the development of uniform porous iron oxide particles and their sustainable recycling. *Materials Advances*, 5(7), 3037. <https://doi.org/10.1039/d3ma01131c>
- Şen, A., Nobre, C., Martins, M., Gonçalves, M., & Pereira, H. (2023). Effect of Bark Biochars on Properties of Steam-Activated Carbons. *Environments*, 11(1), 2. <https://doi.org/10.3390/environments11010002>
- Silva, A. R., Alves, M. M., & Pereira, L. (2021). Progress and prospects of applying carbon-based materials (and nanomaterials) to accelerate anaerobic bioprocesses for the removal of micropollutants. *Microbial Biotechnology*, 15(4), 1073. Wiley. <https://doi.org/10.1111/1751-7915.13822>

- Sklepova, S.-V., Gasyuk, I. M., Ivanichok, N. Ya., Kolkovskiy, P. I., Kotsyubynsky, V., & Rachiy, B. I. (2022). The porous structure of activated carbon-based on waste coffee grounds. *Physics and Chemistry of Solid State*, 23(3), 484. <https://doi.org/10.15330/pcss.23.3.484-490>
- Soest, P. J. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, 74(10), 3583. [https://doi.org/10.3168/jds.s0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.s0022-0302(91)78551-2)
- Sousa, N. F. C., Santos, M. P. F., Barbosa, R. P., Bonomo, R. C. F., Veloso, C. M., & Júnior, E. C. de S. (2024). Pepsin immobilization on activated carbon and functionalized with glutaraldehyde and genipin for the synthesis of antioxidant peptides of goat casein. *Food Research International*, 186, 114161. <https://doi.org/10.1016/j.foodres.2024.114161>
- Sumathirathne, L., & Euler, W. B. (2021). Catalysis of the Thermal Decomposition of Transition Metal Nitrate Hydrates by Poly(vinylidene difluoride). *Polymers*, 13(18), 3112. <https://doi.org/10.3390/polym13183112>
- Suo, H., Xu, L., Xu, C., Qiu, X., Chen, H., Huang, H., & Hu, Y. (2019). Graphene Oxide Nanosheets Shielding of Lipase Immobilized on Magnetic Composites for the Improvement of Enzyme Stability. *ACS Sustainable Chemistry & Engineering*, 7(4), 4486. <https://doi.org/10.1021/acssuschemeng.8b06542>
- Taheri-Shakib, J., Esfandiarian, A., Rajabi-Kochi, M., Kazemzadeh, E., & Karaei, M. A. (2024). Evaluation of rock and fluid intermolecular interaction between asphaltene and sand minerals using electrochemical, analytical spectroscopy and microscopy techniques. *Scientific Reports*, 14(1). <https://doi.org/10.1038/s41598-024-51196-3>
- Toor, S. S., Sharma, K., Klemmt, R., Mamakhel, A., & Achour, A. (2026). Tailored porosity from waste biomass: mesoporous bio-adsorbents for targeted treatment of complex industrial wastewaters. *Frontiers in Fuels*, 3. <https://doi.org/10.3389/ffuel.2025.1716359>
- Vajitha, G., Madan, K., Tewari, C., Sahoo, N. G., & Maliyekkal, S. M. (2023). Carbon-based filters for water and wastewater treatment. In *IWA Publishing eBooks* (p. 155). UWA Publishing. https://doi.org/10.2166/9781789063714_0155

- Vallejo-García, J. L., Cutillo-Foraster, A., Arnáiz, A., Vallejos, S., García, J. M., Santamaría, M. A. M., & Trigo-López, M. (2024). Hydrolysis of Lactose: Conventional Techniques and Enzyme Immobilization Strategies on Polymeric Supports. In *IntechOpen eBooks*. IntechOpen. <https://doi.org/10.5772/intechopen.1005087>
- Venkatachalam, C. D., Ravichandran, S. R., & Sengottian, M. (2021). Lignocellulosic and algal biomass for bio-crude production using hydrothermal liquefaction: Conversion techniques, mechanism and process conditions: A review. *Environmental Engineering Research*, 27(1), 200555. Korean Society of Environmental Engineering. <https://doi.org/10.4491/eer.2020.555>
- Wahab, R. A., Elias, N., Abdullah, F., & Ghoshal, S. K. (2020). On the taught new tricks of enzymes immobilization: An all-inclusive overview. *Reactive and Functional Polymers*, 152, 104613. <https://doi.org/10.1016/j.reactfunctpolym.2020.104613>
- Wahba, M. I. (2024). A comprehensive review on genipin: an efficient natural cross-linker for biopolymers [Review of *A comprehensive review on genipin: an efficient natural cross-linker for biopolymers*]. *Polymer Bulletin*, 81(16), 14251. Springer Science+Business Media. <https://doi.org/10.1007/s00289-024-05406-7>
- Zhang, H., Secundo, F., Sun, J., & Mao, X. (2022). Advances in enzyme biocatalysis for the preparation of functional lipids. *Biotechnology Advances*, 61, 108036. <https://doi.org/10.1016/j.biotechadv.2022.108036>
- Zhang, L., & Zuo, S. (2024). The Significance of Lignocellulosic Raw Materials on the Pore Structure of Activated Carbons Prepared by Steam Activation. *Molecules*, 29(13), 3197. <https://doi.org/10.3390/molecules29133197>
- Zhang, W., Qiu, X., Wang, C., Zhong, L., Fu, F., Zhu, J., Zhang, Z., Qin, Y., Yang, D., & Xu, C. (2022). Lignin derived carbon materials: current status and future trends. *Carbon Research*, 1(1). <https://doi.org/10.1007/s44246-022-00009-1>
- Zhao, D. (2023). Electrospun nanofiber membranes as supports for enzyme immobilization and its application in wastewater treatment. *Research Portal Denmark*, 142.

Sustainable Valorization of Licuri Residues into Functionalized Carbon Supports for Lipase
Immobilization and Licuri Oil Modification

*Manuscrito será submetido à revista: *Bioresource Technology* (ISSN: 1873-297636)

Fator de impacto: 8.2

Sustainable Valorization of Licuri Residues into Functionalized Carbon Supports for Lipase Immobilization and Licuri Oil Modification

Highlights

- Licuri press cake enabled lipase production up to 197.7 U mL⁻¹ via SSF.
- Genipin-functionalized carbon improved immobilization yield (39.2 U mL⁻¹).
- Immobilized lipase efficiently catalyzed the interesterification of licuri oil.
- Enzymatic interesterification modified both the rheological and thermal behavior of licuri oil.
- Residue valorization enables the sustainable production of structured lipids.

ABSTRACT

The sustainable valorization of agro-industrial residues represents an important strategy for developing cost-effective bioprocesses and high-value products. However, the utilization of licuri (*Syagrus coronata*) residues across enzyme production, immobilization support development, and lipid modification remains underexplored. To address this gap, this study evaluated a bioprocess in which licuri cake was used as a substrate for lipase production by *Penicillium roqueforti* via solid-state fermentation, followed by enzyme immobilization on activated carbon and subsequent application in lipid-modification processes. Process optimization resulted in a maximum enzymatic activity of 197.73 U mL⁻¹ under optimized conditions (72 h and 12.5% inducer), representing a marked increase compared to initial experimental conditions (maximum of 66.89 U mL⁻¹ in the Doehlert design). The crude enzymatic extract exhibited optimal activity at pH 7.0 and 40 °C, along with mesophilic thermal stability. Kinetic parameters showed a $V_{\max}$ of 42.68 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ and K_m of 1.36 mM, indicating moderate substrate affinity. Immobilization on the activated carbon demonstrated superior performance for genipin-functionalized support (AC-GF), reaching 39.19 U mL⁻¹ after 6 h. The immobilized enzyme was successfully applied in the interesterification of licuri oil. Rheological analysis showed that crude oil exhibited shear-thinning behavior, whereas all interesterified samples displayed near-Newtonian flow with reduced viscosity variation over the shear-rate range (0.1–100 s⁻¹). Differential scanning calorimetry (DSC) further confirmed structural modifications by revealing changes in the crystallization and melting profiles of the enzymatically modified oils, indicating rearrangement of triacylglycerol molecules. These results indicate that enzymatic

interesterification promoted structural rearrangements in triacylglycerols, thereby modifying their flow behavior. The integrated approach demonstrates a sustainable strategy for producing value-added structured lipids from agro-industrial residues.

Keywords: *Penicillium roqueforti*; agro-industrial waste; biocatalysis; carbon functionalization; lipid restructuring.

1. INTRODUCTION

The rising demand for sustainable and technologically viable industrial solutions is driven by the global transition toward a bioeconomy, which aims to replace harsh chemical catalysts with eco-friendly, highly selective biocatalysts (Abdelaziz et al., 2025). This shift is particularly evident in the food industry, where microbial lipases (EC 3.1.1.3) are employed to improve product quality through the precise hydrolysis, esterification, and transesterification of triacylglycerols (Abdelaziz et al., 2025; Park et al., 2023). These enzymes are widely applied in the food, pharmaceutical, cosmetic, and biofuel industries (Sosa-Martínez et al., 2023). Lipases are classified as hydrolases (EC 3.1.1.3) and preferentially act at oil/water interfaces, which provides advantages in heterogeneous systems and in applications involving organic media (Nascimento et al., 2024; Pinto Brito et al., 2020).

