UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE TECNOLOGIA
DEPARTAMENTO DE ENGENHARIA QUÍMICA
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA QUÍMICA

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EXPLORING THE FEASIBILITY OF ENZYMATIC BIODIESEL PRODUCTION FROM TILAPIA (*OREOCHROMIS NILOTICUS*) WASTE OIL

FORTALEZA
2024

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Dissertação apresentada ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal do Ceará, como requisito à obtenção do título de Mestre em Engenharia Química. Área de concentração: Processos químicos e bioquímicos.

Orientador: Prof. Dr. José Cleiton Sousa dos Santos.
Coorientadora: Profa. Dra. Maria Alexsandra de Sousa Rios

FORTALEZA
2024

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

S698e Sousa, Patrick da Silva.
Exploring the feasibility of enzymatic biodiesel production from tilapia (Oreochromis Niloticus) waste oil / Patrick da Silva Sousa. – 2024.
137 f. : il. color.

Dissertação (mestrado) – Universidade Federal do Ceará, Centro de Tecnologia, Programa de Pós-Graduação em Engenharia Civil: Estruturas e Construção Civil, Fortaleza, 2024.
Orientação: Prof. Dr. José Cleiton Sousa dos Santos.
Coorientação: Prof. Dr. Maria Alexsandra de Sousa Rios.

1. Biodiesel Production. 2. Fish Waste Oil Valorization. 3. Enzymatic Biocatalysis. 4. Molecular Simulation. 5. Green Chemistry. I. Título.

CDD 624.1

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Aprovada em: 11/10/2024.

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A Deus.

A minha família.

AGRADECIMENTOS

Primeiramente, sou grato a Deus e ao Universo por todas as oportunidades que tive de crescimento durante minha vida. O acesso aos estudos, ao conhecimento e a pessoas incríveis que conheci durante a caminhada. É uma crença particular acreditar que para tudo existe um determinado tempo para sua ocorrência debaixo do céu. Ou seja, estar aqui é encerrar um ciclo que comecei há alguns anos e eu tenho muita satisfação em poder estar finalizando esse processo com saúde e espero que com maestria. Há também um ditado em iorubá que sintetiza muito bem tal experiência que diz o seguinte: “Exu matou um pássaro ontem com uma pedra que só jogou hoje”.

A minha família, minha avó (Alaide) e minha mãe (Silvia Helena) que sempre se esforçaram para me dar a melhor educação possível dentro de suas condições. Obrigado pelo apoio, mas também pelo incentivo e por todos os conselhos durante essa caminhada. O amor de vocês é impulso para enfrentar tais adversidades. Espero um dia honrar todo investimento.

Aos meus amigos, Simão, Marliete e Wladilson pela cumplicidade, apoio e companheirismo. Certamente os dias ficaram bem mais leves tendo todo o suporte de vocês.

A Universidade Federal do Ceará (UFC) e ao Programa de Pós-Graduação em Engenharia Química (PPGEQ), por proporcionar educação de qualidade e acreditar que através do conhecimento o mundo pode ser rearranjado. Obrigado por todo suporte e infraestrutura para entrega de pesquisa de qualidade.

Aos professores José Cleiton e Maria Alexsandra pelos conselhos e tempo dedicados a esse trabalho. Obrigado por serem excelentes naquilo que escolheram como profissão e por se tornarem referência não apenas no âmbito acadêmico, mas também como ser humano.

Ao Grupo de Engenharia Enzimática (GENEZ) pela oportunidade de colaborar com o desenvolvimento de pesquisa científica, suporte, infraestrutura e direcionamento desse trabalho. Por compartilhamos metas, ambições e sonhos. Além de ter sido o primeiro contato com a pesquisa cientifica e o começo de uma longa jornada construindo conhecimento e dividindo experiencias. Aos meus amigos: Kaianny, Paulo, Viviane, Dayana, Valdilane, Juliana, Albano e Kátia.

Aos Grupos de Inovações Tecnológicas e Especialidades Químicas (GRINTEQUI/UFC), de Química de Materiais Avançados (GQMAT/UFC) e de Química Teórica (GQT/UFC) por todo incentivo, apoio e infraestrutura necessária para executar com maestria todas as etapas desse projeto.

As agências de fomento que auxiliaram na manutenção das estruturas, equipamentos e reagentes utilizados neste trabalho: Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

A todos que acreditam no poder revolucionário da universidade pública, pesquisa, ensino e extensão!

“Eles combinaram de nos matar, mas nós combinamos de não morrer.” (CONCEIÇÃO EVARISTO)

RESUMO

Os resíduos de pescado, seus subprodutos encontrados na natureza ou gerados pela indústria de processamento representam uma parcela significativa na obtenção de produtos de alto valor. Entre os produtos que podem ser obtidos a partir desses resíduos estão: fertilizantes, biomateriais, cosméticos, biolubrificantes e biodiesel. Dessa maneira, realizou-se uma análise bibliométrica acerca do potencial de produção de biodiesel a partir da utilização do óleo residual de peixe e foi observada uma lacuna a respeito da síntese enzimática de biodiesel possuindo o óleo residual de tilápia (*Oreochromis Niloticus)* como substrato e empregando a Lipase Eversa®Transform 2.0 como catalisador. Em sequência, foi realizado um estudo teórico e experimental sobre a produção biocatalítica de biodiesel através da hidroesterificação enzimática do óleo residual de tilápia. A metodologia Taguchi foi aplicada durante a etapa de esterificação onde foram avaliados os parâmetros: temperatura (25, 40 e 55 ºC), razão molar (1:1, 1:5 e 1:9), porcentagem de biocatalisador (1, 5 e 9%), e o tempo reacional (1, 3 e 5 h). Aplicou-se o delineamento experimental e os resultados de conversão variaram entre 19,3 ± 0,38 % (1:9, 3 h, 55 ºC e 1% de biocatalisador) a 83,8 ± 0,29% (1:5, 3 h, 25 ºC e 9% de biocatalisador). Os fatores que se destacaram foram a quantidade de biocatalisador seguida pela temperatura. Os níveis de reação ideais obtidos após o tratamento estatístico foram 5 h de reação a 25 °C na proporção molar de 1:1 (AGL/etanol) utilizando 9% do biocatalisador indicando uma conversão teórica de 96,73%. Após a realização da reação com os parâmetros indicados obteve-se uma conversão experimental de 89,94 ± 0,09%. Denota-se, que o valor de conversão experimental foi inferior ao da conversão teórica. O bioproduto foi devidamente caracterizado quanto as propriedades de viscosidade e densidade e obtendo os valores de 2,47 cSt e 883,6 Kg/m³, respectivamente. Além disso, foi realizado um estudo de docking e dinâmica molecular que avaliou a estabilidade da Lipase Eversa Transform 2.0 com os AGLs e observou-se o acoplamento do sítio catalítico da enzima com o substrato. Por fim, o óleo residual de tilápia mostrou-se uma matéria-prima alternativa para síntese de bioprodutos e o presente estudo colabora na elaboração de processos bioquímicos sustentáveis.

Palavras-chave: Produção de Biodiesel; Valorização de Óleo Residual de Peixe; Biocatálise Enzimática; Simulação Molecular; Química Verde.

ABSTRACT

Fishing waste and its by-products found in nature or generated by the processing industry represent a significant portion in producing high-value products. Among the products that can be obtained from these residues are: fertilizers, biomaterials, cosmetics, biolubricants, and biodiesel. Thus, a bibliometric analysis was carried out on the potential for biodiesel production from the use of residual fish oil and a gap was observed regarding the enzymatic synthesis of biodiesel using residual tilapia oil (*Oreochromis Niloticus*) using Lipase Eversa®Transform 2.0 as a catalyst. Subsequently, a theoretical and experimental study was carried out on the biocatalytic production of biodiesel through enzymatic hydroesterification of tilapia waste oil. The Taguchi methodology was applied during the esterification step, where the following parameters were evaluated: temperature (25, 40, and 55 ºC), molar ratio (1:1, 1:5, and 1:9), biocatalyst percentage (1, 5, and 9%), and reaction time (1, 3, and 5 h). The experimental design was applied and the conversion results ranged from 19.3 ± 0.38% (1:9, 3 h, 55 ºC, and 1% biocatalyst) to 83.8 ± 0.29% (1:5, 3 h, 25 ºC, and 9% biocatalyst). The factors that stood out were the amount of biocatalyst followed by temperature. The ideal reaction levels obtained after statistical treatment were 5 h of reaction at 25 ºC in a molar ratio of 1:1 (AGL/ethanol) using 9% of the biocatalyst, indicating a theoretical conversion of 96.73%. After carrying out the reaction with the indicated parameters, an experimental conversion of 89.94 ± 0.09% was obtained. It is noted that the experimental conversion value was lower than the theoretical conversion. The bioproduct was properly characterized regarding viscosity and density properties, obtaining values of 2.47 cSt and 883.6 kg/m^3 , respectively. Furthermore, a docking and molecular dynamics study was performed to evaluate the stability of Lipase Eversa Transform 2.0 with FFAs and observed the coupling of the enzyme's catalytic site with the substrate. Finally, tilapia waste oil proved to be an alternative raw material for the synthesis of bioproducts and the present study contributes to the development of sustainable biochemical processes.

Keywords: Biodiesel Production; Fish Waste Oil Valorization; Enzymatic Biocatalysis; Molecular Simulation; Green Chemistry.

LISTA DE FIGURAS

Figure 2. 1 – Products that can be derived from utilizing fish processing waste as a raw material 25
Figure 2. 2 – Operational schematic of the hydroesterification process to produce biodiesel 30
Figure 2. 3 – Transesterification reaction of a triglyceride in the presence of short-chain alcohol (methanol) to obtain fatty acid methyl esters 31
Figure 2. 4 – Esterification reaction between fatty acids and short-chain alcohol to obtain ethyl esters of fatty acids 32

Figure 3. 1 – Number of articles published and citations during the period investigated 45
Figure 3. 2 – The countries with the highest number of scientific publications on the production of residual fish oil 46
Figure 3. 3 – The countries with the highest average number of citations per document 47
Figure 3. 4 – Distribution of global scientific production on the production of biodiesel from residual fish oil. 47
Figure 3. 5 – Global collaboration network between the countries that publish the most articles regarding the production of biodiesel from waste fish oil 48
Figure 3. 6 – Global collaboration network among institutions for the publication of scientific papers on biodiesel production from residual fish oil 49
Figure 3. 7 – Authors who have published the most on the production of biodiesel from waste fish oil 51
Figure 3. 8 – Most cited authors in the production of articles regarding the production of biodiesel using waste oil from the fish processing industry. 51
Figure 3. 9 – Prominent research areas in the publication of articles about the production of biodiesel using fish oil as raw material 55
Figure 3. 10 – Density map of the 20 most prominent keywords in the field of biodiesel production 57
Figure 3. 11 – Network of connections among the most frequently occurring keywords in the investigated research field 59
Figure 3. 12 – Network of connections among prominent keywords in the field of study from 2013 to 2023 59

Figure 4. 1 – Structures in 2D of the fish oil composition 68
Figure 4. 2 — Contour surface plots showing the relationship between the most influential factor and the other parameters. (A) Molar ratio (%) versus Biocatalyst (%). (B) Temperature (ºC) versus Biocatalyst (%). (C) Time versus Biocatalyst (%) 76
Figure 4. 3 — Analysis of the Effect of Investigated Reaction Parameters on the S/N Ratio in Enzymatic Biodiesel Synthesis: Influence of Molar Ratio, Biocatalyst Concentration, Temperature, and Reaction Time 77
Figure 4. 4 — Regular probability graph obtained as a comparison between theoretical and experimental methods 78
Figure 4. 5 – ^{1}H Nuclear Magnetic Resonance (NMR) spectrum for the sample of ester mixture with expansions of ethyl oleate as the major product 80
Figure 4. 6 – HPLC chromatogram of ethyl oleate, the major product in the reaction medium for enzymatic esterification 81
Figure 4. 7 – The active site of Eversa lipase with the catalytic triad Ser 153, His 268, and Asp 206 (A-G) 83
Figure 4. 8 – Root Mean Square Deviation (*RMSD*) for the initial conformation of the

complexes versus the simulation time (nanoseconds) in the production step. Stearic acid (black); Palmitoleic acid (red); Palmitic acid (green); Oleic acid (blue); Myristic acid (orange); Linolenic acid (brown); and Linoleic acid (indigo).....85
Figure 4. 9 – Hydrogen bonds formed between the protein and the ligand during the two 86
Figure 4. 10 – Root Mean Square Fluctuation (RMSF), concerning the initial confirmation of the ligand-enzyme complex versus the simulation time (ns) in the production simulations step of the MD with fish oil composition/Eversa lipase 88
Figure 4. 11 – Solvent accessible surface area (SASA) of the Eversa lipase as a function of time from the MD simulations. The curves are running averages of the raw data with a window of 100 ns.....90

LISTA DE TABELAS

Table 2. 1 – Analysis of enzyme classifications and their pertinent attributes 35

Table 3. 1 – Academic institution ranking in biodiesel research 49
Table 3. 2 – Agencies that stood out in collaboration for the publication of articles on the analyzed topic 50
Table 3. 3 – Ranking of scientific journals with the highest contribution to the publication of research on biodiesel production from residual fish oil 53
Table 3. 4 – Most cited articles on the production of biodiesel from the use of residual fish oil 54
Table 3. 5 – The twenty most frequently used keywords in research and articles on biodiesel synthesis from residual fish oil.....56

Table 4. 1 — Codified levels of experimental design and independent parameter scope 67
Table 4. 2 – L9 orthogonal array of experimental runs accompanied by ethyl ester conversion and signal-to-noise (S/N) ratio 72
Table 4. 3 – Mean S/N ratio for the reaction parameters 74
Table 4. 4 – Results of the ANOVA 75
Table 4. 5 – Molecular docking result with its dock scores. 82
Table 4. 6 – Interactions of ligands with lipase after docking 83

SUMÁRIO

CHAPTER 1 – INTRODUCTION 17
1.1 Introduction 18
1.2 Objectives 19
1.2.1 Main objective 19
1.2.2 Specific objectives 19
CHAPTER 2 – BIBLIOGRAPHIC REVIEW 21
2.1 Biodiesel 22
2.1.1 Raw materials for production 22
2.1.1.1 First generation 23
2.1.1.2 Second generation 23
2.1.1.3 Third generation 23
2.1.1.4 Fourth generation 24
2.2 Fish oil as a feedstock for biodiesel 24
2.2.1.1 Physical method 26
2.2.1.2 Chemical method 26
2.2.1.3 Biological processes 27
2.2.1.3.1 Fermentation 27
2.2.1.3.2 Hydrolysis 27
2.3 Influential reaction parameters in biodiesel synthesis 27
2.3.1 Molar ratio 27
2.3.2 Catalyst concentration 28
2.3.3 Temperature 28
2.3.4 Type of alcohol 28
2.4 Production routes 29
2.4.1 Hydroesterification 29
2.4.2 Transesterification 30
2.4.3 Esterification 31
2.5 Types of Catalysts 32
2.5.1 Homogeneous catalyst 32
2.5.1.1 Alkaline catalysts 32
2.5.1.2 Acid catalysts 33
2.5.2 Heterogeneous catalysts 33
2.6 Enzymes 35
2.6.1 Lipases 36
2.6.1.1 Thermomyces lanuginosus lipase 37
2.6.1.2 Eversa® Transform 2.0 lipase 37
CHAPTER 3 – SUSTAINABLE BIODIESEL PRODUCTION FROM FISH WASTE OIL: A BIBLIOMETRIC REVIEW AND FUTURE PERSPECTIVES 40
3.1 Abstract 41
3.2 Introduction 42
3.3 Methodology 43
3.3.1 Data source 43
3.3.2 Data collection 44
3.3.3 Data extraction 44
3.4 Results and Discussions 45
3.4.1 Bibliometric Analysis 45
3.4.1.1 Trend of global publications and citations 45
3.4.1.2 Contributions of countries/regions 45
3.4.1.3 Contributions of Institutions 48