The growing global demand for microbial lipases underscores the need for efficient and sustainable large-scale production strategies. The global microbial lipase market was valued at USD 557.44 million in 2022 and is projected to expand at a compound annual growth rate (CAGR) of 6.52% from 2023 to 2030, reflecting the increasing industrial demand for these biocatalysts in food, dairy, pharmaceutical, detergent, and other biotechnological applications (Grand View Research, 2023). To meet the growing demand for lipase, solid-state fermentation (SSF) has been widely investigated as an efficient and sustainable strategy for enzyme production. This approach enables the use of agro-industrial residues as low-cost substrates and contributes to both biomass valorization and process sustainability (Agaç et al., 2026; Adebami et al., 2025). This technique involves cultivating microorganisms on solid substrates under low moisture conditions and is particularly suitable for filamentous fungi, such as *Penicillium roqueforti*, which is widely used for lipase production. (Adebami et al., 2025; Borkertas et al., 2025; Ojeda-Hernández et al., 2018). Furthermore, efficient fermentation depends strongly on the choice of substrate.

Lignocellulosic materials, which are rich in carbon and nutrients, including sugarcane bagasse, wheat bran, fruit husks, and oilseed, have been widely studied due to their favorable structures and compositions that support microbial growth and enzyme production (Wang et al., 2023; Xiang et al., 2023). Among these options, licuri cake (*Syagrus coronata*), a by-product of oil extraction from the fruit kernels produced in the semiarid region of Bahia, is particularly noteworthy. Oilseed residues are considered promising substrates for lipase production because their residual lipid content serves as a natural inducer for enzyme synthesis. This underutilized by-product contains high levels of residual lipids, fibers, and proteins, which makes it a suitable substrate for fermentation (Leal et al., 2025; Pereira et al., 2025; Guimarães et al., 2021; Rodrigues et al., 2020).

Despite these advantages, the industrial application of lipases produced by SSF remains limited by the low operational stability, difficult recovery, and limited reusability of free enzymes. Furthermore, purification of the crude enzymatic extract is often required before use. Immobilization on suitable supports offers an attractive alternative, as it can enable direct adsorption of the enzyme from the crude extract while enhancing operational stability and reusability (Chandra et al., 2020; Salgado et al., 2022).

Enzyme immobilization addresses these challenges by enhancing catalytic stability, simplifying enzyme recovery, and enabling repeated use in industrial reactions. Immobilized lipases are widely utilized in biotransformation such as esterification, biodiesel production, and lipid modification, demonstrating improved performance under diverse reaction conditions (Abed et al., 2018; Bai et al., 2025; Louhasakul & Cheirsilp, 2022).

One promising use of immobilized lipases is enzymatic interesterification, which has attracted great attention in the food industry (da Silva et al., 2022; Rohm et al., 2018; Zhang et al., 2021). This process can change the structure and function of lipids without creating unwanted by-products (Dong et al., 2024). This process enables the rearrangement of fatty acids within triacylglycerols, yielding structured lipids with improved nutritional and physicochemical properties (Sivakanthan & Madhujith, 2020; Zhang et al., 2020, 2022). Compared with chemical interesterification, the enzymatic route offers greater selectivity, milder reaction conditions, and reduced environmental impact (Nascimento et al., 2024; Zhang et al., 2021; Zuin et al., 2022).

Although enzymatic interesterification is well established as a sustainable alternative for lipid modification, studies that integrate enzyme production, immobilization, and application into a single residue-based bioprocess remain scarce. To address this gap, this study aimed to develop an integrated biotechnological approach to valorize licuri (*Syagrus coronata*) residues.

Lipase was produced by solid-state fermentation using *Penicillium roqueforti* cultivated on licuri cake, which was also converted into surface-functionalized activated carbon for enzyme immobilization. The crude lipase obtained was immobilized on the developed support and subsequently applied in the enzymatic interesterification of licuri oil. The structural modifications promoted by the reaction were evaluated through rheological and thermal analyses. This approach aimed to develop an integrated biotechnological platform based on the valorization of regional agro-industrial residues. This study contributes to the development of sustainable bioprocesses aligned with the United Nations Sustainable Development Goals, particularly SDG 8 (Decent Work and Economic Growth), SDG 9 (Industry, Innovation and Infrastructure), and SDG 12 (Responsible Consumption and Production), by promoting the valorization of regional resources and the generation of value-added products from agro-industrial residues.

2. MATERIAL AND METHODS

2.1. Reagents and raw materials

All reagents used in this study were of analytical grade PA-ACS. Licuri cake, used as the substrate for solid-state fermentation and crude oil used in interesterification reaction was provided by the COOPES cooperative located in Capim Grosso, Bahia, Brazil (11°22'51" S, 40°00'46" W). Prior to use, the residue was ground and sieved through a 20-mesh sieve to standardize particle size and ensure homogeneous fermentation conditions.

2.2. Microorganisms and inoculum preparation

The microorganism used in this study was *Penicillium roqueforti* ATCC 10110, supplied by the Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, Brazil). The strain was cultivated on Potato Dextrose Agar (PDA) medium and incubated at 25 °C for 7 days to allow spore development. The spore suspension was prepared by adding sterile glass beads and a 0.01% (v/v) Tween 80 solution to the culture plates to facilitate spore detachment. Spore concentration was determined using a Neubauer counting chamber under an optical microscope.

2.3. Solis-state fermentation for lipase production

Solid-state fermentation was carried out in 250 mL Erlenmeyer flasks containing 5 g of dried and sieved licuri cake. The flasks were sterilized in an autoclave at 121 °C for 15 minutes. After cooling, the substrate was inoculated with a spore suspension of *Penicillium roqueforti* ATCC 10110 at a concentration of 5.0×10^7 spores per gram of dry substrate. The spore suspension was previously prepared in 0.01% Tween 80 solution.

The medium's moisture content was adjusted to 70% with sterile distilled water, in accordance with the conditions established in the experimental design. Additionally, different concentrations of inducer (residual palm oil) were incorporated into the substrate according to the levels defined by the Doehlert experimental design.

The flasks were incubated in a bacteriological incubator at 27 °C for 12 to 120 hours, as defined by the experimental design. At the end of the incubation period, the fermented material was collected for the extraction of the crude enzymatic extract.

2.4. Experimental design and statistical analysis

2.4.1. Doehlert design

Preliminary experiments, based on literature data, were conducted to establish suitable ranges for fermentation time, substrate moisture, and medium composition. These initial assays facilitated the assessment of the overall behavior of the solid-state fermentation system by selecting variables and their respective levels. A Doehlert experimental design was selected for its efficiency in evaluating the effects of multiple variables using fewer experiments (Stojanović et al., 2021). The design incorporated two independent factors: fermentation time (X_1) and inducer ratio (X_2 , residual palm oil). Fermentation time was assessed at five coded levels (-1.0, -0.5, 0, 0.5, and 1.0), corresponding to actual values ranging from 12 to 120 hours. The inducer ratio was assessed at three coded levels (-0.866, 0, and 0.866), corresponding to 5, 10, and 15% (w/w), respectively.

Nine experimental runs were conducted to evaluate linear, quadratic, and interaction effects on lipase production. The experimental matrix, including both coded and actual values, is presented in Table 1.

Table 1. Experimental levels (coded and real values) of fermentation time and inducer ratio in the Doehlert design.

Variable		Code	Level				
Factor 1	Time (h)	X_1	-1.00	-0.5	0	0.5	1.00
			12	39	66	93	120
Factor 2	Inductor ratio (%w/w)	X_2	-0.866	0	0.866		
			5	10	15		

2.4.2. Rotatable Central Composite Design (RCCD)

Based on the results obtained from the Doehlert design, a rotational central composite design (RCCD) was subsequently applied to further explore the region of highest enzymatic activity. The RCCD was defined using the same independent variables: fermentation time and inducer ratio.

The experimental matrix consisted of factorial points, axial points, and replicates at the central point, allowing the estimation of second-order polynomial models. Data analysis was performed using SAS OnDemand for Academics software. The significance of the regression model was evaluated using analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$). Model adequacy was assessed using the F-test, coefficient of determination (R^2), and the lack-of-fit test. Response surface methodology (RSM) was used to visualize the effects of the variables and to identify trends in lipase production.

2.5. Extraction of crude enzyme

The crude enzymatic extract was obtained by adding 30 mL of 0.1 mol L⁻¹ sodium phosphate buffer (pH 7.0) containing 6% (w/v) NaCl to the fermented substrate. The mixture was agitated for 40 minutes at room temperature on an orbital shaker operating at 200 rpm.

The liquid phase was separated using sterile gauze and subsequently centrifuged at 3000 rpm for 15 minutes at 4 °C. The supernatant was collected and stored for subsequent enzymatic analyses.

2.6. Lipase activity assay

Lipase activity was determined using a spectrophotometric method based on the hydrolysis of *p*-nitrophenyl palmitate (*p*-NPP). The preparation of substrate solutions followed a previously described methodology.

Two stock solutions were prepared. Solution A consisted of 60 mg of *p*-nitrophenyl palmitate dissolved in 20 mL of isopropyl alcohol. Solution B was prepared by dissolving 4 g of Triton X-100 and 0.4 g of gum arabic in 200 mL of 0.1 M sodium phosphate buffer (pH 7.0). The enzymatic reaction mixture consisted of 500 µL of Solution B, 125 µL of Solution A, and 125 µL of the enzymatic sample (crude enzymatic extract). The reaction mixture was incubated at 40 °C for 15 minutes. The reaction was stopped by heating the mixture at 100 °C for 1 minute, followed by adding 2.0 mL of acetone.

The hydrolysis of *p*-NPP by lipase releases *p*-nitrophenol, producing a yellow coloration whose intensity was measured by absorbance at 410 nm (Ikhwan et al., 2024). Quantification was performed using a standard calibration curve constructed with known concentrations of *p*-

nitrophenol. One unit of lipase activity (U.mL^{-1}) was defined as the amount of enzyme capable of releasing 1 μmol of p-nitrophenol per milliliter per minute under the assay conditions. Total enzymatic activity (U.mL^{-1}) was calculated according to the method described by Nascimento et al. (2021) using Equation 1.

$$U = \frac{C_{pNP} \times DF \times V_T}{V_E \times t} \quad (1)$$

Where: C_{pNP} = concentration of p-nitrophenol ($\mu\text{mol.mL}^{-1}$); DF = dilution factor; V_T = total reaction volume (mL); V_E = volume of enzymatic extract used (mL); t = reaction time (min).

Specific enzymatic activity (U.mg^{-1}) was obtained by dividing the total enzymatic activity (U.mL^{-1}) by the protein content of the extract (mg), which was determined using the Bradford method (Bradford, 1976)

2.7. Enzymatic characterization

2.7.1. Determination of optimum temperature and pH

The optimum pH and temperature of the enzyme were determined by performing activity assays, as described in Section 2.6, at pH values ranging from 4.0 to 11.0 and temperatures between 40 and 60 °C. First, tests were performed to determine the optimum pH with the reaction temperature fixed at 40 °C. The only modification in the protocol was the buffer system used to adjust the pH of the reaction medium: 0.1 M sodium acetate (pH 4-5), 0.1 M sodium citrate (pH 6.0), 0.1 M sodium phosphate (pH 7.0), 0.1M Tris-HCl (pH 8.0), and 0.1 M glycine-NaOH (pH 9-11). To determine the optimum temperature, enzymatic tests were performed at the previously established optimum pH

2.7.2. Enzyme stability

The thermal stability of the enzymatic extract was evaluated by incubating the enzyme at the optimal pH determined previously and at different temperatures (30, 40, 50, and 60 °C). For each temperature condition, aliquots of the enzymatic extract were collected after incubation periods of 0, 30, 60, 90, and 120 minutes. After each incubation period, the residual enzymatic activity was determined using the standard p-NPP assay described (item 2.6). The results were expressed as residual activity relative to the initial activity measured at time zero.

2.7.3. Determination of kinetic parameters

The kinetic parameters of the lipase were determined by measuring enzymatic activity at different concentrations of the substrate, p-nitrophenyl palmitate (p-NPP). The substrate concentrations used were 1.0, 2.5, 5.0, 7.5, and 10.0 mM. The Michaelis & Menten constant (K_m) and maximum velocity (V_{max}) were determined using Lineweaver-Burk graphs (Lineweaver & Burk, 1934).

2.8. Immobilization of the crude enzymatic extract

The enzymatic extract was immobilized on different activated carbon supports, including non-functionalized activated carbon (AC), genipin-functionalized activated carbon (AC-GF), and iron-functionalized activated carbon (AC-MF), previously synthesized. For immobilization, 0.1 g of each support was added to 5 mL of the crude enzymatic extract, presenting a protein concentration of $5 \text{ mg} \cdot \text{mL}^{-1}$ as determined by the Bradford method (Elhazar et al., 2023). The mixture was maintained under constant agitation at room temperature for different contact times (2, 4, and 6 h) to evaluate the effect of immobilization time. After each period, the solid phase was separated, and the immobilized enzyme was recovered and used to evaluate activity. The immobilization time yielding the highest enzymatic activity was selected for subsequent interesterification assays. The immobilized biocatalyst was stored at 4°C until further use.

The immobilization capacity of each carbon was determined as the difference between the initial and final protein concentrations using Equation (2).

$$C_{Im} = \frac{V(C_{in}-C)}{m_s} \quad (2)$$

Where: C_{Im} is the immobilization capacity ($\text{mg} \cdot \text{g}^{-1}$); V is the volume of solution (mL); C_{in} is the initial concentration of the solution ($\text{mg} \cdot \text{L}^{-1}$); C is the concentration of solution at equilibrium ($\text{mg} \cdot \text{L}^{-1}$); m_s is the mass of the support (g).

2.9. Enzymatic interesterification

Enzymatic interesterification reactions were conducted using an immobilized biocatalyst selected for its high enzymatic activity during immobilization. Reactions were performed at 40, 50, and 60°C for 12 hours with constant agitation at 150 rpm in a BOD incubator.

The reaction system included licuri oil and immobilized biocatalyst at a 15% (w/w) ratio relative to the oil mass, with 0.1 g of immobilized material used per assay. Following the reaction, the biocatalyst was separated by centrifugation. The oil phase was collected, refrigerated, and protected from light and oxygen to prevent oxidative degradation before

further analyses. Control assays were conducted under identical conditions in the absence of the biocatalyst to assess potential non-enzymatic changes. The experimental conditions were defined based on previously reported studies on enzymatic interesterification of oils (Zhang et al., 2021; Sivakanthan et al., 2019).

2.10. Rheological analysis

The rheological behavior of licuri oil before and after enzymatic treatment was evaluated using a rotational rheometer (HR20, TA Instruments, UK) equipped with a parallel plate geometry (40 mm diameter, 0.07 mm gap). Approximately 2 g of sample was carefully placed onto the lower plate, ensuring complete coverage of the measuring surface. Measurements were performed at a controlled temperature of 25 °C, over a shear rate range of 0.1-100 s⁻¹. The apparent viscosity was recorded as a function of shear rate to evaluate changes in the oil's flow behavior after enzymatic interesterification.

2.11. Thermal characterization by Differential Scanning Calorimetry

The thermal behavior of crude licuri oil and enzymatically interesterified samples obtained at 40, 50, and 60 °C was evaluated using differential scanning calorimetry (DSC) (TA Instruments DSC250, New Castle, DE, USA). The instrument was calibrated using indium as the reference standard, with nitrogen as the purge gas.

Approximately 13 mg of the sample was weighed into hermetically sealed aluminum pans, with an empty pan used as the reference. To eliminate thermal history and ensure complete melting of lipid crystals, samples were initially heated from 5 to 80 °C at 5 °C min⁻¹ and then maintained at 80 °C for 10 min. Crystallization profiles were then obtained by cooling the samples from 80 to -30 °C at 5 °C min⁻¹, followed by a 10 min isothermal period at -30 °C. Subsequently, the samples were reheated from -30 to 80 °C at the same rate to obtain melting profiles.

The thermal transitions were evaluated from the crystallization and melting thermograms by determining the onset temperature (T_{onset}), peak temperature (T_p), and transition enthalpy (ΔH) when applicable.

3. RESULTS AND DISCUSSION

3.1. Lipase production and process optimization by solid-state fermentation

Lipase production via solid-state fermentation was assessed using a Doehlert experimental design to examine the effects of fermentation time and inducer ratio on enzymatic activity. The results (Table 2) indicated that all tested conditions resulted in detectable lipase activity, thereby confirming licuri cake as a suitable substrate for enzyme production.

Table 2. Experimental results of lipase production by solid-state fermentation according to the Doehlert design.

Run	Time (h)	Inducer ratio (w/w%)	Protein concentration (mg/mL)	Enzymatic activity (U/mL)	Specific activity (U/mg)
1	39	15	2.509	19.59	7.81
2	93	15	2.675	66.89	25.01
3	12	10	3.588	31.12	8.67
4	120	10	1.802	10.02	5.56
5	39	5	2.402	28.23	11.75
6	93	5	2.895	17.12	5.91
7C	66	10	2.432	43.55	17.91
8C	66	10	2.319	46.82	20.19
9C	66	10	2.230	48.56	21.78

Center point (C)

Both variables had a significant influence: higher enzymatic activities were generally associated with intermediate-to-high fermentation times and elevated inducer concentrations. The maximum experimental activity ($66.89 \text{ U}\cdot\text{mL}^{-1}$) occurred at 93 hours with 15% inducer, indicating that lipid supplementation is a key factor in inducing lipase synthesis. This behavior is consistent with previous studies, which report that the presence of lipid substrates enhances lipase production by serving as both a carbon source and a metabolic inducer (Santos et al., 2021; Silva et al., 2022). Reduced enzymatic activity was observed under extreme fermentation conditions, particularly with prolonged fermentation time (120 hours), resulting in a decrease in activity ($10.02 \text{ U}\cdot\text{mL}^{-1}$). Extended incubation may lead to enzyme denaturation and nutrient depletion, thereby impairing catalytic performance. In contrast, triplicate experiments conducted at the central point (66 h and 10% inducer) yielded highly reproducible activity values, indicating satisfactory experimental precision. The observed variability is consistent with the expected behavior of biological systems and reflects the inherent heterogeneity of solid-state fermentation processes (Sánchez et al., 2024).

The effects of the investigated variables on lipase production were further assessed using analysis of variance (ANOVA) (Table 3). The results demonstrated that both the quadratic term for fermentation time ($p = 0.0080$) and the linear effect of the inducer ratio ($p = 0.0150$) were statistically significant for enzymatic activity. Additionally, the interaction between

fermentation time and inducer ratio was significant ($p = 0.0075$), indicating that the effect of one factor depends on the level of the other.