3.4.1.4 Contributions of Funding Agencies 50
3.4.1.5 Contributions of Authors 50
3.4.1.6 Journal Analysis 52
3.4.1.7 Most articles cited 53
3.4.1.8 Research area 55
3.4.2 Hot research topics 56
3.4.2.1 Keyword analysis 56
3.5 Conclusions 60
CHAPTER 4 – A STUDY ON BIODIESEL PRODUCTION FROM TILAPIA (*OREOCHROMIS NILOTICUS*) WASTE OIL USING EVERSA® TRANSFORM 2.0 LIQUID LIPASE: THEORETICAL AND EXPERIMENTAL APPROACHES 62
4.1 Abstract 63
4.2 Introduction 64
4.3 Methodology 64
4.3.1 Materials 66
4.3.2.2 Acidity index 67
4.3.2.3 Statistical analysis and experimental matrix (Taguchi Methodology) 67
4.3.2.4 Theoretical study 68
4.3.2.5. Physicochemical characterization of the bioproduct produced 71
4.3.2.5.1 Kinematic viscosity at 40 ºC 71
4.3.2.5.2 Density at 20ºC 71
4.3.2.5.3 Gas Chromatography-Mass Spectrometry (GC/MS) 71
4.3.2.5.4 High-performance liquid chromatography (HPLC) 71
4.3.2.5.5 Nuclear Magnetic Resonance (NMR) 71
4.4 Results and discussion 72
4.4.1 Enzymatic hydrolysis 72
4.4.2 Parameter optimization using the Taguchi method 72
4.4.2.1 Signal-to-Noise (S/N) Analysis 74
4.4.2.2 Analysis of Variance (ANOVA) 75
4.4.3 Conversion Statistical Analysis 76
4.4.4 Analysis and characterization of the obtained bioproduct 78
4.4.4.1 Density and viscosity 78
4.4.4.3 High-performance liquid chromatography (HPLC) 81
4.4.5. In silico study 81
4.4.5.1 Molecular docking 81
4.4.5.2 Molecular dynamics simulations 84
4.4.5.3 RMSF 88
4.4.5.4 SASA calculations 89
4.6 Conclusions 90
CHAPTER 5 – FINAL CONSIDERATIONS 92
5.1 Final considerations 93
REFERENCES 94

CHAPTER 1

CHAPTER 1 – INTRODUCTION

1.1 Introduction

In recent years, there has been a notable global emphasis on the sustainability of fuel and energy sources, primarily attributed to the limited availability of petroleum reserves (Hassan *et al.*, 2024; Holechek *et al.*, 2022; Sharma; Shrestha, 2023). The increasing attention towards biodiesel is driven by pressing concerns regarding the sustainability of current energy consumption and supply practices (Ali Ijaz Malik *et al.*, 2024; Gonçalves *et al.*, 2023; Suhara *et al.*, 2024). Biodiesel is a versatile fuel that functions both as a blending component and a direct alternative to conventional petrodiesel, easily used in current engines and produced from widely available bio-based resources (Benti *et al.*, 2023; Maheshwari *et al.*, 2022).

Biodiesel, produced from vegetable oils or animal fats, provides important environmental benefits (Brahma *et al.*, 2022; Demirbas; Karslioglu, 2007; Issariyakul; Dalai, 2014). These include reduced greenhouse gas emissions and lower toxicity. However, the use of traditional raw materials like edible vegetable oils raises concerns about competition with the food chain and long-term sustainability (Abdullah *et al.*, 2024). Therefore, alternative sources, such as waste oils, have become more important.

In Brazil, tilapia (*Oreochromis niloticus*) stands out as the leading fish species in aquaculture (Carvalho *et al.*, 2022; Valenti *et al.*, 2021). In 2023, farmed fish production reached approximately 887,029 tons, a 3.1% increase compared to 2022, according to the Brazilian Association of Aquaculture. Among the most cultivated species, tilapia leads with 579,080 tons, accounting for 65.3%. States such as Paraná, São Paulo, Minas Gerais, Santa Catarina, Mato Grosso do Sul, and Pernambuco are the top producers, contributing a significant portion of this output (Associação Brasileira de Piscicultura, 2023). With a large amount of waste generated by this activity, tilapia residual oil emerges as a potentially underutilized feedstock for biodiesel production.

In recent times, enzymatic biodiesel production has garnered significant attention owing to its potential to mitigate environmental impacts and bolster process efficiency (Kamal Pasha *et al.*, 2024; Xia *et al.*, 2024). Additionally, the utilization of non-traditional raw materials, such as waste oils derived from animals, aligns with prevailing global principles of circular economy and sustainability (Sundaram *et al.*, 2024). Thus, examining tilapia residual oil for enzymatic biodiesel production presents a distinct opportunity to foster innovation in both the biofuel industry and aquaculture waste management.

In this research, we aim to assess the potential for producing biodiesel from tilapia waste oil using enzymatic processes. We will analyze the process yield, evaluate the efficiency of the lipases utilized, and investigate the sustainability of this innovative approach. Chapter 2

will feature a comprehensive literature review encompassing relevant topics to provide a thorough contextualization and enhance comprehension of the study.

In Chapter 3, a bibliometric analysis was carried out of scientific works published on the potential of residual fish oil in biodiesel production from 2010 to 2024. This analysis highlights the countries that contributed the most publications, the main organizations involved in the development of the research, the journals with the largest number of publications on the subject, the most productive authors, in addition to an analysis of the interactions between organizations, authors, journals and countries.

In Chapter 4, a theoretical and experimental study of the enzymatic biodiesel production from tilapia (*Oreochromis niloticus*) waste oil is presented. Initially, enzymatic hydrolysis of the substrate was performed and, subsequently, the experimental planning was outlined, and a free enzymatic catalyst was used to conduct the reaction. The experimental design was based on the Taguchi methodology. The physicochemical characterization of the bioproduct was performed aiming at future applications. In addition, a theoretical docking and molecular dynamics study was conducted to evaluate the coupling of the catalytic site between the enzyme and the substrate. In Chapter 5, the final considerations on the chapters discussed are presented.

1.2 Objectives

1.2.1 Main objective

Conduct a theoretical and experimental study on the production of biodiesel via enzymatic route, using residual tilapia (*Oreochromis Niloticus*) oil as a substrate and the Eversa® Transform 2.0 enzyme as a biocatalyst, to evaluate the process efficiency and the properties of the biodiesel produced.

1.2.2 Specific objectives

a. To conduct a bibliometric analysis of the scientific production related to biodiesel production from fish waste oil, identifying major research trends, leading authors, institutions, countries, and key journals, as well as research gaps and future perspectives on the topic;
b. To perform the physicochemical characterization of residual tilapia oil;
c. To synthesize biodiesel via the enzymatic route using residual tilapia oil and Eversa® Transform 2.0 as a biocatalyst;

d. To evaluate the influence of temperature, molar ratio, reaction time, and biocatalyst concentration on process efficiency;

e. To report the physicochemical properties of ethyl esters derived from residual tilapia oil;

f. To develop and validate the structure of Eversa® Transform 2.0 through homology modeling;

g. To conduct in silico analyses to theoretically evaluate molecular docking and calculate the interaction energies between the enzyme and the substrate;

h. To investigate the stability of lipase-ligand complexes through molecular dynamics simulations.

CHAPTER 2

CHAPTER 2 – BIBLIOGRAPHIC REVIEW

2.1 Biodiesel

The global energy and environmental crisis have driven the development of renewable energy sources, therefore, aiming to reduce the amount of carbon released into the atmosphere due to the worsening greenhouse effect (Alves *et al.*, 2024; Ismaeel *et al.*, 2024; Sánchez-Solís *et al.*, 2024). Thus, biodiesel, a mixture of fatty acids, presents itself as an alternative to traditional fuels from non-renewable and highly polluting sources (Bhattacharyya, 2022). Biodiesel has advantages such as lower toxicity, lower corrosion, and greater biodegradability (Cruz *et al.*, 2019). The combustion of biodiesel produces smaller amounts of carbon dioxide, one of the main greenhouse gases, compared to traditional diesel (Rathore *et al.*, 2022). This reduction is because the carbon dioxide released during the combustion of biodiesel is part of the natural carbon cycle being originally absorbed by the plants used to produce the raw material (Monika; Banga; Pathak, 2023).

These triglycerides can be obtained from raw materials of organic and renewable origin. Renewable lipids such as microalgae, vegetable oils, residual cooking oil, and animal fats may be turned into biodiesel (Fayyazi *et al.*, 2015; López-Yerena *et al.*, 2022). One of its primary benefits is the ability to blend biodiesel and petroleum diesel in certain ratios to give sustainable fuel choices. Blends with varying percentages of biodiesel blended with petroleum diesel are known as B5, B10, B20, and B100 (Tamrat *et al.*, 2024). By including biodiesel into the fuel blend, we can reduce our reliance on fossil fuels and mitigate the environmental damage caused by traditional diesel use (Babadi *et al.*, 2022).

2.1.1 Raw materials for production

The choice of primary material is subject to climatic conditions specific to the national territory, and selecting the right raw material can save production costs and streamline the process, with free fatty acids (FFA) being an important component of raw materials converted into biodiesel (Mizik; Gyarmati, 2021; P. Ramos *et al.*, 2017). An excess of free fatty acids can negatively impact biodiesel production, leading to undesirable characteristics such as saponification (Babadi *et al.*, 2022). The raw components include vegetables, algae, animal fats, and microbial oil. It is important to note that the purity and content of biodiesel vary according to the type of feedstock employed, with cost, composition, conversion, and local considerations all influencing the material choice (Li *et al.*, 2016).

Biodiesel may be produced using a variety of raw materials. Thus, four generations are organized to group substrates with similar characteristics.

2.1.1.1 First generation

The first generation of biodiesels was produced from edible feedstocks such as rapeseed oil, soybean oil, coconut oil, corn oil, palm oil, mustard oil, olive oil, rice oil, and others during the first stage of biodiesel synthesis (Mahdavi; Abedini; Darabi, 2015). Because these feedstocks were readily available and easily converted, they were employed widely. Nevertheless, a significant disadvantage of employing edible feedstocks is the significant chance of reducing the food supply, which eventually raises the price of food goods (Aransiola *et al.*, 2014). Additionally, there aren't many places available for growing these feedstocks, the cost of producing biodiesel from them is somewhat expensive, and their environmental adaptability hampers the production of biodiesel from edible feedstocks (Tariq; Ali; Khalid, 2012).

2.1.1.2 Second generation

Non-edible feedstocks such neem oil, jatropha oil, nagchampa oil, karanja oil, Calophyllum inophyllum oil, rubber seed oil, Mahua indica oil, and others are used in the manufacturing of second-generation biodiesels (Peer *et al.*, 2017; Roy; Abedin, 2022; Shameer; Ramesh, 2017). Non-edible feedstocks have several advantages over first-generation feedstocks, such as being less expensive, more environmentally friendly, and not causing food disparity(Abdul Hakim Shaah *et al.*, 2021). Additionally, they do not require farmland for cultivation. However, the yields of non-edible plants like Jatropha oil, Jojoba oil, and Karanja oil are lower, which can impact the economy and food production. To address these socioeconomic issues, researchers are exploring economically feasible and easily accessible alternate solutions. One drawback to note is the increased requirement for alcohol in second generation biodiesel production (Mofijur *et al.*, 2021).

2.1.1.3 Third generation

The biodiesel produced from microalgae and waste oils, termed as third-generation biodiesel, offers significant benefits, including reduced greenhouse emissions, faster growth rates, and higher productivity, less reliance on farming land, and a higher percentage of oil content, all with minimal impact on the food supply (Chintagunta *et al.*, 2021; Ma *et al.*, 2018). However, there are also some disadvantages to consider, such as the need for significant investment, sunlight requirements, large-scale production issues, and oil extraction challenges (Chaos-Hernández *et al.*, 2023; Mofijur *et al.*, 2019). Currently, researchers are exploring ways to improve the production rate and extraction process for biodiesel from algal biomass, with

primary sources for third-generation biodiesel including fish oil, animal fat, microalgae, and waste cooking oil (Xie, 2017).

The third generation of biodiesel resources presents a viable solution to the issues posed by earlier-generation feedstock that affect the food chain, availability, flexibility with environmental parameters, and economic feasibility. In dire situations, certain algal species can survive and produce high lipid content, making microalgae a promising future source for third-generation biodiesel production (Thanigaivel *et al.*, 2022). Waste oils, such as used cooking oil, waste fish oil, and waste animal tallow oil, also serve as sources for third-generation biodiesel, while simultaneously reducing the burden on waste handling facilities and mitigating water pollution (Zulqarnain *et al.*, 2021). Currently, animal fats, such as pork, beef, goat, and poultry, are emerging as a promising and dependable source for biodiesel production (Neupane, 2022).

2.1.1.4 Fourth generation

The fourth generation of biodiesel includes photobiological solar fuels and electro-fuels, produced by converting solar energy into biodiesel using readily available and inexpensive raw materials (Mahapatra *et al.*, 2021). This innovative method of conversion is still a new area of research. Synthetic biology is an essential technology enabling this transformation. To achieve sustainable development, we must discover new-to-nature solutions that can create synthetic living forms and stylish microorganisms to efficiently and directly convert solar energy into fuel, with another promising method being the combination of photovoltaic or inorganic water-splitting catalysts with metabolically engineered microbial fuel for efficient development and liquid fuel storage (Singh, Narender *et al.*, 2024).