Table 3. Analysis of variance (ANOVA) for lipase production by solid-state fermentation using licuri coconut cake as substrate based on the Doehlert design.

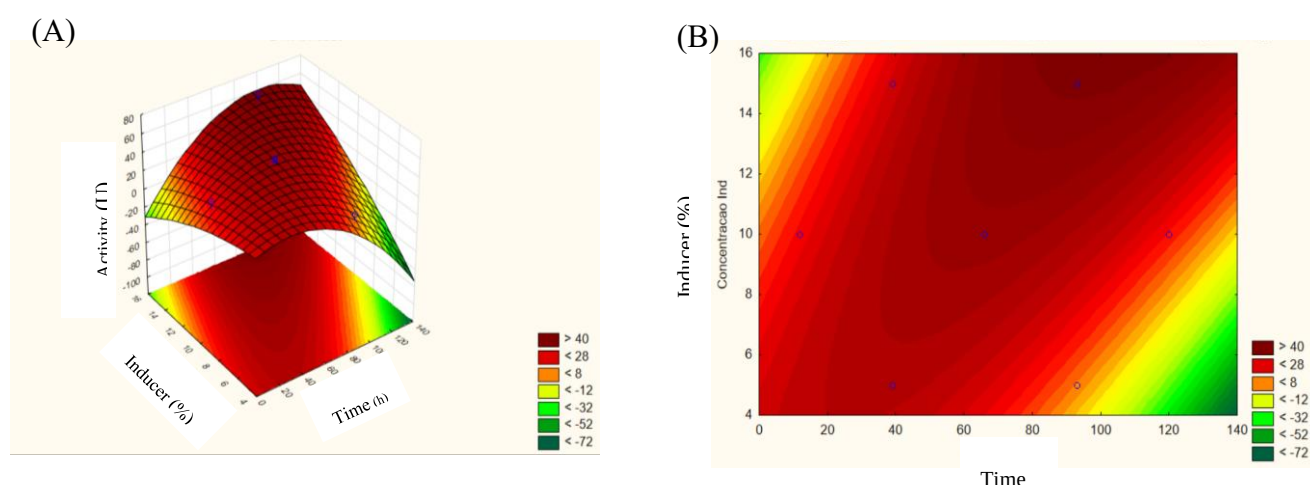
Source of variation	Sum of squares	Degree of freedom	Mean square	F - value	p-value
Time ²	795.020	1	795.020	122.50	0.0080
Inducer	423.213	1	423.212	65.21	0.0150
Inducer ²	102.023	1	102.023	15.72	0.0581
TimexInducer	852.782	1	852.782	131.40	0.0075
Lack of fit	547.250	1	547.250	84.32	0.0117
Pure error	12.980	2	6.490	-	-
Total	2654.984	8			

In contrast, the quadratic term for the inducer ratio was not statistically significant ($p = 0.0581$), although it exhibited a tendency to affect the response. The significance of the quadratic term for fermentation time indicates curvature in the response surface, which is characteristic of biological systems such as solid-state fermentation. This observation aligns with the findings of Lima et al. (2021), who reported that lipase production in solid-state systems generally peaks within 72–120 hours, followed by a decline due to metabolic limitations and enzyme instability. Similarly, Kumar et al. (2023) showed that oily carbon sources can enhance lipase production by acting as inducers, highlighting the strong dependence of enzymatic activity on both fermentation time and substrate composition.

Although certain model terms were significant, the analysis identified a significant lack of fit ($p = 0.0117$), indicating that the model does not adequately represent the experimental data. This limitation may result from the limited number of experimental runs and the inherent variability of solid-state fermentation systems, which are characterized by heterogeneity and sensitivity to minor environmental changes. Additionally, the adjusted coefficient of determination ($R^2 = 0.437$) indicates limited predictive capability. Therefore, although the model is useful for identifying general trends and significant factors, it is not suitable for precise predictions or for defining a definitive optimal condition. Thus, the results were interpreted by focusing on regions of higher enzymatic activity rather than a single optimal point.

The response surface analysis (Figure 1) visually represents the combined effects of fermentation time and inducer ratio on lipase production, thereby complementing the statistical results from the ANOVA. The surface plot demonstrates pronounced curvature, which aligns with the significant quadratic term for fermentation time and identifies a region of increased enzymatic activity at intermediate to high fermentation times and inducer concentrations.

Figure 1. Contour surface(A) Response surface (B) of *Penicillium roqueforti* lipase activity as a function of the effect of fermentation time (h) and inducer ratio (%).



The highest enzymatic activities were observed at fermentation times between 90 and 100 h and inducer concentrations ranging from 12 to 15%. These results closely match the experimental maximum observed at 93 hours and 15% inducer (66.89 U.mL⁻¹). These results confirm the synergistic interaction between the variables, as indicated by the significant interaction term ($p = 0.0075$). The contour plot (Figure 1B) suggests that the optimal region is near the upper boundary of the studied domain, as no well-defined peak appears within the experimental range. The response surface analysis corroborates the statistical results from ANOVA and highlights interactions among process variables, supporting the identification of a favorable region for lipase production rather than a single optimal point.

The response surface analysis identified a favorable region for lipase production at high fermentation times and inducer concentrations. However, because the predicted optimum was located at the edge of the experimental domain, the Doehlert design could not reliably establish the true optimum conditions, indicating the need for further optimization. Therefore, a Rotational Central Composite Design (RCCD) was subsequently employed to refine and validate the optimal region, allowing a more detailed exploration of the response surface

around the most promising conditions. The experimental results obtained with the RCCD are presented in Table 4.

Table 4. Experimental results obtained from the rotational central composite design (RCCD) for lipase production.

Experiment	Time (h)	Inductor ratio (%)	Enzimatic Activity (U/mL)
1	24	5.0	184.64
2	24	20.0	166.46
3	120	5.0	191.91
4	120	20.0	97.85
5	72	12.5	197.73
6	96	5.0	149.73
7	96	20.0	166.22
8	48	10.0	108.03
9	48	15.0	189.73
10	120	12.5	46.70
11	24	12.5	174.94
12	72	5.0	106.82
13	96	12.5	100.03
14	96	12.5	115.79
15	96	12.5	116.76

The data indicate that both variables significantly affected enzymatic activity, with higher values observed at intermediate fermentation times and moderate inducer levels. The maximum activity (197.73 U mL^{-1}) was obtained at 72 h with 12.5% inducer. Comparable activity levels were also observed within the ranges of 48-96 h and 10-15% inducer, defining a well-defined region of enhanced enzymatic performance.

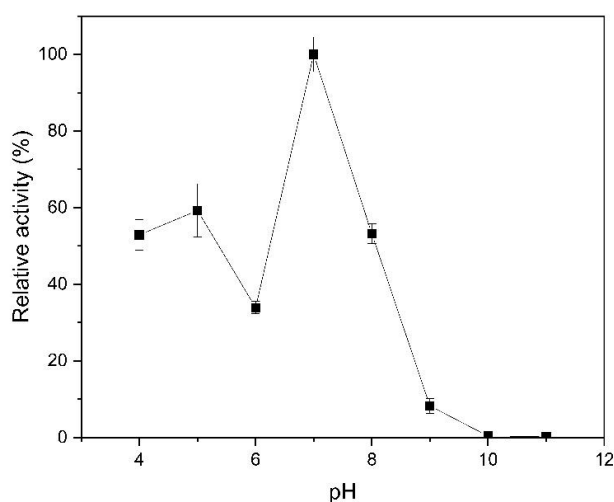
The RCCD model predicted an optimal condition at 96.2 hours and 14.8% inducer, consistent with the region identified in the Doehlert design. This condition was selected for subsequent experiments because it lies at the center of the optimal range. Selecting a central point prioritizes process robustness and reproducibility, as extreme experimental values may not represent stable operational conditions. Operating within this range improves control over fermentation parameters and facilitates process scalability, thereby reinforcing the suitability of the selected condition for further applications.

3.2. Effect of pH on lipase activity

The enzymatic lipase extract exhibited maximal activity at pH 7.0, with high activity maintained between pH 5 and 8 (Figure 2). A pronounced decline in activity was observed at

alkaline pH values above 9. Enzymatic activity is influenced by the ionization state of catalytic residues, typically serine, histidine, and aspartate. At acidic pH, excess protonation disrupts the organization of the active site, whereas at alkaline pH, excessive deprotonation leads to a loss of critical interactions (Roy et al., 2026). Similar behavior has been reported for lipases produced by *Penicillium* species, which typically present optimal activity in slightly acidic to neutral conditions. A recent study on lipase production by *Bacillus* spp. reported the highest activity ($6.90 \pm 0.10 \text{ U.mL}^{-1}$) at pH 6, indicating a preference for slightly acidic to neutral conditions (Hadiza et al., 2025). Likewise, Pandey et al. (2016) reported an optimal pH of 6.8 for lipase produced by *Penicillium aurantiogriseum*, with an activity of $16.718 \text{ IU mL}^{-1} \text{ min}^{-1}$. These results reinforce that lipases from both bacterial and fungal sources commonly exhibit maximum activity near neutral pH, which is associated with the optimal ionization state of catalytic residues at the active site (Sari et al., 2026). The preference for neutral pH is advantageous for potential applications in lipid modification processes, including enzymatic interesterification, since most edible oils are processed under near-neutral conditions. Operating close to the optimum pH minimizes enzyme deactivation and enhances catalytic efficiency in biotransformation reactions (Hadiza et al., 2025).

Figure 2. Effect of pH on the enzymatic activity of lipase at 40 °C.

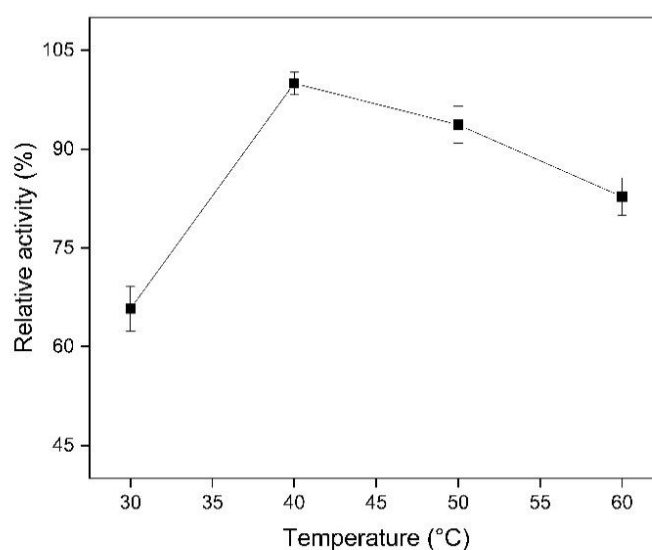


3.3. Effect of temperature on lipase activity

The enzymatic lipase extract exhibited maximal activity at 40°C, as illustrated in Figure 3. Temperature influences both the kinetic energy and the tertiary structure of enzymes. The increased activity at 40 °C may be attributed to enhanced enzyme-substrate collisions, which

facilitate activation. In contrast, elevated temperatures often result in denaturation of the protein's tertiary structure and disruption of non-covalent interactions such as hydrogen bonds and hydrophobic interactions (Riyad et al., 2024; Ali et al., 2023). Previous studies indicate that lipases typically exhibit optimal activity between 35 °C and 50 °C, with progressive denaturation at higher temperatures; these findings are consistent with those of the present study (Sari et al., 2026; Agaç et al., 2026). The observed optimum temperature of 40 °C is particularly advantageous for enzymatic interesterification and other lipid-modification processes. Operating under moderate temperatures reduces energy consumption and minimizes thermal degradation of oils, preserving sensitive compounds and maintaining product quality. In addition, mild reaction conditions enhance enzyme stability, increasing the feasibility of biocatalytic processes as sustainable alternatives to conventional chemical modifications, which typically require higher temperatures and harsher operating conditions (Sari et al., 2026; Riyad et al., 2024).

Figure 3. Effect of temperature on the enzymatic activity of lipase.

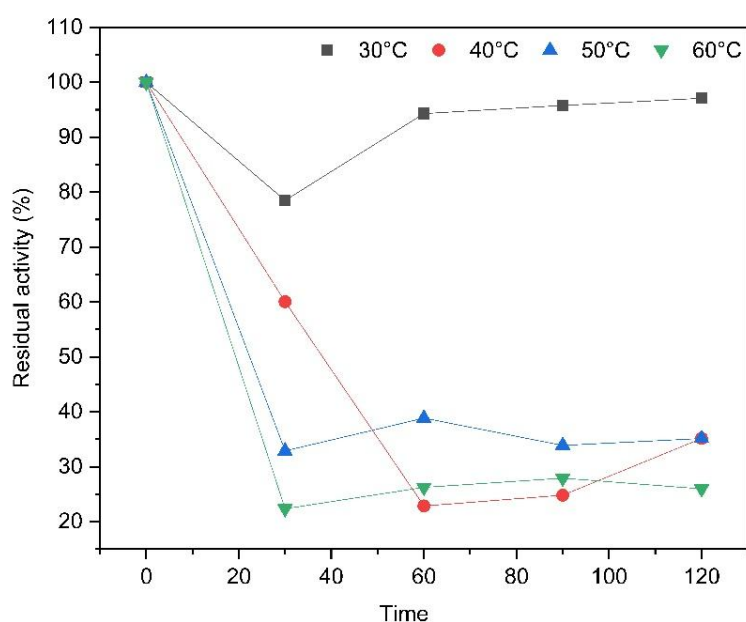


3.4. Thermal stability of the enzyme

The thermal stability profile of the enzyme extract showed mesophilic characteristics (Figure 4), with higher activity at moderate temperatures (30-40°C) and reduced activity at elevated temperatures (60°C). Mesophilic enzymes typically exhibit optimal performance at 25-45°C (Agaç et al., 2026; Ali et al., 2023). In this study, although the highest relative activity was initially observed at 40 °C (Figure 3), the thermal stability assay revealed a pronounced

loss of residual activity during incubation at this temperature (Figure 4). In contrast, at 30 °C the enzyme maintained approximately 100% of its residual activity throughout the 120 min incubation period, demonstrating superior operational stability. This finding indicates that the temperature associated with maximum catalytic activity is not necessarily the most suitable for prolonged operation, since higher temperatures accelerate thermal inactivation (Sari et al., 2026; Turati et al., 2019). The marked decline observed at 40 °C suggests that the enzyme undergoes progressive conformational changes that reduce catalytic efficiency over time, whereas the stability observed at 30 °C indicates preservation of its native structure under these conditions. Similar behavior has been reported for other fungal lipases, in which moderate temperatures ensure greater structural stability despite slightly lower (Kumar et al., 2023; Ali et al., 2023).

Figure 4. Thermal stability of the enzymatic extract at different temperatures (30, 40, 50, and 60 °C) over time (0–120 min). Residual activity was determined under optimal conditions and expressed as relative activity (%).



3.5. Enzyme kinetic parameters

The kinetic parameters K_m and V_{max} for lipase produced by *Penicillium roqueforti* were determined using p-nitrophenyl palmitate as the substrate. The kinetic data (Table 5) demonstrated a strong fit to the Michaelis-Menten model ($R^2=0.912$), indicating that the model sufficiently describes the enzymatic behavior (Edabami et al., 2025; Radwan et al., 2025). The

use of crude extract may result in interference from extraneous proteins, increased system heterogeneity, and potential diffusion limitations. The observed K_m value indicates moderate enzyme-substrate affinity, consistent with previously reported values for microbial lipases acting on p-nitrophenyl esters (Romo-Silva et al., 2024). A comparable behavior was reported by Popoola and Olateru (2021), who obtained a K_m value of 1.25 mg mL⁻¹ for a partially purified lipase from *Pseudomonas fluorescens*. Although the units and substrates used differ, both studies indicate moderate substrate affinity, reinforcing the notion that lipase, particularly in crude or partially purified systems, tends to exhibit higher K_m values due to structural complexity, substrate heterogeneity, and mass-transfer limitations. V_{max} represents the enzyme's catalytic capacity under the experimental conditions and is consistent with previously reported values for crude or partially purified lipase extracts. For instance, a lipase from *Bacillus subtilis kubl4* exhibited a V_{max} of 38.46 $\mu\text{M min}^{-1}$ using p-nitrophenyl palmitate as substrate, which is comparable to the value obtained in this study (42.68 $\mu\text{M min}^{-1}$) (Nadaf et al., 2025). These results indicate that the enzymatic extract exhibits significant catalytic capacity despite being a crude system. Variations in V_{max} values across studies are commonly attributed to enzyme source, purification level, and experimental conditions, with purified enzymes typically showing higher catalytic rates due to reduced mass-transfer limitations and the absence of interfering compounds (Zhao et al., 2021).

Table 5. Kinetic parameters obtained from the Michaelis–Menten model for the crude enzymatic extract.

Parameter	Value	Standard Error
V_{max} ($\mu\text{mol.min}^{-1}.\text{mg}^{-1}$)	42.68	± 3.69
K_m (mM)	1.36	± 0.47
R^2	0.913	—
Adjusted R^2	0.884	—

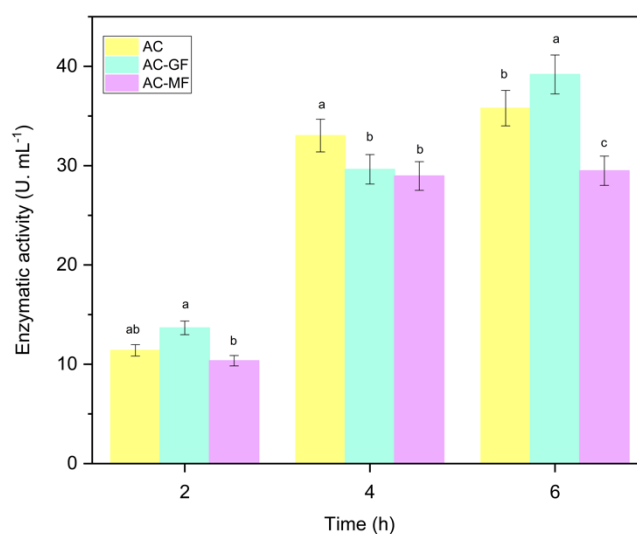
3.6. Immobilization of crude extract lipase on activated carbon: effect of support type and contact time

The effects of immobilization time and support functionalization were evaluated to identify the most effective biocatalyst for enzymatic interesterification. Beyond maximizing

catalytic activity, the study also considered process efficiency, aiming to reduce operational time and energy demand. Non-functionalized and functionalized activated carbons were evaluated across different immobilization times to determine optimal conditions that maximize enzymatic performance and ensure process feasibility.