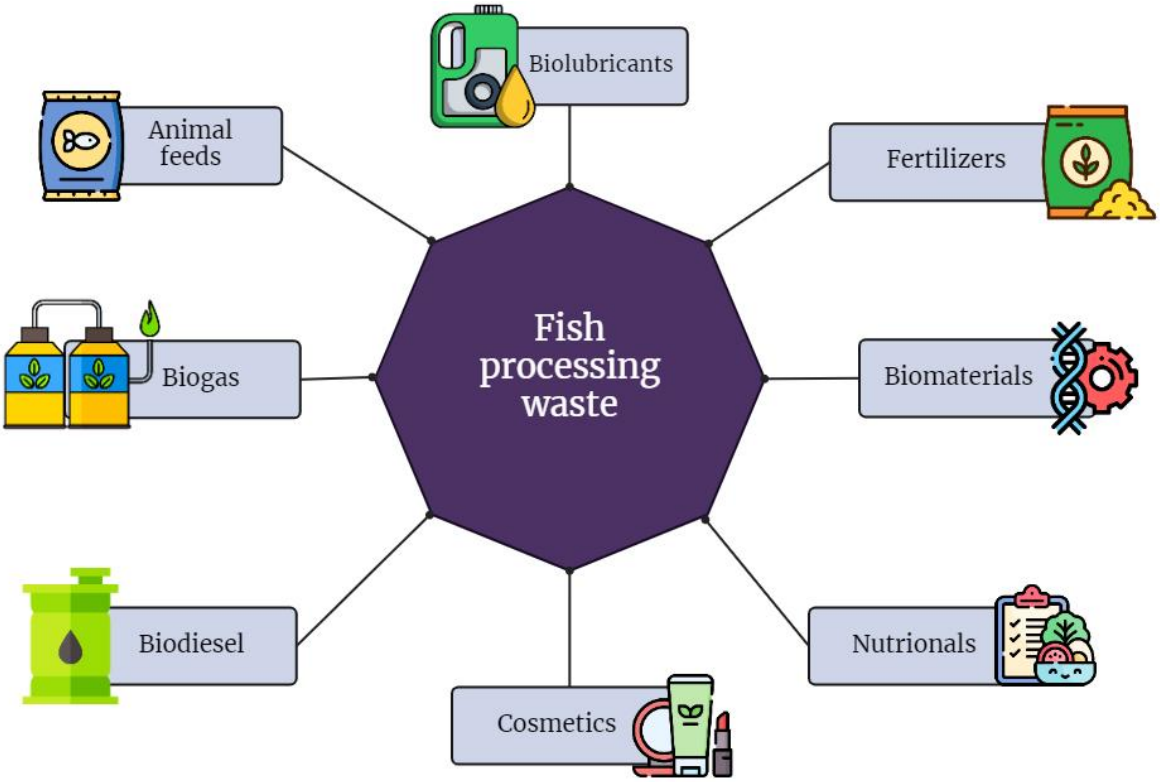
2.2 Fish oil as a feedstock for biodiesel

Farmed fish production in the Northeast of Brazil has shown steady growth, consolidating the region as the second-largest producer in the country. In 2023, production reached 170,933 tons, an increase of 0.51% compared to the previous year, with the state of Ceará standing out by registering 8,300 tons, 21% more than in 2022. Tilapia is the predominant species in Ceará's fish farming, accounting for 8,200 tons of total production(Associação Brasileira de Piscicultura, 2023). This growth in fish production, especially tilapia, generates a significant amount of aquaculture waste, creating opportunities for valorizing these byproducts in sectors such as biofuels.

In the context of biodiesel production, waste generated by aquaculture, such as that from fish processing plants and cuts from the filleting process, can be used as raw material for

the synthesis of biofuels (Ambaye *et al.*, 2021). While vegetable oils and animal fats are traditionally used as reagents, utilizing fish waste, such as residual tilapia oil, represents a promising alternative (Anu Prasanna *et al.*, 2023). This waste offers a renewable and sustainable source for biodiesel production, in addition to providing a solution for managing by-products from the fishing industry, thereby promoting a more circular and ecological economy. Figure 2.1 presents some of the products that can be derived from using such waste as raw material.

Figure 2. 1 – Products that can be derived from utilizing fish processing waste as a raw material



Source: Author (2024).

Aquaculture production grew due to satisfactory results obtained after the insertion of advanced practices to supply the global food chain. Therefore, there was a substantial increase in the supply of fish, which grew due to the increase in per capita consumption from 9.0 kg in 1961 to 20.5 in 2015, showing the relevance of fishing activity in maintaining the global food chain (Anu Prasanna *et al.*, 2023). Fish is a source of animal protein containing value-added compounds, such as enzymes, bioactive peptides, oils, and biopolymers. Additionally, collagen, chitin, and polyunsaturated fatty acids (PUFAs) – long-chain omega-3 fatty acids.

It is reported that 2/3 of fish is discarded with residue showing potential to be added to the circular economy as raw material for the synthesis of high value-added products (Borges

et al., 2023). Globally, it is estimated that 250 billion tons of fish processing waste are generated and is expected to increase annually, making it a promising agent for sustainable development (Yahyaee; Ghobadian; Najafi, 2013).

Free fatty acid (FFA) content is a critical parameter in the conversion of fish oils to methyl esters (Unugul *et al.*, 2020). Fish oil, characterized by a high content of polyunsaturated fatty acids, typically exhibits longer carbon chains compared to common vegetable oils, which primarily consist of palmitic, oleic, linoleic, and linolenic acids (Lin; Li, 2009). The increased cetane number of biodiesel derived from fish oil enhances engine performance and lowers pollutant emissions (Gharehghani; Mirsalim; Hosseini, 2017)

Mixing fish oil with diesel oil could help reduce emissions of particulate matter (PM), carbon monoxide (CO) and sulfur dioxide (SO2) by more than 60%, 30% and 75%, respectively, when compared to fuels of fossil origin alone. (Preto; Zhang; Wang, 2008). Preto *et al* (2008) obtained similar results showing that fish oil has good combustion properties offering significant advantages in the environmental and economic sector.

2.2.1 Extraction of fish oil

In the context of fish oil manufacturing, physical extraction encompasses wet rendering techniques, namely pressing, homogenization, heating, and filtering, widely utilized across the industry. Chemical extraction involves the use of organic solvents, while biological processes involve leveraging residual enzymes from fish viscera, enzymatic extractions from oil (autolysis), and other sources of enzymes (hydrolysis).

2.2.1.1 Physical method

In industrial manufacturing, the physical method is widely used and involves four main stages: frying, pressing, decantation, and centrifugation (Pudtikajorn; Benjakul, 2020). It is critical to note that the increased temperatures and pressures used in these techniques may have a minor impact on polyunsaturated fatty acid levels due to protein coagulation and oil release, potentially promoting breakdown processes such as oxidation and hydrolysis (Linder; Fanni; Parmentier, 2005; Mbatia *et al.*, 2010). Understanding these effects is crucial to maintaining the desired quality in the profile of biodiesel.

2.2.1.2 Chemical method

Chemical extraction techniques use solvents to separate fats from other components by taking advantage of fats' solubility in organic solvents (Atabani *et al.*, 2013). Fats are insoluble in water, which aids their segregation. However, solvents have limitations, notably

their acceptability for use in the food sector, restricting their applicability to analytical rather than industrial-scale manufacturing (Torres-Valenzuela; Ballesteros-Gómez; Rubio, 2020).

2.2.1.3 Biological processes

Biological approaches are used to concentrate proteins on a big scale and produce huge amounts of oil (de Jesus; Filho, 2020; Saini *et al.*, 2021). The absence of solvents or high temperatures is a significant benefit of this technique, since it decreases microbiological and physicochemical changes, allowing better efficiency in the elimination process while avoiding undesired processes such as fat oxidation. Furthermore, this technology enables the recovery of functional components such as collagen and protein hydrolyzate, as well as essential fatty acids (Rigogliuso *et al.*, 2023; Robalo *et al.*, 2024).

2.2.1.3.1 Fermentation

This biological process comprises the conversion of minced fish into silage and oil using bacteria, carbohydrate resources (such as molasses or sugar), or organic acids (such as lactic acid) (Zhao *et al.*, 2019). The level of effectiveness of this process is dependent on microorganisms producing lactic acid, which must reach significant quantities to drop the pH of the process to around 4.5 and remain stable throughout storage to limit bacterial development (Marti-Quijal *et al.*, 2020; Rai *et al.*, 2010). Centrifugation is then used to extract the oil generated from the fish components during the fermentation process (Ma *et al.*, 2022). However, silage is prone to oxidation, especially when the oil has a high concentration of polyunsaturated fatty acids (PUFA)(Koch; Kampschulte; Schebb, 2022).

2.2.1.3.2 Hydrolysis

Enzymatic hydrolysis is a widely used method in the fish processing industry to extract protein from fish (Qian *et al.*, 2023). This process involves the breakdown of fish muscle using enzymes (Zhang *et al.*, 2019). Fish silage production, a common method involving the autolysis of fish muscle, uses fish visceral enzymes to stabilize fish digestion, resulting in an aqueous solution rich in small peptides and amino acids, with enzymes facilitating the release of oil (FAO, 2018).

2.3 Influential reaction parameters in biodiesel synthesis

2.3.1 Molar ratio

The molar ratio between oil and alcohol in the biodiesel synthesis reaction is a

significant parameter since it directly affects the conversion efficiency and consequently the reaction yield (Musa, 2016). Therefore, the molar ratio must exceed the stoichiometric one, corroborating the miscibility and contact between the alcohol and the triglyceride/acid (Verma; Sharma, 2016). However, the excessive amount of alcohol must be carefully evaluated, because despite indicating high conversions in relatively short periods, the amount of reagent used will affect the production costs of biodiesel, making it competitive or not with other energy sources.

2.3.2 Catalyst concentration

The concentration of the catalyst used in the synthesis of biodiesel is a crucial parameter, as it affects the reaction rate and conversion efficiency since high catalyst concentrations will present a greater amount of available active sites, facilitating the reaction and subsequently accelerating the conversion of triglycerides, therefore, the yield (Atadashi *et al.*, 2013). However, the growth of the reaction rate decreases with the disproportionate increase in the use of the catalyst, which may lead to the generation of undesirable by-products (soaps when the reaction occurs in the presence of an alkaline catalyst), making the separation and purification of biodiesel difficult. Therefore, optimal catalyst concentration values must be analyzed to optimize the process and obtain significant conversions with reduced operating costs and without the generation of undesirable waste, mitigating possible environmental impacts (Kosuru *et al.*, 2024).

2.3.3 Temperature

Temperature is a relevant parameter in biodiesel production because it affects the reaction rate, affecting the conversion of reactants and, consequently, the overall efficiency of the process (Weldeslase *et al.*, 2023). The reaction rate increases as the temperature increases, because according to chemical kinetics, the reactant molecules gain more energy, resulting in more frequent and effective collisions, facilitating the reaction. Esterification or transesterification reactions are classified as endothermic, as they are favored by increased temperature (Sarve; Varma; Sonawane, 2016). In addition, high temperatures favor the chemical equilibrium of the reaction to obtain products; however, this increase must be evaluated, as excessively high temperatures can cause the decomposition of reactants/products, the generation of unwanted co-products and greater energy consumption, increasing operating costs.

2.3.4 Type of alcohol

The yield of biodiesel is determined by the amount of alcohol used, as it implies

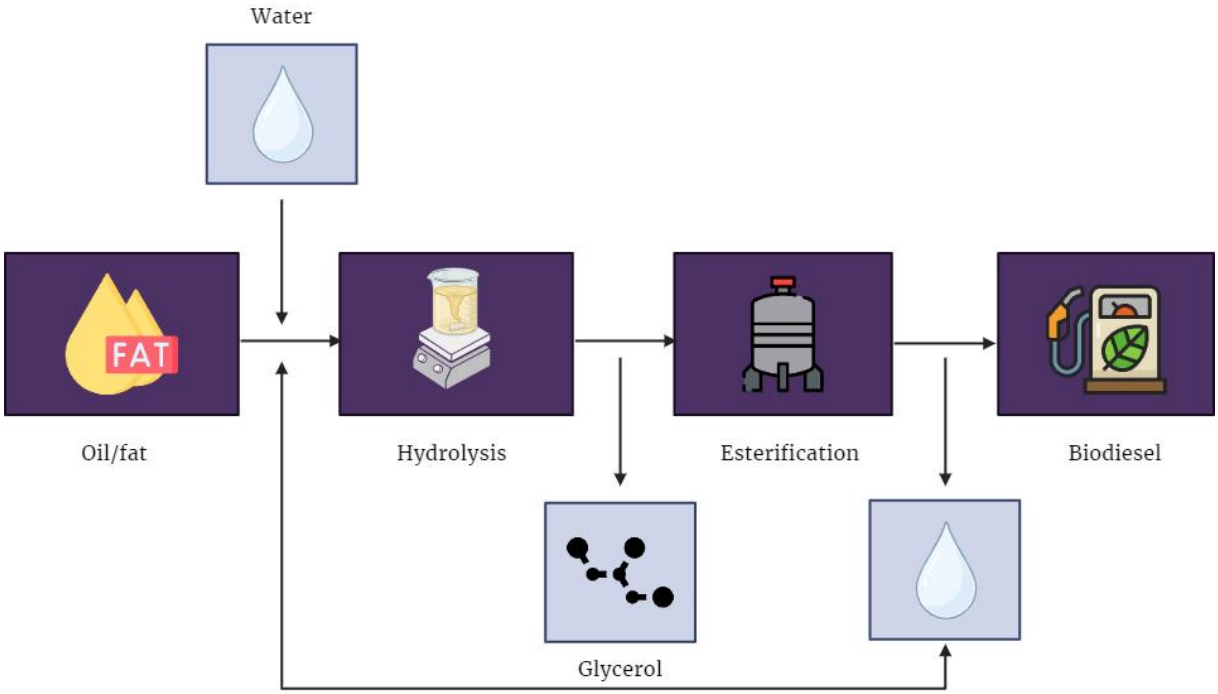
that the reaction is not reversible (Miyuranga *et al.*, 2023). Excess alcohol increases productivity and reduces time, since large amounts of alcohol will aid in the dissociation between triglycerides and fatty acids. Therefore, the type of alcohol used must be investigated and evaluated. Among the alcohols used are methanol, ethanol, propanol, butanol, and tert-butanol. Methanol is generally used in the production of biodiesel due to its high reactivity, availability, and low cost. In addition, it has a simple and small molecular structure, facilitating the occurrence of the reaction, resulting in significant conversion rates. Ethanol, despite being less reactive than methanol, is a sustainable alternative, as it can be obtained from renewable sources (sugar cane) and is less toxic. Therefore, it is the alcohol used in this process.

2.4 Production routes

2.4.1 Hydroesterification

Hydroesterification presents a viable solution for addressing the challenges of producing biodiesel from raw materials characterized by elevated levels of free fatty acids (FFA) and water (Pourzolfaghar *et al.*, 2016). This approach is commonly utilized to tackle issues in the traditional production of second-generation substrates, such as animal fat and a range of non-edible oils (Guedes Júnior *et al.*, 2022). The production of biodiesel through hydroesterification involves a two-stage process, comprising successive hydrolysis and esterification reactions (Domingues *et al.*, 2022; dos Santos *et al.*, 2019). Upon initial processing, mono- and triacylglycerols undergo hydrolysis, yielding fatty acids and glycerol. In the subsequent stage, the isolated fatty acids are subject to esterification, there by converting them into biodiesel (See Figure 2.2) (Costa *et al.*, 2020).

Figure 2. 2 – Operational schematic of the hydroesterification process to produce biodiesel



Source: Author (2024)

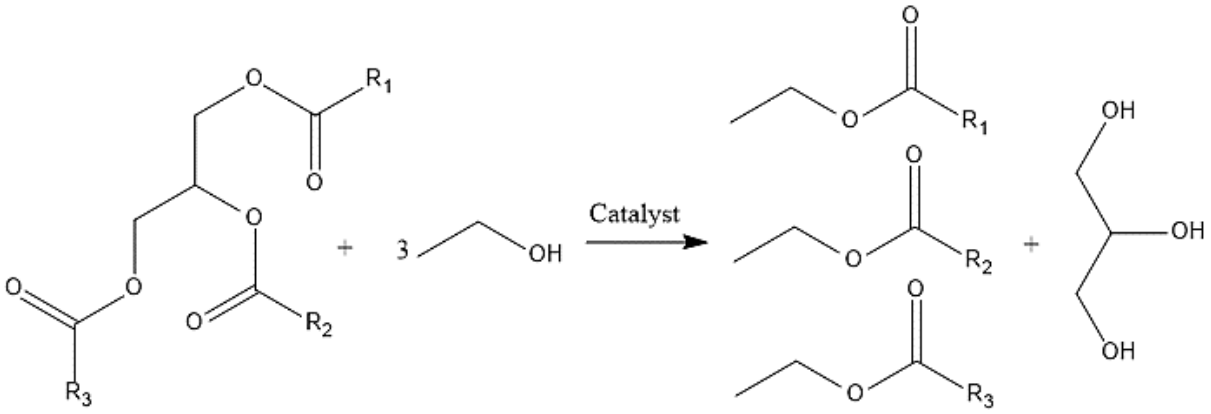
This process, known as hydroesterification, can be achieved through catalyst-free reactions (under supercritical temperature and pressure conditions), combined enzymatic and chemical catalysts (hybrid catalysts), or reactions using only enzymatic catalysts (Ketzer *et al.*, 2022). The hydroesterification process offers the advantage of producing glycerol devoid of contaminants like methanol and salts, resulting in high-quality glycerol for industrial applications (Wancura *et al.*, 2023). Additionally, this process expands the potential for using inexpensive raw materials such as animal fats containing significant amounts of water, fatty acids, and unreacted esters.

2.4.2 Transesterification

The transesterification process entails the reaction of a vegetable oil or animal fat with a short-chain alcohol, such as methanol or ethanol, facilitated by a specific catalyst to yield ethyl esters (biodiesel) and glycerol (Figure 2.3) (Orege *et al.*, 2022). Another name for the transesterification process is alcoholysis the reaction of the original ester with alcohol. The transesterification reaction may be facilitated by either chemical or enzymatic catalysts, wherein the quantity of alcohol utilized exerts influence over the chemical equilibrium of the reaction, thereby amplifying the conversion of the product (Nayab *et al.*, 2022). Transesterification, being a reversible reaction, requires a rapid separation process to avoid the possibility of reversal (Salaheldeen *et al.*, 2021). After the reaction, the resulting products are

typically transferred to a separatory funnel. The biodiesel phase rises to the top while the resulting glycerol settles to the bottom.

Figure 2. 3 – Transesterification reaction of a triglyceride in the presence of short-chain alcohol (methanol) to obtain fatty acid methyl esters



Source: Author (2024)

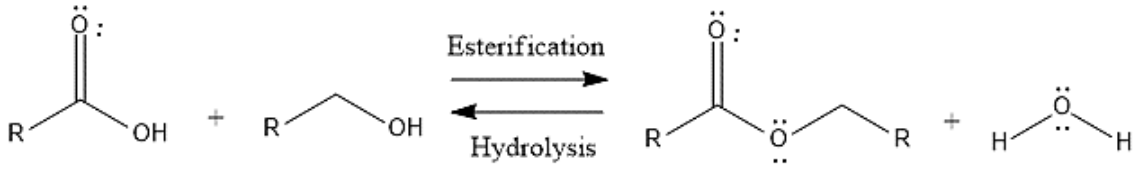
It is important to highlight those alcohols such as ethanol, propanol, butanol and pentanol are also used in the transesterification process (Kant Bhatia *et al.*, 2021). However, the most used are methanol and ethanol (Mofijur *et al.*, 2021). Methanol stands out due to its physical and chemical properties, high polarity and short chain. Biodiesel produced from the use of methanol is called fatty acid methyl ester (FAME). In contrast, ethanol also stands out for its high enthalpy, low toxicity and significantly favorable effect on the cetane number (a property relevant to the commercialization of the bioproduct). Biodiesel obtained from the reaction of free fatty acids with ethanol is called fatty acid ethyl ester (FAEE).

2.4.3 Esterification

Esterification is an important industrial process used in the pharmaceutical, food, flavoring, and biofuel (biodiesel) industries (Khan *et al.*, 2021). Esterification consists of a reaction between free fatty acids obtained from lipid sources, a short-chain alcohol (such as ethanol or methanol), and a catalyst, which can be either chemical or biochemical (Ahmed; Huddersman, 2022). Figure 2.4 shows the esterification reaction between fatty acids and alcohol, where esters and water are produced. This method offers advantages over transesterification as it uses fatty acids instead of triglycerides, enabling the use of low-cost raw materials such as waste oils (Perumal *et al.*, 2024). It is noteworthy that glycerol is not produced. However, the conversion is directly influenced by the acidity level of the raw material used.

Additionally, the availability of the waste materials employed will affect the feasibility of large-scale production.

Figure 2. 4 – Esterification reaction between fatty acids and short-chain alcohol to obtain ethyl esters of fatty acids



Source: Author (2024).

2.5 Types of Catalysts

2.5.1 Homogeneous catalyst

In the field of biodiesel synthesis, homogeneous catalysts— which work in the same phase as the reactants— are essential for promoting reactions. One key benefit is their ability to effectively convert triglycerides into esters that may be used as fuels. Homogeneous catalysts, with their high catalytic activity, guarantee efficient conversion rates. Furthermore, the need for extremely high or low pressures is lessened when they operate under mild reaction circumstances. Because of these intrinsic qualities, homogeneous catalysts are a highly advantageous choice in the biodiesel production environment, supporting both economic viability and operational efficiency.

2.5.1.1 Alkaline catalysts

In the industrial production of biodiesel, strong alkaline catalysts like sodium hydroxide (NaOH) and potassium hydroxide (KOH) are widely used (Tubino; Junior; Bauerfeldt, 2014). These catalysts are highly effective in facilitating the transesterification process, which involves converting vegetable oils or animal fats into biodiesel using alcohols such as methanol or ethanol (Farouk *et al.*, 2024). During this reaction, hydroxide ions (OH^-) break down the ester bonds in the fats or oils, resulting in the formation of methyl or ethyl esters of fatty acids— the primary components of biodiesel. Additionally, glycerol is produced as a by-product. The use of alkaline catalysts is favored due to their high reaction rates and relatively mild operating conditions, making the production process more efficient and cost-effective (Granjo; Duarte; Oliveira, 2009). However, careful handling and precise control of reaction

conditions are essential to minimize soap formation and ensure high yield and purity of the biodiesel (Chanakaewsomboon *et al.*, 2023).

2.5.1.2 Acid catalysts

Acid catalysts such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) are common in biodiesel production (Ryu *et al.*, 2018; Su; Chou, 2019). These catalysts are particularly effective for processing raw materials with high acidity or high-water content, as they remain unreactive with water and free fatty acids (Rizwanul Fattah *et al.*, 2020). This characteristic makes acid catalysts suitable for a broader range of feedstocks, including those that might be less refined or of lower quality (Esmi; Borugadda; Dalai, 2022). However, it is important to acknowledge that acid catalysts have their drawbacks. They tend to exhibit lower selectivity, which can result in the formation of unwanted byproducts (Wang, Baohua *et al.*, 2023). This necessitates additional purification steps, potentially increasing the overall cost and complexity of the biodiesel production process (Hamza *et al.*, 2021). Therefore, while acid catalysts are valuable for their versatility, their use must be carefully managed to balance efficiency and product purity.

2.5.2 Heterogeneous catalysts

Heterogeneous catalysts are characterized by having a phase distinct from the matter relating to the reaction medium (Semwal *et al.*, 2011). Heterogeneous catalysts present themselves as an alternative to the barriers faced in homogeneous reactions such as the presence of water, limitation in the content of free fatty acids, and difficulties in the purification process (Maroa; Inambao, 2021). Since heterogeneous catalysts reduce problems related to saponification and contribute to the synthesis of pure biodiesel and glycerol. Therefore, ideal solid catalysts would present characteristics such as a network of pores connected, a hydrophobic surface, and the ability to control the hydrophobicity of the surface to interrupt the deactivation process (Abou-Elyazed *et al.*, 2020). Furthermore, heterogeneous catalysts offer the benefit of facilitating the separation of biodiesel and enabling recovery and reuse, resulting in increased efficiency and reduced costs.

2.5.2.1 Alkaline catalysts

The use of alkaline solid derivatives in biodiesel synthesis has shown significant results (Maleki; Ashraf Talesh; Mansouri, 2022). Since these discussions present superior catalytic potential about the solid acid aspects (Mandari; Devarai, 2022). Furthermore, alkaline

solid investors have activity composed of their homogeneous counterparts (Faruque; Razzak; Hossain, 2020). Another aspect to be highlighted is the economy and non-corrosion of the equipment (Gupta; Pal Singh, 2023). However, some consequences must be measured, such as the vulnerability of the finding to air occurring upon its deactivation, the increase in the molar ratio (alcohol: oil) and its sensitivity to primary materials containing higher levels of free fatty acids and the possibility of leaching. of active sites (Aderibigbe *et al.*, 2021). Therefore, damaging the purity and making it necessary to add product purification steps (Maleki; Ashraf Talesh; Mansouri, 2022). Examples include hydrotalcite, basic zeolites, and alkaline earth metal oxides (MUKHTAR et al., 2022).

2.5.2.2 Acid catalysts

Solid acid catalysts are used in making biodiesel because they can handle high levels of free fatty acids and moisture in input oils (Šánek *et al.*, 2016). This means that low-quality and low-cost raw materials can be used without the need for acid pre-treatment(Chouhan; Sarma, 2011). Additionally, the use of solid acid catalysts results in the production of biodiesel and high-quality glycerol (Sreeprasanth *et al.*, 2006). Switching from homogeneous to heterogeneous catalysts also eliminates concerns about equipment corrosion (Esmi; Borugadda; Dalai, 2022). An ideal heterogeneous catalyst should have a significant pore size, strong acidic site specifications, and hydrophobic characteristics (Mukhtar *et al.*, 2022). Promising options include metal oxides known for their high acidity (Guo *et al.*, 2023). Some examples of solid acid catalysts are zeolites, mixed oxides, and sulfated metal oxides (Okechukwu *et al.*, 2022).

2.5.3 Enzyme catalysts

Enzymatic results are biological proteins that act in specific chemical reactions (Cavalcante *et al.*, 2021). They stand out for being highly selective, resulting in the reduction of unwanted by-products and consequently the formation of clean products. Another advantage of conventional chemical catalysts is their ability to be operational at moderate reaction parameters (Pasha *et al.*, 2021). Therefore, production processes are expanded and reduced costs are involved in maintaining and controlling reaction conditions (Kalita *et al.*, 2022). The use of enzymes as catalysts in biodiesel synthesis is attractive, since their applicability will assist in the product's superior characteristics regarding purity and stability, meeting the standards set in normative acts, by industry and by environmental regulatory institutions (Arcus *et al.*, 2020).

2.6 Enzymes

Enzymes are biological macromolecules that aim to increase the speed of recurring chemical reactions in cells or organisms without altering themselves (Zhang; Narlikar; Kutateladze, 2021). Therefore, the molecules involved in reactions catalyzed by enzymes are known as substrates. Each enzyme has a high degree of specificity to act on an individual substrate and convert it into a specific product (Barelli *et al.*, 2021). This specificity is essential in biochemical processes, as it brings security to the results and operational control of the enzymatic system (Costa *et al.*, 2023). Enzymes have several advantages when compared to chemical catalysts due to their region and stereoselectivity, resulting in the obtaining of enantiomerically pure products (Monteiro *et al.*, 2021).

These biocatalysts are efficient from an energy point of view, as they operate at moderate reaction parameters (pressure and temperature) and act in a moderate range of pH values (Garcia-Silvera *et al.*, 2023). Enzymes accelerate the reaction without altering the chemical equilibrium characteristic of each reaction and accelerate reversible reactions in both directions (Katsimpouras; Stephanopoulos, 2021). Therefore, the reaction speed is equivalent to converting some moles of substrate per unit of time (Chukwuma *et al.*, 2020). Measuring the enzymatic activity of an enzyme is crucial to characterize the enzymatic reaction system since enzymes have characteristic activity under different operating conditions (Victorino da Silva Amatto *et al.*, 2022).

Given the reported advantages and the importance of implementing biological catalysts in industrial processes using biotechnological tools, Table 2.1 presents the classification of enzyme types according to the International Union of Biochemistry (IUB).

Table 2. 1 – Analysis of enzyme classifications and their pertinent attributes

Class	Name	Characteristic	Reference
1	Oxidoredutases	The oxidation or reduction process involves the transfer of hydrogen or electrons, or the use of acceptor groups to modify substrates.	(Athiappan *et al.*, 2022)
2	Transferases	The process involves the removal of substrate groups, followed by their transfer to acceptor molecules.	(Paul; Sangeetha; Deepika, 2019)
3	Hydrolases	The involvement of water in the hydrolysis of substrate covalent bonds.	(JAEGER; EGGERT, 2004)
4	Lyases	Participation of water in the cleavage of	(Manubolu *et al.*,

		substrate covalent bonds.	2018)
5	Isomerases	They promote the isomerization of substrates.	(Yan *et al.*, 2024)
6	Ligases	Catalysis of covalent bonds between two molecules.	(Manubolu *et al.*, 2018)

Source: Author (2024).

2.6.1 Lipases

The global industrial enzymes market has seen significant expansion from $6.4 billion in 2021 to an expected $8.7 billion in 2026 (Mesbah, 2022). Lipases are the third most widely used enzyme type, after amylases and proteases (Gonçalves Filho; Silva; Guidini, 2019). Lipases, also known as triacylglycerol hydrolases (EC 3.1.1.3), are a subclass of hydrolases and are used in the hydrolysis of free fatty acids from long-chain triacylglycerols by breaking the ester bond between glycerol and the fatty acid of triacylglycerides (Ishak *et al.*, 2024). These enzymes are attractive to industry because of their versatility in acting in various transesterification, esterification, and interesterification reactions (Ferreira Mota *et al.*, 2022). In addition, they can operate under mild reaction parameters such as temperature, pressure, and pH.

The properties of interface activation are unique to lipases. Given this, it is crucial to emphasize that the active sites are protected by an amphipathic α-helix, referred to as a "lid" that can open to let substrates enter the site when it encounters the water-oil interface component that makes up the enzyme's active ingredient (de Sousa *et al.*, 2023). Moreover, most of its substrates are fat-soluble, yet it like to dissolve in an aqueous medium. A broad area of the water-oil contact is crucial for lipase-catalyzed processes to be as effective as possible (Cavalcante *et al.*, 2021).