At an immobilization time of 2 h, all evaluated supports, including non-functionalized activated carbon (AC), genipin-functionalized activated carbon (AC-GF), and iron-functionalized activated carbon (AC-MF), exhibited comparable enzymatic activities, with AC-GF showing a slight (13.67 U mL^{-1}), as illustrated in Figure 5. Increasing the immobilization time to 4 h resulted in higher activity across all supports, a trend that continued at 6 h, suggesting progressive enzyme adsorption toward equilibrium. Among the tested materials, AC-GF demonstrated the highest activity, whereas AC-MF exhibited the lowest, reaching 29.49 U.mL^{-1} at 6 h. The observed immobilization behavior can be explained by the physicochemical properties of the supports (Zhang et al., 2022; Santos et al., 2021). The enzymatic extract, derived from solid-state fermentation, was prepared in phosphate buffer at pH 7.0, which exceeds the point of zero charge (pHpzc) for the evaluated materials (5.32, 5.30, and 6.37 for AC, AC-GF, and AC-MF, respectively). Under these conditions, the surfaces of the supports are predominantly negatively charged, thereby reducing electrostatic interactions with the enzyme, which also tends to carry a negative charge at neutral pH (Bilal et al., 2021; Sheldon & Brady, 2022). Consequently, hydrophobic interactions and physical adsorption mechanisms likely play a more significant role in the immobilization process (de Oliveira et al., 2020; Rodrigues et al., 2023).

Figure 5. Effect of immobilization time (2, 4, and 6 h) and support type (AC, AC-GF, and AC-MF) on the enzymatic activity of immobilized lipase. Error bars represent standard deviation. Different letters above the bars indicate statistically significant differences between means according to Tukey's test ($p \leq 0.05$).



However, the differences in enzymatic activity observed among the supports cannot be explained solely by surface charge effects. Although AC and AC-GF exhibited very similar pH_{pzc} values, AC-GF consistently showed higher catalytic activity throughout the immobilization experiments. This result suggests that the functionalization strategy influenced the enzyme-support interaction beyond electrostatic considerations (Zhou et al., 2021; Li et al., 2023). Lipases possess a characteristic movable lid that covers the catalytic triad in aqueous media. Upon contact with hydrophobic surfaces, this lid undergoes conformational opening, exposing the catalytic site through the mechanism known as interfacial activation. Consequently, hydrophobic supports tend to immobilize lipases preferentially in their open and catalytically active conformation, often resulting in higher activity recovery than supports whose interactions are predominantly hydrophilic (Rodrigues et al., 2019; Zdarta et al., 2018). In the case of AC-GF, the presence of genipin likely promoted not only favorable hydrophobic interactions but also covalent stabilization through reactions with amino groups located on the enzyme surface. This combination may have stabilized the open conformation of the lipase while reducing enzyme desorption during immobilization, contributing to the higher catalytic activity observed for this support. In contrast, functionalization with Fe³⁺ ions and iminodiacetic acid introduces coordination sites capable of establishing stronger interactions with the enzyme. Although these interactions enhance enzyme anchoring, they also increase the hydrophilic character of the support due to the hydration shell surrounding the coordinated metal ions. Under these conditions, lipase molecules may adsorb preferentially in a closed-lid conformation, reducing accessibility of the catalytic pocket to the substrate. Furthermore, excessive restriction of conformational mobility may limit the structural flexibility required for

efficient catalysis, explaining the lower activity observed despite efficient immobilization.. Therefore, the distinct performances observed for AC, AC-GF, and AC-MF likely result from differences in enzyme-support interactions rather than from surface charge effects alone (Wang et al., 2022). Based on these results, AC-GF with an immobilization time of 6 h was selected for subsequent interesterification assays, as it exhibited the highest enzymatic activity (39.19 U. mL⁻¹) while maintaining practical process duration.

3.7. Rheological behavior of interesterified licuri oil using immobilized lipase

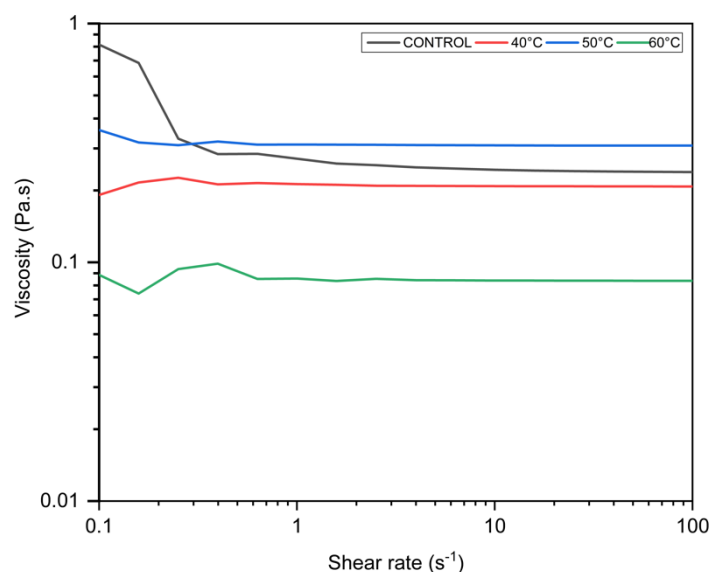
The rheological parameters indicate a marked change in the flow behavior of licuri oil following enzymatic treatment. The values for the flow behavior index (n), consistency index (K), and apparent viscosity are summarized in Table 6.

Table 6. Rheological parameters (consistency index, K, flow behavior index, n, and apparent viscosity) of crude and enzymatically modified licuri oil samples. ICG40, ICG50, and ICG60 - lipase immobilized on genipin-functionalized activated carbon, followed by the reaction temperature.

Sample	K (Pa·s ⁿ)	n	Apparent viscosity at 10 s ⁻¹ (Pa·s)	Flow behavior
Control	0.420	0.55	0.0303	Shear-thinning
ICG40	0.032	0.98	0.0315	Near-Newtonian
ICG50	0.034	0.97	0.0327	Near-Newtonian
ICG60	0.030	0.99	0.0300	Newtonian-like

As shown in Table 6, the crude oil exhibited a pronounced shear-thinning behavior (n=0.55). This is visually confirmed in Figure 6, where the high initial viscosity decays rapidly as the shear rate increases. This non-Newtonian behavior is typical of structured lipid matrices, where fat crystal aggregates are disrupted by mechanical shear (Nikolaev et al., 2026; Zhong et al., 2025).

Figure 6. Apparent viscosity as a function of shear rate for crude and enzymatically interesterified licuri oil samples



In contrast, all enzymatically interesterified samples transitioned to a near-Newtonian flow ($n \approx 0.97$ - 0.99), showing nearly horizontal lines in Figure 6. This transition, as discussed previously, results from the redistribution of fatty acids within the triacylglycerols, which prevents the formation of large, shear-sensitive crystalline networks (Silva, Stahl, Grimaldi, & Ribeiro, 2025; Liu, et al., 2025). This reorganization of triglyceride species impacts the crystallization profile, leading to a more homogeneous lipid distribution and reduced shear sensitivity (Batista, Macêdo, Luccas, & Macedo, 2025; Chen et al., 2025; Liu et al., 2025). Consequently, the stabilization of viscosity across the evaluated range observed in samples ICG40, ICG50, and ICG60 reflects more predictable flow behavior, which is essential for industrial processing (Singh et al., 2021).

These results indicate that enzymatic interesterification induces molecular rearrangements within triacylglycerols, yielding a more homogeneous distribution of molecular species, reduced shear sensitivity, and diminished shear-thinning with viscosity stabilization. These changes reflect altered intermolecular interactions resulting from repositioned fatty acids and modified packing within the lipid matrix. This reorganization, specifically the interchange of fatty acid moieties within and among triglyceride species, impacts the crystallization profile and physical characteristics of the oil blend (Singh et al., 2021), while reducing structured lipid domains or fat crystal networks responsible for non-Newtonian behavior (Iwe et al., 2023), explaining the Newtonian transition and viscosity variations among treated samples.

Additional studies are advised to more fully elucidate the thermal and structural consequences of these alterations. Methods such as differential scanning calorimetry and crystallization studies would yield important data on melting profiles, solid fat content, and polymorphism in interesterified lipids. Such evaluations would facilitate a thorough assessment of the effects of enzymatic interesterification on the physicochemical and functional attributes of licuri oil, particularly for applications in structured lipid development.

3.8. Thermal characterization by Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was utilized to examine the thermal behavior of both crude and enzymatically interesterified licuri oils. This analytical method yields detailed information on crystallization and melting transitions, facilitating the assessment of structural modifications resulting from enzymatic alteration of triacylglycerols. In lipid systems, crystallization and melting profiles are primarily determined by fatty acid composition and the specific distribution of fatty acids within the glycerol backbone. Consequently, DSC is frequently used to assess changes in crystal formation, thermal stability, and molecular organization following interesterification (Marangoni & Ghazani, 2018; Liu et al., 2025). Table 7 presents the crystallization parameters of crude licuri oil (OP) and enzymatically interesterified samples (ICG40, ICG50, and ICG60). The crystallization peak temperature (T_c) remained nearly constant across all samples, ranging from $-7.75\text{ }^{\circ}\text{C}$ to $-6.96\text{ }^{\circ}\text{C}$. This minimal variation indicates that enzymatic interesterification did not significantly affect the temperature of the primary crystallization event. Comparable results have been observed in enzymatically modified vegetable oils, where fatty acid redistribution influences crystal organization more than the initiation of crystallization (Batista et al., 2025; Chen et al., 2025).