Therefore, these enzymes are important for several sectors, such as the food, pharmaceutical and chemical industries. In the food industry, they are used in several applications, such as the manufacture of dairy products, oil processing and the formulation of various dietary supplements (Ortega-Requena *et al.*, 2024). In the pharmaceutical industry, they are a marker enzyme in the prevention of disease occurrence (Wang, Hong hai *et al.*, 2023). In the chemical industry, they are used for washing due to their hydrolysis ability (Freije García; García Liñares, 2024). In addition, they are widely used in the synthesis of biodiesel, which is the interest of the present study.

Lipases can be obtained from several sources: plants, animals, and bacteria (Bullo

et al., 2024). However, in biodiesel production, microbial lipases (obtained from bacteria, yeasts and fungi) stand out when compared to other sources due to their specificity, ease of growth and thermal and pH stability (Ali *et al.*, 2023; Singh; Jana, 2023; Zhao, Junxin *et al.*, 2024). Among the frequently used microbial lipases are: *Rhizopus oryzae*, *Aspergillus oryzae, Aspergillus niger*, *Thermomyces lanuginosus*, *Geobacillus thermocatenulatus* and *Burkholderia cepacia*. In this study, two lipases will be used for the synthesis of ethyl esters via enzymatic hydroesterification, *Thermomyces lanuginosus* (during hydrolysis) and Eversa Transform 2.0 lipase (during esterification).

2.6.1.1 Thermomyces lanuginosus lipase

Thermomyces laguginosus lipase (TLL) is a basophilic and thermostable enzyme. Commercially available in soluble and immobilized form, TLL is the active enzyme in Lipolase®, a commercial soluble lipase preparation from Novozymes, and is also available in immobilized form (Lipozyme TL IM®) (Fernandez-Lafuente, 2010). Composed of 269 amino acids, TLL has a molecular weight of 31,700 g/mol and an isoelectric point of 4.4. The active site presents the typical catalytic triad Ser-His-Asp (Abreu Silveira *et al.*, 2019).

2.6.1.2 Eversa® Transform 2.0 lipase

Eversa Transform 2.0 (ET2) lipase is a genetically modified enzyme industrially derived from *Thermomyces lanuginosus* lipase (TLL), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain (Monteiro *et al.*, 2021). ET2 was designed primarily to produce biodiesel using feedstocks with any free fatty acid content, i.e., from low acidity oils to high acidity oils such as residual oils. ET2 has 269 amino acid residues, a molecular weight corresponding to 31.5 kDa and has an activity of 9100 IU/mL (Acherki; Bouaid; Marchetti, 2022). Its catalytic triad is composed of serine (153), aspartic acid (206) and histidine (268) like other natural lipases (Brandão *et al.*, 2021; Ding *et al.*, 2019; Xu *et al.*, 2021). Its catalytic site is in a cavity protected from the external solvent, resulting in a selective interaction with substrates.

2.7 Taguchi methodology

The Taguchi methodology is a technique that uses orthogonal experimental design matrices to evaluate significant quantities of variables, reducing the number of experiments to be performed (Li *et al.*, 2019). It is important to emphasize that the conclusions obtained from small-scale experimental executions are valid for the experimental region covered by their control factors and settings (Abou-Taleb *et al.*, 2016). The Taguchi method evaluates the signal-

to-noise (S/N) obtained from a logarithmic function of the desired result and serves for optimization, data analysis, and prediction of optimal results (Aziri *et al.*, 2024). The S/N ratios are divided according to their characteristics: smaller-is-better, larger-is-better, and nominal-is-better (Neag *et al.*, 2022). Given the advantages reported, the aforementioned method is used in this work to optimize biodiesel production by evaluating significant parameters and reducing the number of reagents to be used.

2.8 Theoretical study

Theoretical study is a relevant approach in the academic community that involves the formulation of concepts, hypotheses, and models without the urgency of practical experiments (López *et al.*, 2022). Thus, this technique enables the exploration and understanding of complex phenomena through analysis and synthesis of existing information (Jain *et al.*, 2023). In the development of theoretical approaches, a wide range of conditions are used to predict results. Therefore, several reactions can be studied under severe operational conditions and possible results obtained can be evaluated (Karimi *et al.*, 2023; Kaya; Kökkülünk, 2023). Among the computational tools widely used to evaluate routes, behaviors, and results in chemical or biochemical processes are molecular docking and molecular dynamics.

2.8.1 Molecular docking

Molecular docking is a computational tool that aims to predict the coupling between a ligand (small molecules) and a protein (receptor) (Naqvi *et al.*, 2018). In our study, an enzyme. Therefore, this process performs a simulation of the interaction between the ligand and the protein to determine the binding position and affinity of the complex (Morris; Corte, 2021). The applicability of this tool includes drug discovery, protein engineering, and understanding of protein-ligand interactions. In drug discovery, it is widely used in screening potential drug candidates about target proteins to identify promising leads (Agu *et al.*, 2023). In protein engineering, coupling is evaluated to optimize proteins in the search for better interactions with ligands or to design new proteins with required properties and/or functions (Modenutti *et al.*, 2019). Furthermore, it makes it possible to elucidate the mechanisms involved in reactions and understand the interaction that occurs between the ligands and the target protein, which is the objective of this work.

2.8.2 Molecular dynamics

Another tool widely used in the development of biotechnological processes is molecular dynamics, which consists of a method for computational simulation to evaluate the physical movements of atoms and molecules in a given space of time (Singh; Bani Baker; Singh,

2022). Therefore, in an approach with classical mechanics, molecular dynamics enables simulations of time-dependent behaviors in molecular systems, enabling researchers to observe the interaction of molecules, their movements and changes and conformation in a dynamic environment (Filipe; Loura, 2022). Applications of this tool include protein folding, studying protein-ligand interactions, exploring conformational changes, and investigating allosteric regulation (Vidal-Limon; Aguilar-Toalá; Liceaga, 2022).

This tool is used in protein folding to study the processes involved in this phenomenon and to help understand the range of their functional conformations (Melo *et al.*, 2024b). In the study of protein-ligand interactions, this technique provides relevant information about the dynamics and stability of protein-ligand complexes by evaluating the effects of solvents and ions (Jin; Wei, 2024). While for exploring conformational changes, the method is used to understand the alterations of proteins or other biological molecules during biological processes (Cieślak; Kabelka; Bartuzi, 2024; Zhao, Zhen *et al.*, 2024). Finally, in the investigation of allosteric regulation, molecular dynamics is used to explore allosteric sites and the effects of ligand binding on protein function (Kairys *et al.*, 2024).

CHAPTER 3

CHAPTER 3 – SUSTAINABLE BIODIESEL PRODUCTION FROM FISH WASTE OIL: A BIBLIOMETRIC REVIEW AND FUTURE PERSPECTIVES

3.1 Abstract

Biodiesel, generally obtained from the transesterification of vegetable oils, is an alternative to the use of fossil fuels. Among the advantages of its use: lower toxicity, resistance to corrosion, greater biodegradability and a reduced carbon footprint, since the CO_2 (carbon dioxide) released during combustion is part of the natural carbon cycle and is absorbed by raw material plants. It is worth noting that several raw materials are used, such as vegetable oils (soybean, palm, sunflower and pequi), animal fats and residual oils. Because of this, residual fish oil, a byproduct of the fishing industry, has gained notoriety due to its low cost and high potential in the production of biodiesel. The conventional method used is chemical transesterification, however, the higher content of free fatty acids can complicate this process, making pre-treatment steps such as esterification necessary. Therefore, research and development of alternative routes become relevant to guarantee the efficiency and guidelines of the production process. Thus, the present study aims to carry out a bibliometric analysis, a tool conventionally used to quantitatively and qualitatively investigate scientific productions on a given topic, about biodiesel derived from residual fish oil and the production routes commonly employed. The investigation period was delimited between the years 2010 to 2024, where the keywords “biodiesel production”, “fish oil” or “residual fish oil” were used, where the types of scientific documents used to construct the database were articles, conference papers and review articles only in the English language. Among the countries that published the most on the topic investigated are Brazil (20 documents), India (17 documents) and Iraq (16 documents). In addition, collaboration between the countries was observed: Brazil, Spain, Canada, England and India. The University of Mosul, located in Mosul, Iraq; leads the list of institutions that published the most regarding the production of biodiesel obtained from residual fish oil (15 documents). Meanwhile, the National Council for Scientific and Technological Development (CNPQ), located in Brazil, stands out for the number of funded works (13 documents), followed by the Coordination for the Improvement of Higher Education Personnel (CAPES) (10 documents) and finally the Science and Technology Foundation (FCT) (6 documents). Therefore, this study highlights the potential of residual fish oil and its relevance for synthesizing and developing routes to produce biofuels, such as biodiesel and biolubricants.

Keywords: Biodiesel. Fish waste oil. Bibliometric analysis.

3.2 Introduction

The production of biodiesel involves the utilization of various raw materials, including vegetable oils, animal fats, and residual oils (Brahma *et al.*, 2022). Vegetable oils like soybean, palm, sunflower, castor, and pequi are notable for their availability and unique properties (Al-jabiri; Balla; Al-zuhairy, 2025; Ghesti *et al.*, 2022; Santos *et al.*, 2024). Soybean oil is widely used due to its high supply and concentration of fatty acids, while palm oil stands out for its high productivity, being popular in tropical regions (Ezeorba *et al.*, 2024). Sunflower oil contributes to production with lower pollutant emissions, and castor oil is valued for its resistance to arid climates (Berman; Nizri; Wiesman, 2011; Wang, Lu *et al.*, 2023). Animal fats, such as beef and pork tallow, and residual cooking oil, are low-cost raw materials, obtained as by-products of the meat industry, with the potential to produce high-quality biodiesel, although they require pre-treatment because they contain high levels of saturated fatty acids (Andreo-Martínez *et al.*, 2022; Cunha *et al.*, 2013). Waste cooking oil is advantageous due to its availability and low cost, in addition to helping to mitigate environmental problems related to its disposal (Cordero-Ravelo; Schallenberg-Rodriguez, 2018; Hosseinzadeh-Bandbafha *et al.*, 2022).

Waste fish oil has emerged as a promising source for biodiesel production. This oil is obtained from the by-products of the fishing industry and is often underutilized or improperly discarded, making it an attractive candidate due to its low cost and potential for utilization (Thamarai Kannan *et al.*, 2022). Biodiesel production from fish oil can be carried out using both traditional and innovative methods. Chemical transesterification is the most used conventional method, where the oil is mixed with an alcohol and alkaline catalysts, forming fatty acid esters and glycerol (Parida *et al.*, 2024). However, the high content of free fatty acids in fish oil can result in unwanted soap formation, complicating the process (Anu Prasanna *et al.*, 2023). To address this issue, esterification is applied before transesterification.

Among alternative methods, enzymatic transesterification stands out, characterized using lipases to prevent soap formation, although it comes with higher costs (Bueso *et al.*, 2015). Other interesting methods include the use of heterogeneous catalysis with reusable solid catalysts, as well as technologies like microwaves and ultrasound, which accelerate the transesterification process, reducing reaction time and energy consumption (Mandari; Devarai, 2022; Poppe *et al.*, 2018).

The use of fish oil as a raw material offers advantages such as the reuse of waste from the fishing industry and the improvement of biodiesel quality due to the presence of unsaturated fatty acids (Cubas *et al.*, 2016; Jaiswal *et al.*, 2024). However, there are specific

challenges, such as the high free fatty acids content, which hinders traditional transesterification, and the oxidative instability of biodiesel, requiring antioxidants to extend its shelf life (Nambiraj; Suresh Kumar, 2024; Pullen; Saeed, 2012). Additionally, the strong odor of fish oil and the variability in its composition can affect the quality and commercial acceptance of biodiesel, requiring proper treatment to overcome these limitations (Abdul Hakim Shaah *et al.*, 2021; Mahdi *et al.*, 2023).

Given the challenges and advantages discussed, bibliometric analysis emerges as a tool that uses quantitative methods to assess the scientific production of a specific scientific topic (de Castro Bizerra *et al.*, 2024b). Therefore, it is concerned with analyzing the number of publications, citations, authors and reference sources (Dari *et al.*, 2024). To this end, bibliographic data obtained from scientific databases is collected and metrics are applied to verify the impact and relevance of articles, authors or research topics (de Castro Bizerra *et al.*, 2024a). Bibliometrics is widely used to identify the main trends in each area of knowledge, mapping the development of science over time (Melo *et al.*, 2024a).

In this context, bibliometric analysis becomes crucial in recognizing emerging trends as well as in identifying new technologies and catalysis methods. By examining publication patterns, we can identify research gaps and areas that are still not well studied, as well as underexplored technologies (da Silva Aires *et al.*, 2024). This study aims to conduct a bibliometric analysis of scientific research on the use of residual fish oil as a raw material for biodiesel production. The focus is on collecting data from scientific documents published in English from 2010 to 2024. This analysis aims to provide insights into the current state of research worldwide.

3.3 Methodology

3.3.1 Data source

The study uses bibliometric analysis as a key methodology for measuring scientific research, offering a thorough and accessible approach to gathering and evaluating data. The analysis includes information on authors, citations, journals, institutions, and other new scientific works related to biodiesel synthesis using residual fish oil. To conduct the bibliometric analysis, an important part of the study involves using the database obtained from publications collected on the Web of Science website, which is currently owned by Clarivate Analytics and contains around 74.8 million academic publications as of 2020. This platform is a highly respected and crucial data source in the educational field and was used to access numerous

scientific literature productions on the topic under consideration.

3.3.2 Data collection

The initial phase involved the acquisition of academic data on scientific publications related to biodiesel production from waste fish oil. This was done using the keywords “biodiesel production”, “fish oil” or “waste fish oil” within the period 2010 to 2024 to conduct a quantitative survey of publications in this domain. The scope of the search was deliberately confined to the above-mentioned terms and the English language to mitigate the influences of update bias in the database. This method was employed to construct a comprehensive synthesis of the scientific publications on the proposed topic, considering the current scenario.

3.3.3 Data extraction

Upon obtaining the requisite database for the study, all gathered material was imported into Microsoft Excel 2023 software (Microsoft Corporation, Redmond, Washington, WA, United States) for subsequent processing and analysis to present statistical results. The analysis encompasses the frequency of citations, the prominent countries in the field of study, scientific production regarding the utilization of residual fish oil for biodiesel production, authorship details, annual publication figures, funding agencies, and participating institutions. Qualitative analysis data were sourced from the Citation Reports (JCR) website (https://www.thomsonreuters.com/journal-citation-reports/). Throughout the analysis, the collected data underwent manual filtering and processing within Excel.

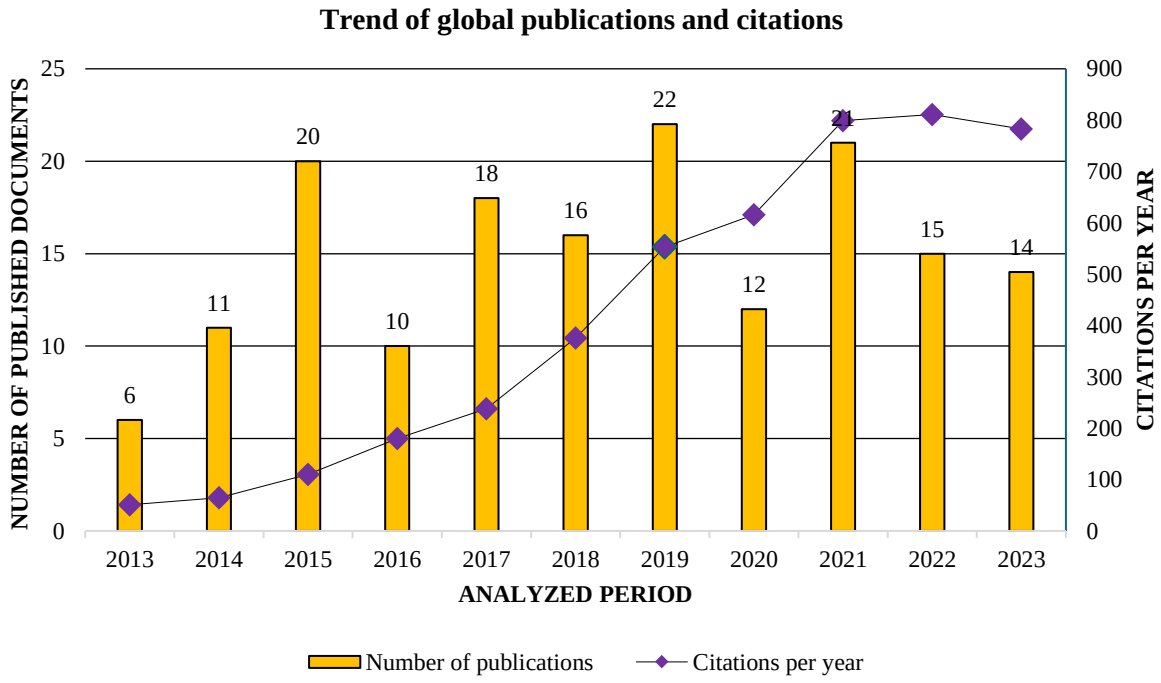
3.4 Results and Discussions

3.4.1 Bibliometric Analysis

3.4.1.1 Trend of global publications and citations

Bibliometric analysis is a tool that offers the opportunity to identify and evaluate scientific publications related to a given field of research. Therefore, Figure 3.1 shows the number of publications and citations over the period investigated (2010 to 2024). The number of scientific documents published corresponds to yellow columns while the number of citations per year is represented by dots and a purple line. Given this, it is observed that during the period analyzed the number of publications increased consistently, reaching a peak of 22 documents in 2019. After 2019 there is a reduction in the number of publications, however, the level remains high. In contrast, the number of citations appears to be continuous throughout the period investigated, revealing the impact of the publications.

Figure 3. 1 – Number of articles published and citations during the period investigated



Source: Author (2024)

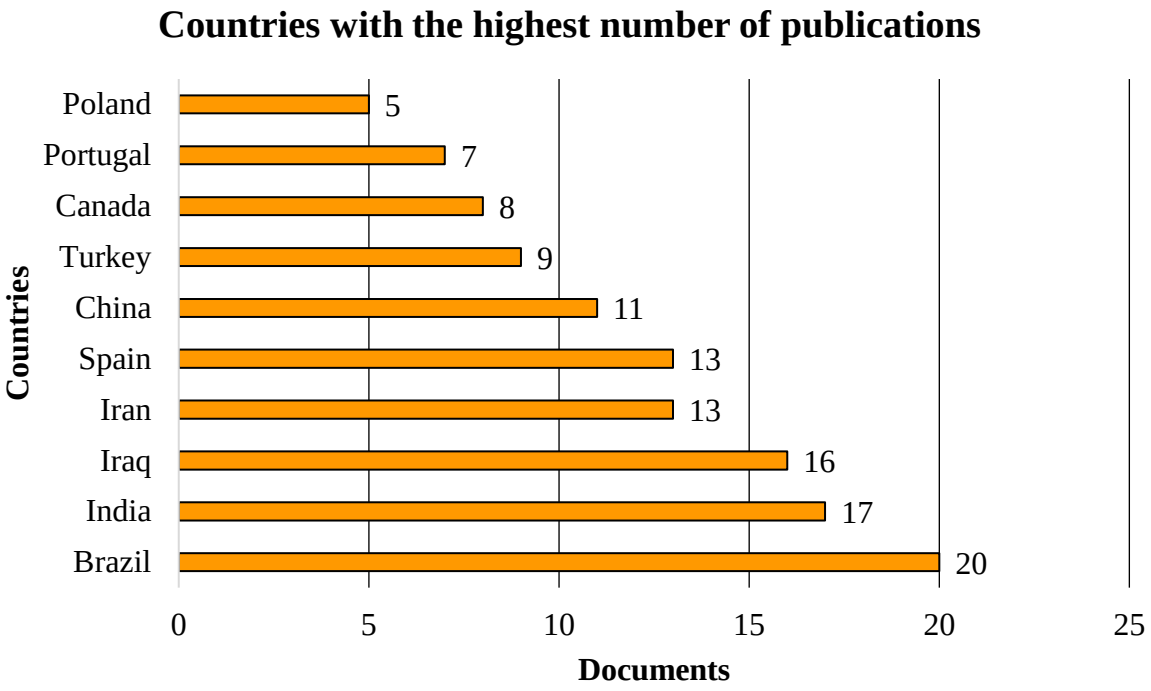
3.4.1.2 Contributions of countries/regions

Analyzing the number of publications by country is essential to assess the concentration of research on a specific topic and to visualize collaboration between nations. In

this context, Figure 3.2 highlights the 10 leading countries in publications on the synthesis of biodiesel from fish oil. It is worth noting that Brazil leads with the largest number of publications (20), followed by India (17) and Iraq (16).

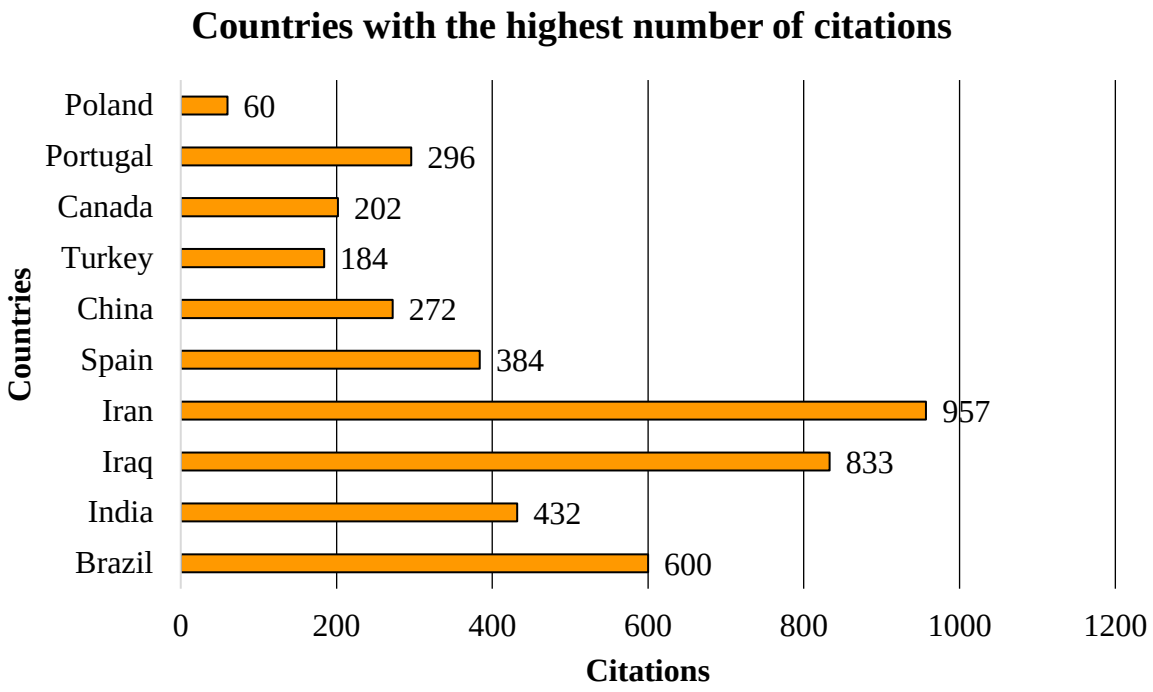
Furthermore, it is essential to investigate which countries are references in this field, measuring the number of citations received by their publications (Figure 3.3). When analyzing the citations, we observe that Iran stands out significantly, accumulating a total of 957 citations during the period analyzed. Iraq comes next, with 833 citations, and Brazil with 600. This analysis not only reveals the productivity of the countries in terms of publications but also the influence and impact of their research in the field of biodiesel from fish oil.

Figure 3. 2 – The countries with the highest number of scientific publications on the production of residual fish oil



Source: Author (2024)

Figure 3. 3 – The countries with the highest average number of citations per document



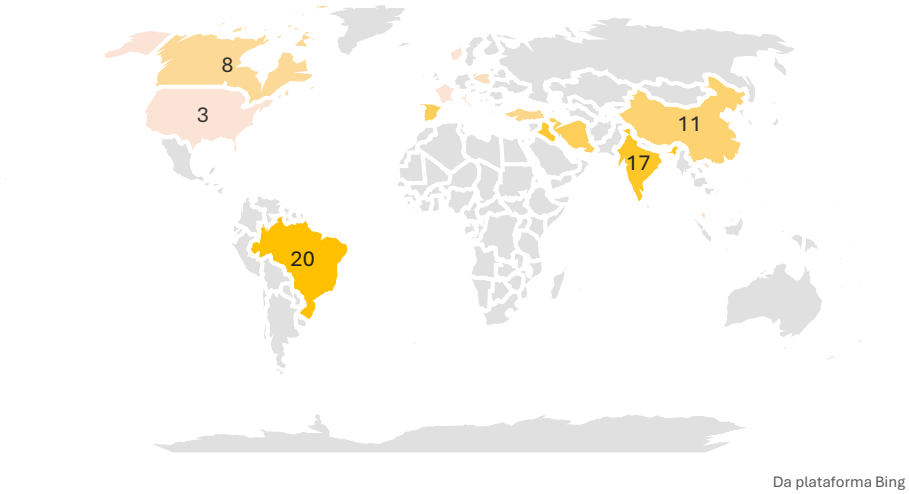
Source: Autor (2024).

Figure 3.4 shows the worldwide distribution of publications on the topic investigated. Meanwhile, Figure 3.5 presents a network of connections between several countries in clusters, which are identified by different colors and node sizes that suggest degrees of relevance or influence within the group. The red cluster around India includes countries such as Saudi Arabia, Pakistan and Ethiopia. While the green cluster: England, France, Finland and Scotland. Spain, Norway and Turkey are within the yellow cluster. India has an extensive fishing industry and is rich in marine biodiversity, which allows the generation of large quantities of fish waste. Brazil is connected to Angola and Canada, indicating possible commercial or technological relations in the use of residual fish oil for biodiesel production.

Figure 3. 4 – Distribution of global scientific production on the production of biodiesel from

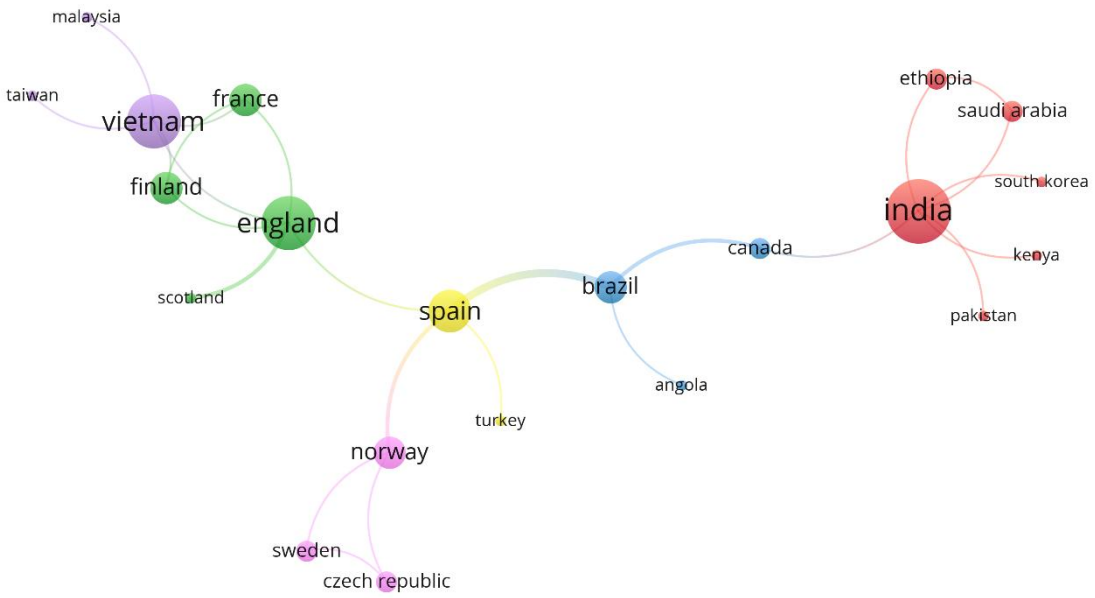
residual fish oil.

Global distribution of scientific production



Source: Author (2024).

Figure 3. 5 – Global collaboration network between the countries that publish the most articles regarding the production of biodiesel from waste fish oil



Source: Author (2024).

3.4.1.3 Contributions of Institutions

Following the collection and refinement of data for this study, a total of 197 institutions were identified, each corresponding to the number of publications concerning the study's topic. Subsequently, the study defined institutions that had published at least one work

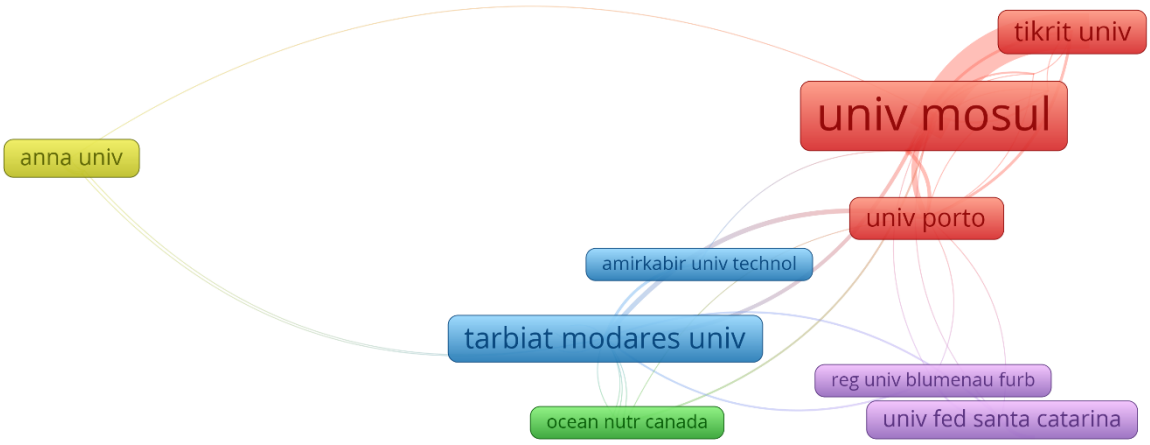
and obtained a minimum of 100 citations. Table 3.1 presents the ranking of the top 10 institutions that have made relevant contributions, their respective countries of origin, and the number of publications on fish oil biodiesel production. Notably, the University of Mosul emerged as the institution with the highest number of publications (15 papers), followed by Tarbiat Modares University (7 papers) and the University of Tikrit (6 papers), respectively. Subsequently, Figure 3.6 presents the collaboration network between the institutions.