Table 7. Thermal properties of crude licuri oil and enzymatically interesterified samples determined by differential scanning calorimetry (DSC).

Sample	T_c ($^{\circ}\text{C}$)	$T_{\text{onset, c}}$ ($^{\circ}\text{C}$)	ΔH_c (J.g^{-1})	T_m ($^{\circ}\text{C}$)	$T_{\text{onset, m}}$ ($^{\circ}\text{C}$)	ΔH_m (J.g^{-1})
Control	-7.46	-0.96	85.00	17.94	4.27	89.82
ICG40	-7.75	-5.99	91.98	17.08	3.43	95.33
ICG50	-6.96	-5.78	96.17	17.27	3.05	100.48
ICG60	-7.39	-6.11	108.00	16.44	9.80	111.00

T_c = crystallization peak temperature; $T_{\text{onset, c}}$ = crystallization onset temperature; ΔH_c = crystallization enthalpy; T_m = melting peak temperature; $T_{\text{onset, m}}$ = melting onset temperature; ΔH_m = melting enthalpy.

Despite the stability of T_c values, considerable differences were identified in the crystallization enthalpy (ΔH_c). The crude oil demonstrated a crystallization enthalpy of approximately 85 J. g^{-1} , while the interesterified samples exhibited progressively higher values, reaching about 108 J. g^{-1} for ICG60. The observed increase in crystallization enthalpy indicates that enzymatic interesterification facilitated the development of a more organized crystalline structure, necessitating greater energy release during crystal formation. This phenomenon is likely related to the redistribution of fatty acids among triacylglycerol molecules, resulting in improved molecular packing and enhanced crystallization efficiency (Silva et al., 2025; Liu et al., 2025).

The onset crystallization temperatures (T_{onset}) showed only minor variations among samples. These results indicate that the enzymatic treatment had a limited effect on the initiation of crystal formation but influenced the extent and organization of crystallization, as evidenced by the enthalpy values.

The melting thermograms also demonstrated differences between crude and interesterified oils. The melting peak temperature (T_m) decreased slightly after enzymatic treatment. This reduction suggests modifications in triacylglycerol composition and crystal stability, resulting in structures that melt at slightly lower temperatures. Similar reductions in melting temperature have been reported following enzymatic interesterification of vegetable oils and are generally attributed to the generation of new triacylglycerol species with different crystallization and melting characteristics (Norizzah et al., 2018; Liu et al., 2025).

More pronounced changes were observed in melting enthalpy (ΔH_m). The crude oil exhibited a melting enthalpy of 89.82 J. g^{-1} , whereas the interesterified samples presented progressively higher values, reaching 111 J. g^{-1} in ICG60. The increase in ΔH_m indicates that greater energy was required to disrupt the crystalline structures formed after interesterification, suggesting a more organized arrangement of lipid molecules. This observation is consistent with the crystallization results and supports the hypothesis that enzymatic rearrangement of fatty acids promoted modifications in crystal packing and molecular interactions within the lipid matrix.

Among the evaluated conditions, ICG60 exhibited the highest crystallization and melting enthalpies, indicating the most pronounced structural modification. The higher thermal transition enthalpies suggest that interesterification conducted at 60°C favored the formation of triacylglycerol capable of establishing more organized crystalline domains. This finding is particularly relevant because it aligns with the rheological results, which showed that interesterified samples exhibited a transition from pseudoplastic to near-Newtonian behavior.

Together, the DSC and rheological analyses indicate that enzymatic interesterification promoted molecular rearrangements that altered both the thermal properties and flow characteristics of licuri oil.

From an industrial perspective, these changes may be advantageous for applications requiring more predictable melting behavior and improved processability, such as lipid-based food formulations, confectionery fats, bakery products, and structured lipid systems. However, complementary analyses, including determination of solid fat content and polymorphic characterization, would be valuable for further elucidating the relationship between thermal behavior and crystalline structure in the interesterified oils.

4. Conclusion

This study validated a residue-based bioprocess in which licuri (*Syagrus coronata*) was used as a substrate for lipase production and a precursor for the synthesis of an immobilization support. The combination of solid-state fermentation, support functionalization, enzyme immobilization, and enzymatic interesterification demonstrates a circular approach to biomass utilization, maximizing the value obtained from a single agro-industrial residue. Beyond demonstrating the technical feasibility of the process, the results highlight the potential of licuri residues as versatile resources for sustainable biotechnological applications, supporting enzyme production, the development of immobilization supports, and lipid modification within a circular bioeconomy framework.

5. References

Abdelaziz, A., Abo-Kamar, A. M., Elkotb, E. S., & Al-Madboly, L. A. (2025). Microbial lipases: advances in production, purification, biochemical characterization, and multifaceted applications in industry and medicine. *Microbial Cell Factories*, 24(1), 40. <https://doi.org/10.1186/s12934-025-02664-6>

Adebami, G. E., Adebayo-Tayo, B. C., Kuila, A., Babalola, B. A., & Abiala, M. A. (2025). Biosourcing and optimization of fungal lipase production from cheap agro waste via solid state fermentation. *Scientific Reports*, 15(1), 20967. <https://doi.org/10.1038/s41598-025-06505-9>

Agaç, A., Dag, N., Tekin, S., Ozogui, Y. (2026). Optimization of culture conditions for microbial lipase production using fish processing waste as a substrate. *Bioresource Technology Reports*, 34(6), 102664. 10.1016/j.biteb.2026.102664

Anidiobu, V. O., Oladeji, B. S., & Anidiobu, C. O. (2025). Rheological, Physicochemical, and GC-MS of Fatty acid Composition of Palm Oil from Cross River State, Nigeria. *Turkish Journal of Agriculture - Food Science and Technology*, 13(10), 2906. <https://doi.org/10.24925/turjaf.v13i10.2906-2915.7802>

Batista, D., Macêdo, G. A., Luccas, V., & Macedo, J. A. (2025). Enzymatic Interesterification: An Innovative Strategy for Lower-Calorie Lipid Production From Refined Peanut Oil. *Journal of Food Science*, 90(10). <https://doi.org/10.1111/1750-3841.70603>

M. M. Bradford, “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding”, 1976.

Borkertas, S., Viškelis, J., Viškelis, P., Štreimikytė, P., Gasiunaite, U., & Urbonavičienė, D. (2025). Fungal Biomass Fermentation: Valorizing the Food Industry’s Waste. *Fermentation*, 11(6), 351. <https://doi.org/10.3390/fermentation11060351>

Chen, J., Zou, S., Mao, Y., Lee, Y., Lou, W., Sonwai, S., Wang, Y., & Zhang, Z. (2025). Effects of fatty acid positional rearrangement on palm-based oils properties during enzymatic glycerolysis modification. *Food Chemistry*, 498, 147106. <https://doi.org/10.1016/j.foodchem.2025.147106>

Elhazar, T., Kaya, B., & Caf, F. (2023). Determination of anti-cancer and antioxidant properties of protein extracts obtained from aquatic Helophorus (Coleoptera: Helophoridae) insects. *Ege Journal of Fisheries and Aquatic Sciences*, 40(1), 35. <https://doi.org/10.12714/egejfas.40.1.05>

Ikhwani, A. Z. N., Idris, I., Elfirta, R. R., & Ferdian, P. R. (2024). Production and Activity Characterization of Lipase from *Bacillus flexus* InaCC-B486. *Journal of the Turkish Chemical Society Section A Chemistry*, 11(2), 397. <https://doi.org/10.18596/jotcsa.1333916>

Iwe, M. O., Chibor, B. S., & Obasi, N. E. (2023). Optimization of the Chemical Interesterification of *Allanblackia* (*Allanblackia floribunda*), African Walnut (*Tetracarpidium conophorum*) and Fluted Pumpkin (*Telfairia occidentalis*) Seed Oil Blend for use in Bakery Shortening Production, a Response Surface Approach. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-2633267/v1>

Kumar, A., Verma, V., Dubey, V. K., Srivastava, A. K., Garg, S., Singh, V. P., & Arora, P. K. (2023). Industrial applications of fungal lipases: a review [Review of *Industrial*

applications of fungal lipases: a review]. *Frontiers in Microbiology*, 14. Frontiers Media. <https://doi.org/10.3389/fmicb.2023.1142536>

Leal, G. F., Cruz, M. de S., Santos, J. C. C., Sousa, H. M. S., Martins, G. A. de S., & Queiroz, F. (2025). *Syagrus coronata*: A bibliometric and critical review on nutritional composition, bioactive compounds, and future applications. *Trends in Food Science & Technology*, 166, 105384. <https://doi.org/10.1016/j.tifs.2025.105384>

Lima, A. C. M., Santos, C. M. D., Santos, I. L. D., Bastos, L. T. A. de, Santos, A. C. dos, Elias, F. C. de P., & Almeida, A. F. de. (2021). Use of agro-industrial residues for lipase production by *Candida viswanathii* in a solid-state cultivation system. *Scientia Plena*, 17(8). <https://doi.org/10.14808/sci.plena.2021.081513>