Table 3. 1 – Academic institution ranking in biodiesel research

Rank	Institutions	Regions/Country	Number of publications
1	University of Mosul	Mossul, Iraq	15
2	Tarbiat Modares University	Tehran, Ira	7
3	University of Tikrit	Tikrit, Iraq	6
4	Universidade do Porto	Porto, Portugal	5
5	Universidade Federal de Santa Catarina	Santa Catarina, Brazi	4
6	Anna University	Madras, Indía	4
7	University of Granada	Granada, Spain	3
8	Vellore Institute of Technology	Vellore, India	3
9	Aarhus University	Aarhus, Denmark	3
10	K.S Rangasamy College of Technology	India	3

Source: Autor (2024).

Figure 3. 6 – Global collaboration network among institutions for the publication of scientific papers on biodiesel production from residual fish oil



Source: Author (2024)

3.4.1.4 Contributions of Funding Agencies

Table 3.2 presents the funding agencies for research development, the country of origin, the number of works funded within the period analyzed, and the percentage of the total number of articles. It is observed that the National Council for Scientific and Technological Development (CNPQ) stands out for the number of financed works (13), followed by the Coordination of Higher Education Personnel (CAPES) (10) and finally the Foundation for Science and Technology (FCT) (6). The three institutions that financed the most work on the topic during the period analyzed are in Brazil, which is also the country that stood out in the number of published works. Therefore, the significant role of funding agencies in encouraging and developing scientific research is identified, corroborating the influence of countries in certain fields of research.

Table 3. 2 – Agencies that stood out in collaboration for the publication of articles on the analyzed topic

Funding Agencies	Regions/Country	Count	Percentage (%)
National Council for Scientific and Technological Development	Brazil	13	7.34
Coordination for the Improvement of Higher Education Personnel	Brazil	10	5.65
Foundation for Science and Technology	Brazil	6	3.39
Spanish Government	Spain	5	2.82
Natural Sciences and Engineering Research Council of Canada	Canada	4	2.25
European Union	Europe	3	1.69
São Paulo Research Foundation	Brazil	3	1.69
National Natural Science Foundation of China	China	3	1.69
Soja de Portugal	Portugal	3	1.69
Engineering and Physical Sciences Research Council	United Kingdom	2	1.12

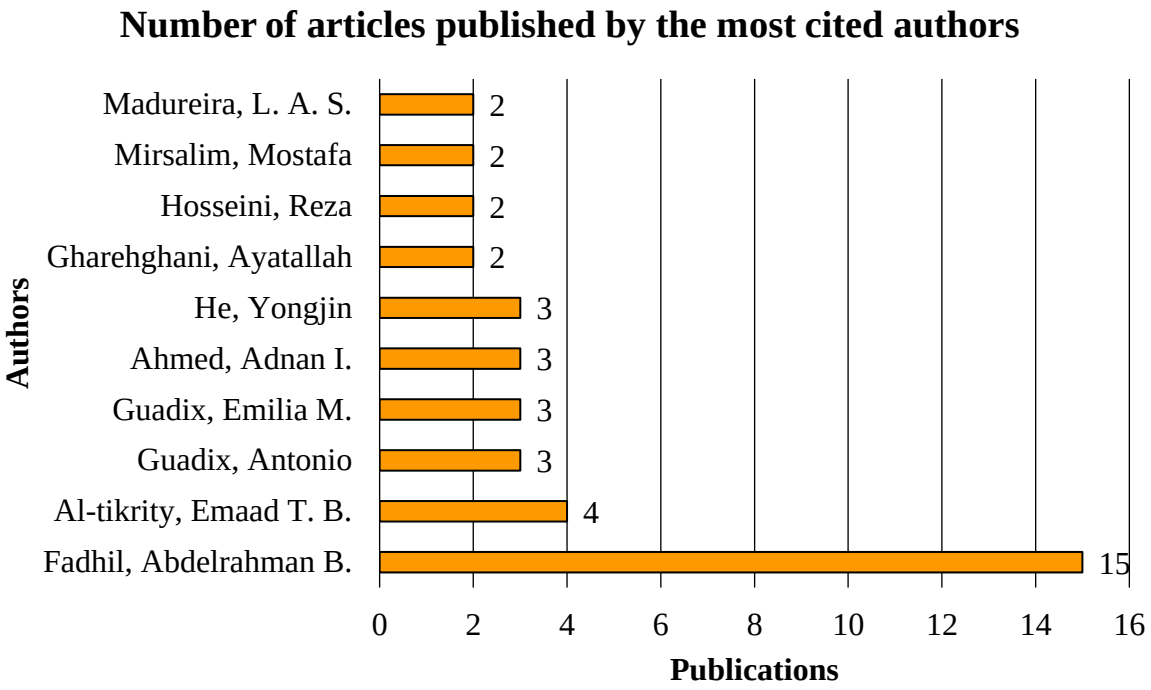
Source: Author (2024)

3.4.1.5 Contributions of Authors

The contribution of the authors was also investigated, where 502 authors were reported, but after the design where the author should publish at least 1 work and have 100 citations, only 40 authors met this criterion. Therefore, Figure 3.7 ranks the authors who published the most articles. The author Abdelrahman B. Fadhil, from the University of Mosul,

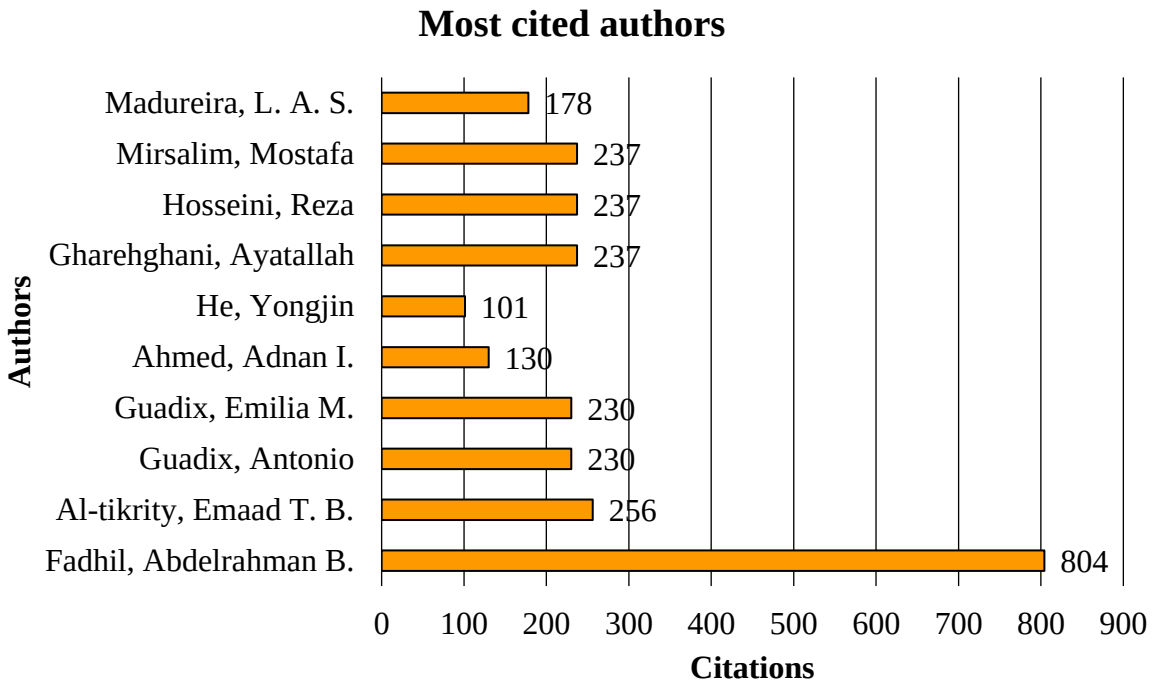
holds the largest number of published works (15). Next, the author Emaad T. B. Al-tikrity (4). Subsequently, the authors Antonio Guadix, Emilia M. Guadix, Adnan I Ahmed and Youngjin He with 3 published works each. Finally, the authors Ayatallah Ghareghani, Reza Hosseini, Mostafa Mirsalim and L. A. S. Madureita with 2 publications.

Figure 3. 7 – Authors who have published the most on the production of biodiesel from waste fish oil



Source: Author (2024)

Figure 3. 8 – Most cited authors in the production of articles regarding the production of biodiesel using waste oil from the fish processing industry.



Source: Author (2024)

3.4.1.6 Journal Analysis

Table 3.3 delineates the top 10 publications that extensively cover the topic of biodiesel production utilizing fish oil as a primary raw material. It also provides comprehensive details on the publications' country of origin, number of publications (NP), number of citations (NC), percentage of documents published by the journal on this topic, and the impact factor (IF).

The journal called "Fuel" accounts for 9.03% of all published papers and holds an impact factor (IF) of 7.4. It focuses on publishing articles related to the science and technology of fuels and energy, covering a wide range of topics including fuel cells, biofuels, synthetic fuels, oils, and gases.[1]. The journal "Renewable Energy" (7.64%) and IF: 8.7 is dedicated to the dissemination of knowledge about various aspects of renewable energy system technologies and components. Its scope encompasses biomass conversion, hydrogen production technology, climatology and meteorology, as well as socio-economic and political factors related to renewable energy.[2]. "Energy" (3.47%) and IF: 9, is an international, multidisciplinary magazine focusing on engineering and energy research. The journal publishes analyses, reviews, and evaluations of energy-related topics, as well as research in mechanical engineering and thermal sciences with an emphasis on energy analysis, modeling, and forecasting. Additionally, the

[1] Available at: https://www.sciencedirect.com/journal/fuel. Accessed on: 7/9/2024 3:55 PM
[2] Available at: https://www.sciencedirect.com/journal/renewable-energy. Accessed on: 7/9/2024 3:56 PM

journal welcomes articles on energy conservation, biomass and bioenergy, energy storage, and other relevant topics within its multidisciplinary scope.

Table 3. 3 – Ranking of scientific journals with the highest contribution to the publication of research on biodiesel production from residual fish oil

Rank	Journal title	Country	NP	NC	Percentage (%)	IF
1	Fuel	Netherlands	13	776	9.03	7.4
2	Renewable Energy	United Kingdom	11	634	7.64	8.7
3	Energy	United Kingdom	5	293	3.47	9
4	Energy Conversion and Management	United Kingdom	4	284	2.78	10.4
5	Energy Source, Art A: Recovery Utilization and Environmental Effects	United Kingdom	4	125	2.78	2.49
6	Renewable sustainable energy reviews	United Kingdom	4	468	2.78	15.9
7	Bioresource technology	United Kingdom	3	122	2.08	11.4
8	Catalysts	Switzerland	3	41	2.08	3.9
9	Energy & Fuels	United States	3	36	2.08	5.3
10	Fermentation	Switzerland	3	17	2.08	3.7

Source: Author (2024)

3.4.1.7 Most articles cited

Bibliometric analysis is a relevant tool to allow a quantitative and qualitative analysis of published works on a given topic. Therefore, Table 3.4 presents the ranking of the most cited articles on biodiesel production using residual oil from fish industry processing. It becomes possible to identify the most influential works during the period analyzed. It also allows the visualization of relevance metrics, and recognition of trends and emerging areas in the research field.

"A review on the prospects of sustainable biodiesel production: A global scenario with an emphasis on waste-oil biodiesel utilization", published in 2017 by the journal Renewable & Sustainable Energy Reviews, has a total of 320 citations and is the most cited article in the database. The authors discussed the controversies surrounding the use of edible and non-edible oils for biodiesel synthesis. This study presents a comprehensive literature review on the use of waste oils for biofuel production to ensure the production and consumption

of an economic and ecological bioproduct (Hajjari *et al.*, 2017).

"Biodiesel production Processes and Sustainable Raw Materials", published in 2019 by the Energies magazine has a total of 153 citations and its scope also presents a literature review on the possibility, advantages and disadvantages of replacing homogeneous catalyzed processes with heterogeneous processes to enable strategies for improving and sustaining biodiesel. It is worth noting that the article in question is the most recent among the most cited, indicating the relevance of the topic in the academic community and its impact on the industrial sector, as they are emerging improvements for promoting sustainable development (Ramos *et al.*, 2019).

"Effects of waste fish oil biodiesel on diesel engine combustion characteristics and emission", published in 2017 by the Renewable Energy magazine has a total of 145 citations and is an experimental article that aims to evaluate the performance characteristics between fish oil biodiesel and pure diesel. The authors concluded that biodiesel reduced variations from cycle to cycle, presenting superior gross thermal efficiency (2.92%) and lower combustion loss (15.2%) compared to diesel (Gharehghani; Mirsalim; Hosseini, 2017). In this way, contributing to the applicability of the generated bioproduct.