Liu, Y., Gao, P., Yang, Y., Liu, C., Zhong, W., Yin, J., & Reaney, M. J. T. (2025). Enzymatically Interesterified *Triadica sebifera* Oil: A Novel Shortening for Enhanced Nutritional Quality and Sustainability. *Foods*, 14(4), 590. <https://doi.org/10.3390/foods14040590>

Marangoni, A. G., & Ghazani, S. (2018). Facile lipase-catalyzes synthesis of a chocolate fat mimetic. *Scientific Reports*, 8. <https://doi.org/10.1038/s41598-018-33600-x>

Menezes, L. H. S. de, Santo, E. L. do E., Santos, M. M. O. dos, Tavares, I. M. de C., Mendes, A. A., Franco, M., & Oliveira, J. R. de. (2021). *Candida rugosa* lipase immobilized on hydrophobic support Accurel MP 1000 in the synthesis of emollient esters. *Biotechnology Letters*, 44(1), 89. <https://doi.org/10.1007/s10529-021-03196-w>

Moentamaria, D., Irfin, Z., Chumaidi, A., Widjaja, A., Widjaja, T., Muharja, M., & Darmayanti, R. F. (2024). Development of a Novel Support Modification for Efficient Lipase Immobilization: Preparation, Characterization, and Application for Bio-flavor Production. *Bulletin of chemical reaction engineering and catalysis*, 19(2), 215. <https://doi.org/10.9767/bcrec.20130>

Nascimento, P. A., Alves, A., Dos Santos, K. A., Veloso, C. M., Fontan, R. C. I., Sampaio, V. S., Bonomo, R. C. F. (2021). Optimization of lipase extraction from pequi Seed (*Caryocar brasiliense* Camb.). *Journal of Food Processing and Preservation*, 38(4). <https://doi.org/10.1111/jfpp.15616-6>.

Nikolaev, A., Goluntsov, A., & Plotnikova, K. (2026). A physically based rheological model for predicting the flow behavior of non-newtonian crude oil mixtures under cold climate conditions. *Frontiers in Chemical Engineering*, 8. <https://doi.org/10.3389/fceng.2026.1736520>

Ojeda-hernández, D., Cosío-Cuadros, R., Sandoval, G., Rodriguez-González, J., Mateos-Días, J. (2018). Solid-state fermentation as an economic production method of lipases. *Methods in Molecular Biology*, 1835, p. 217–228. https://doi.org/10.1007/978-1-4939-8672-9_12

Park, J., Yu, H., Charalampopoulos, D., Park, K., & Chang, P. (2023). Recent advances on erythorbyl fatty acid esters as multi-functional food emulsifiers. *Food Chemistry*, 432, 137242. Elsevier BV. <https://doi.org/10.1016/j.foodchem.2023.137242>

Reis, W. de S. M., Preto, A. O., Santana, G. de O., Tessaro, Í., Ferreira, A. L. G., Pereira, E. B., & Carvalho, A. K. F. de. (2025). Sustainable Alternative Media for the Production of Lipolytic Cells and Fatty Acid Concentrates: Integration of the Enzyme and Food Industries. *Foods*, 14(6), 990. <https://doi.org/10.3390/foods14060990>

Roy, T. K., Rubio, P., Cruz, J., Wauer, N., Hettiarachchi, E., Amaro, R. E., & Grassian, V. H. (2026). Revealing the Impact of pH on Lipase Structure and Surface Propensity at the Air–Water Interface and in Aqueous Aerosols. *The Journal of Physical Chemistry Letters*, 17(3), 818. <https://doi.org/10.1021/acs.jpclett.5c03315>

Sánchez, A., Oiza, N., Artola, A., Font, X., Barrena, R., Moral-Vico, J., & Gea, T. (2024). Solid-state fermentation: a review of its opportunities and challenges in the framework of circular bioeconomy. *Deleted Journal*, 81(601), 51. <https://doi.org/10.55815/424209>

Sari, N., Nurhasanah, Haryatu, T. (2026). Partial purification and characterization characterization acidophilic lipase from *Bacillus cereus* ALP E1. *Protein Expression and Purification*, 239, 106870. [10.1016/j.pep.2025.106870](https://doi.org/10.1016/j.pep.2025.106870)

Silva, T. P., Araujo, S. C., Santo, E. L. E., Gonçalves, M. S., Sampaio, I. C. F., Pereira, H. J. V., Irfan, M., Ferreira, M. L. O., Da Silva, E. G. P., De Oliveira, J. R., Franco, M. (2024). From byproducts to bioresources: cocoa shell solid-state fermentation lipase as a low-cost esterification catalyst. *Biomass Conversion and Biorefinery*, v. 15, p. 22603-22613. <https://doi.org/10.1007/s13399-024-05280-3>

Silva, T. T., da, Stahl, M. A., Grimaldi, R., & Ribeiro, A. P. (2025). Enzymatic interesterification of palm olein and hydrogenated palm oil: Effects on physicochemical

properties. *Food Research International*, 221, 117369.
<https://doi.org/10.1016/j.foodres.2025.117369>

Singh, P. K., Chopra, R., Garg, M., Dhiman, A., & Dhyani, A. (2022). Enzymatic Interesterification of Vegetable Oil: A Review on Physicochemical and Functional Properties, and Its Health Effects. *Journal of Oleo Science*, 71(12), 1697. Japan Oil Chemists' Society.
<https://doi.org/10.5650/jos.ess22118>

Sousa, F., Safri, A., & Gueciouer, A. (2025). Comparative flow behavior of oil sludge and crude oils from algerian storage tanks. *Studia Universitatis Babeş-Bolyai Chemia*, 177.
<https://doi.org/10.24193/subbchem.2025.3.12>

Stanciu, I. (2025). Rheological Behavior of Linseed (*Linum usitatissimum*) Oil. *Oriental Journal Of Chemistry*, 41(1), 282. <https://doi.org/10.13005/ojc/410133>

Stojanović, J., Krmar, J., Protić, A., Svrkota, B., Đajić, N., & Otašević, B. (2021). Experimental design in HPLC separation of pharmaceuticals. *Arhiv Za Farmaciju*, 71(4), 279.
<https://doi.org/10.5937/arhfarm71-32480>

Yang, D., Lee, Y., Lu, Y., Huang, Y., Lou, Z., Wang, Y., & Zhang, Z. (2025). Improved compatibility of palm olein and palm kernel stearin via enzymatic interesterification: Effect of newly-formed triacylglycerols. *Food Research International*, 213, 116558.
<https://doi.org/10.1016/j.foodres.2025.116558>

Zhong, M., Niu, Z., Fan, J., Huang, H., Li, N., Jia, L., & Zhang, H. (2025). Establishing the relationship between heavy oil viscosity and molecular markers using an enhanced neural network model. *Scientific Reports*, 15(1). <https://doi.org/10.1038/s41598-025-18561-2>

CAPÍTULO 6

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

Os resultados obtidos ao longo desta tese demonstram que os resíduos do licuri possuem elevado potencial de aplicação em processos biotecnológicos integrados, atuando tanto como substrato para a produção de enzimas quanto como matéria-prima para o desenvolvimento de suportes de imobilização enzimática.

A produção de lipase por fermentação em estado sólido, utilizando a torta de licuri, mostrou-se eficiente e sustentável, com elevada atividade enzimática alcançada sob condições otimizadas. A caracterização do extrato enzimático confirmou que ele é adequado para aplicações industriais, especialmente as que exigem condições operacionais moderadas.

A conversão da casca do licuri em carvão ativado funcionalizado mostrou-se uma estratégia eficaz para a obtenção de suportes com propriedades adequadas à imobilização enzimática. A funcionalização com genipina destacou-se por promover maior estabilidade e melhor desempenho catalítico, evidenciando a importância das interações químicas para a eficiência do biocatalisador.

Quando aplicada à interesterificação do óleo de licuri, a lipase imobilizada promoveu modificações estruturais confirmadas tanto por análise reológica quanto por calorimetria exploratória diferencial (DSC). A transição do comportamento pseudoplástico para próximo ao newtoniano, associada às alterações nos perfis térmicos de cristalização e fusão, evidenciou rearranjos na organização dos triacilgliceróis, confirmando o potencial do sistema desenvolvido para a produção de lipídios estruturados.

De modo geral, o estudo demonstrou ser possível integrar diferentes etapas da produção da enzima, do desenvolvimento do suporte e da aplicação catalítica em uma estratégia alinhada aos princípios da economia circular e da sustentabilidade. Além disso, contribui para a valorização dos recursos regionais do semiárido brasileiro, com potencial de impacto econômico e tecnológico.

Como perspectivas futuras, recomenda-se a realização de análises complementares, como a determinação do conteúdo de gordura sólida e a caracterização dos polimorfos cristalinos, que seriam importantes para aprofundar a compreensão da relação entre o comportamento térmico e a organização cristalina dos óleos interesterificados e para a melhor compreensão das alterações físico-químicas dos lipídios modificados. Sugere-se também a realização de estudos de reuso do biocatalisador, o escalonamento do processo e a avaliação

funcional dos lipídios estruturados, o que poderá ampliar a aplicabilidade industrial do sistema proposto.