Table 3. 4 – Most cited articles on the production of biodiesel from the use of residual fish oil

Rank	Title	Journal	Year	Citations
1	A review on the prospects of sustainable biodiesel production: A global scenario with an emphasis on waste-oil biodiesel utilization	Renewable & Sustainable Energy Reviews	2017	320
2	Biodiesel Production Processes and Sustainable Raw Materials	Energies	2019	153
3	Effects of waste fish oil biodiesel on diesel engine combustion characteristics and emission	Renewable Energy	2017	145
4	Biodiesel production from *Silybum marianum* L. seed oil with high FFA content using sulfonated carbon catalyst for esterification and base catalyst for transesterification	Energy Conversion and Management	2016	143
5	Covalent immobilization of *Candida antarctica* lipase on core-shell magnetic nanoparticles for production of biodiesel from waste cooking oil	Renewable energy	2017	133

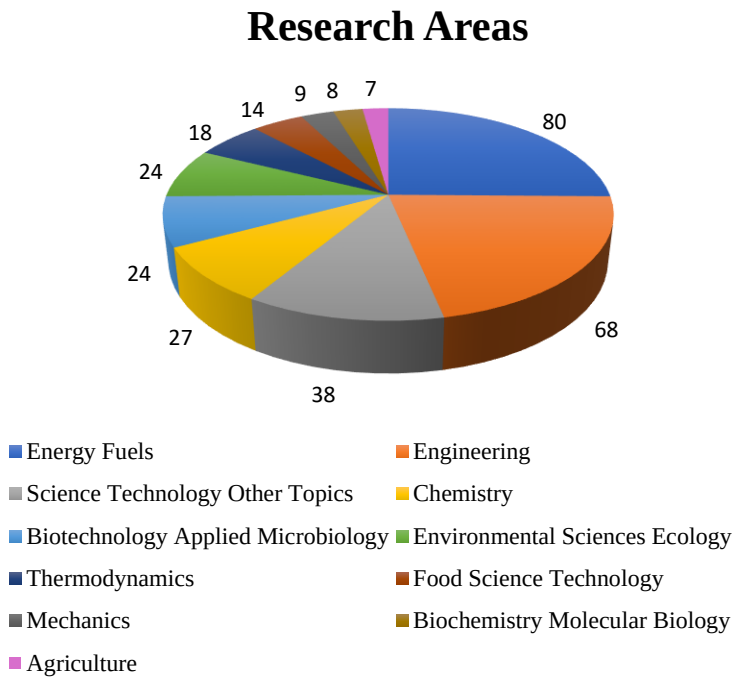
6	Influence of injection timing on performance, emission and combustion characteristics of a diesel engine running on fish oil biodiesel	Energy	2016	129
7	Biodiesel production from mixed non-edible oils, castor seed oil and waste fish oil	Fuel	2017	117
8	Waste fish oil biodiesel as a source of renewable fuel in Iran	Renewable & Sustainable Energy Reviews	2013	108
9	Biodiesel production from mixtures of waste fish oil, palm oil and waste frying oil: Optimization of fuel properties	Fuel Processing Technology	2015	98
10	Biofuels from waste fish oil pyrolysis: Continuous production in a pilot plant	Fuel	2009	97

Source: Author (2024)

3.4.1.8 Research area

Given the emergence of development and improvement of processes related to biodiesel production and the diversification of raw materials used, it becomes relevant to evaluate prominent research areas to identify trends and gaps. Therefore, Figure 3.9 shows the research areas where there were high amounts of publications. Therefore, “Energy Fuels” presents 80 publications showing the importance of expanding raw materials of renewable origin in the synthesis of liquid fuels. Next, “Engineering” totals 68 publications indicating the contribution of engineering in the development, advancements, and improvements of industrial processes with emphasis on the area of energy security. In contrast, “Agriculture” has only 7 publications, being the area with the smallest number of publications in the archives evaluated. Another promising area “Science Technology Other Topics” presents 38 published documents contributing to dissemination in the academic world and industrial sector.

Figure 3. 9 – Prominent research areas in the publication of articles about the production of biodiesel using fish oil as raw material



Source: Author (2024).

3.4.2 Hot research topics

3.4.2.1 Keyword analysis

Table 3.5 ranks the keywords that presented the highest number of occurrences accompanied by Total link strength. “Biodiesel production” leads the list with a total of 53 occurrences among the articles analyzed. Next came “Biodiesel” and “Transesterification” with 52 and 48 occurrences respectively. Analyzing the occurrence of keywords makes it possible to visualize the most active and emerging research areas, identifying trends and topics in each field. In this sense, Figure 3.10 presents a density map of the most prominent keywords in the evaluated study area. Furthermore, it enables the creation of knowledge maps where different concepts interconnect, facilitating the visualization of the knowledge structure (Figure 3.11), gaps or underexplored areas. In this way, it serves as a tool for strategic research design. Figure 3.12 presents an evaluation of the use of prominent keywords throughout the period investigated, corroborating the understanding of the relationship between different concepts pertinent to biodiesel production at different times.

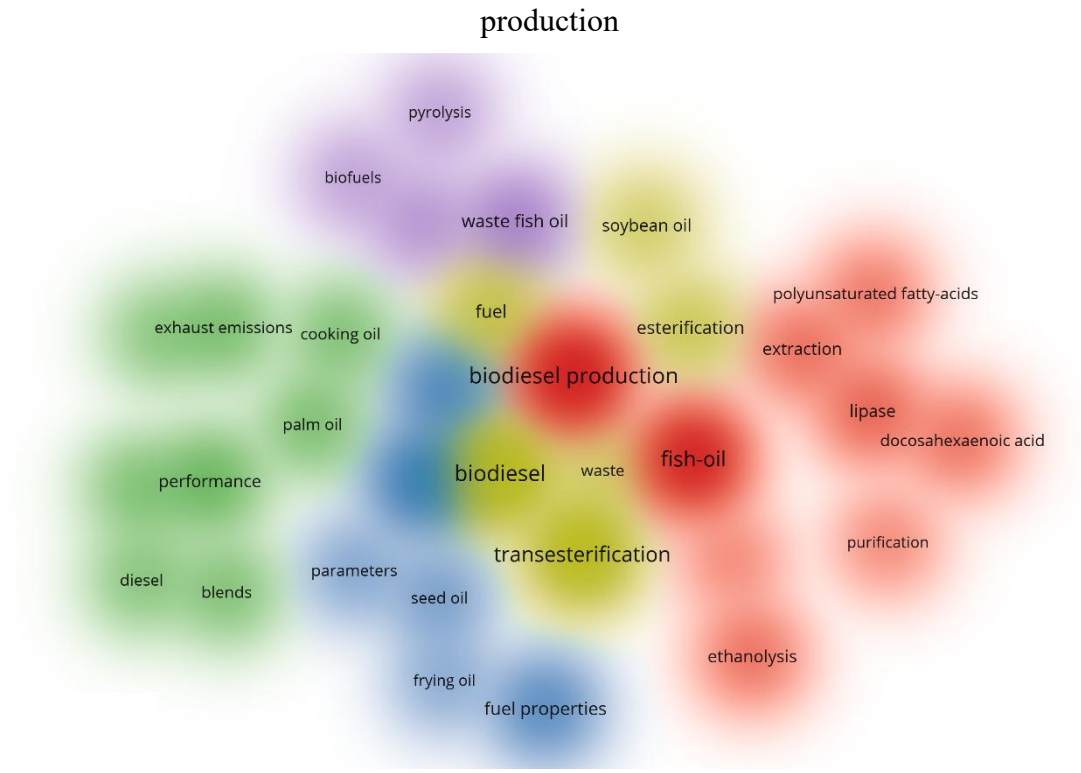
Table 3. 5 – The twenty most frequently used keywords in research and articles on biodiesel synthesis from residual fish oil

Rank	Keyword	F	Total link Strength	Rank	Keyword	F	Total link Strength

1	Biodiesel production	53	157	**11**	Lipase	16	54
2	Biodiesel	52	166	**12**	Performance	15	44
3	Transesterification	48	164	**13**	Ethanolysis	13	54
4	Fish-oil	46	155	**14**	Soybean oil	13	49
5	Optimization	31	126	**15**	Extraction	13	44
6	Fuel	22	71	**16**	Waste	12	48
7	Fish oil	21	68	**17**	Docosahexaenoic acid	12	35
8	Fuel properties	19	67	**18**	Seed oil	11	46
9	Esterification	18	61	**19**	Palm oil	11	42
10	Waste fish oil	18	45	**20**	Polyunsaturated fatty-acids	11	42

Source: Author (2024)

Figure 3. 10 – Density map of the 20 most prominent keywords in the field of biodiesel



Source: Author (2024)

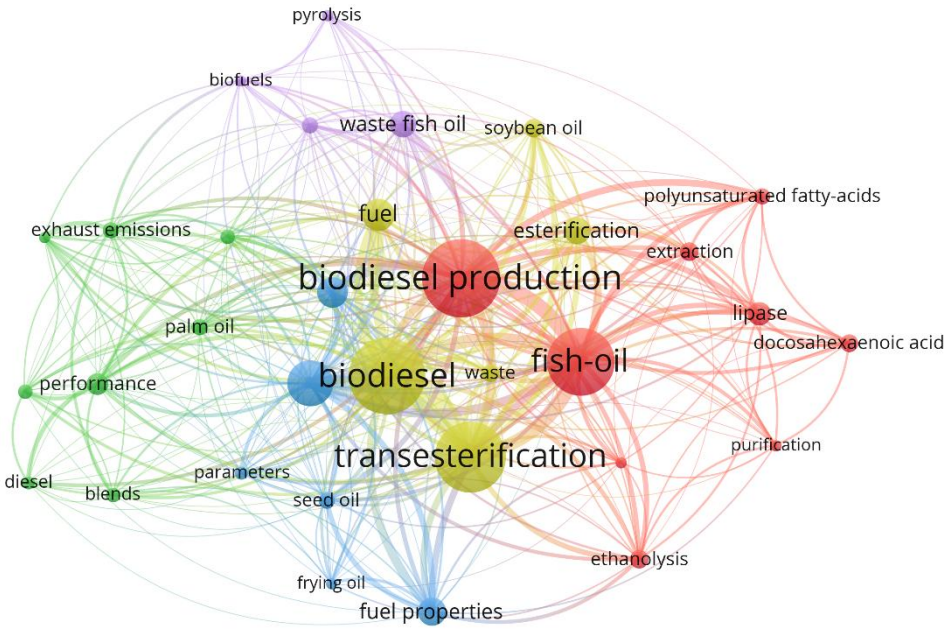
Figure 3.11 depicts a network of links that illustrates significant subjects and interrelations in the field of biodiesel generation from waste oils, with a focus on fish oils. The network's most essential terms, such as biodiesel production, biodiesel, fish oil, and transesterification, reflect the importance of these themes in literature. The pivotal location and extent of the links between these keywords emphasize their significance as foundational subjects for study and development in the field. Transesterification is one of the most used biodiesel production procedures, because to its effectiveness in turning waste oils into biodiesel (Yue *et al.*, 2024; Yusuf; Oladepo; Ganiyu, 2024; Zhang *et al.*, 2024). The strong connection between "biodiesel production" and "transesterification" highlights that much of the research in the field has focused on the optimization of this reaction, involving different catalysts and experimental conditions (Jayabal, 2024; Quispe; Coronado; Carvalho Jr., 2013; Ullah *et al.*, 2024).

The term fish-oil presents a dense network of connections with other terms such as waste fish oil, polyunsaturated fatty acids and docosahexaenoic acid. This demonstrates the growing interest in exploring fish oil as an alternative feedstock for biodiesel production, not only as a solution for industrial waste management but also because of its unique composition of polyunsaturated fatty acids, which can influence the properties of the biodiesel produced (Anu Prasanna *et al.*, 2023; Karia *et al.*, 2024; Tian *et al.*, 2024). Other connected terms, such

as exhaust emissions, fuel properties and performance, reflect environmental and technological concerns associated with the use of biodiesel derived from waste oils (Adhithan; Sachdeva, 2023; Ormond *et al.*, 2024; Taheri-Garavand *et al.*, 2022). Optimizing fuel properties to meet the demands of modern engines and reduce greenhouse gas emissions has been a priority for researchers in the last decade (Algayyim *et al.*, 2024; Islam Rony *et al.*, 2023; Togun *et al.*, 2024).

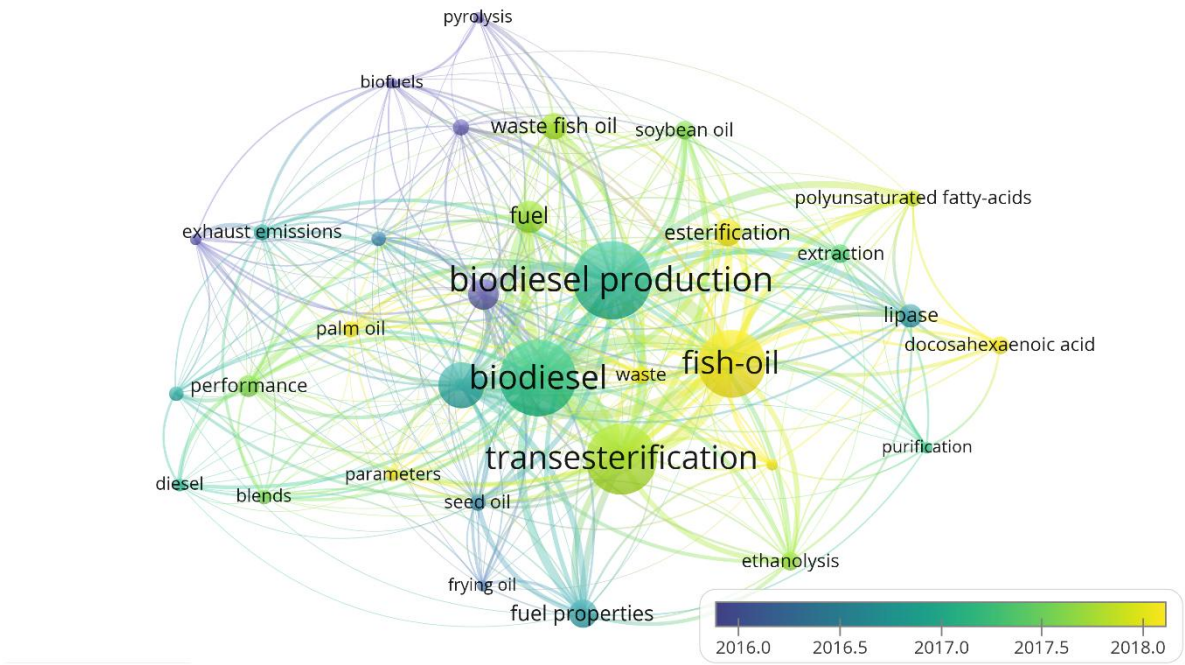
The coloring of the nodes, representing the period 2013 to 2023, suggests that themes related to fish oil and waste fish oil have gained relevance more recently. This may be related to the increased concern about industrial waste management and the search for renewable sources of biodiesel in response to pressure for more sustainable practices.

Figure 3. 11 – Network of connections among the most frequently occurring keywords in the investigated research field



Source: Author (2024).

Figure 3. 12 – Network of connections among prominent keywords in the field of study from 2013 to 2023



Source: Author (2024).

3.5 Conclusions

After conducting bibliometric analysis on the production of biodiesel from residual fish oil, it is possible to infer the growing relevance that this topic has in the scientific community. The production of biodiesel from fish waste has gained prominence, both for its potential to add value to byproducts of the fishing industry and for its contribution to the circular economy and environmental sustainability. The bibliometric analysis reveals that Brazil stands out as the main producer of publications in this area, which is directly related to the fact that the country is one of the largest fish producers in the world, having abundant residual raw material for this type of research.

Among the advantages identified, it is worth highlighting the use of a resource that would otherwise be discarded, promoting the reduction of waste and generating an alternative source of renewable energy. In addition, biodiesel from waste fish oils can present physical and chemical properties suitable for fuels, being compatible with the specifications required for use in diesel engines.

However, there are also significant challenges to be overcome, such as the variability in the composition of residual oils, which can impact the efficiency of transesterification processes and the final quality of biodiesel. Another obstacle involves the need to develop economically viable purification and refinement processes that ensure a competitive final product on the market. In addition, the logistics of collecting and storing this

type of waste can be complex, especially in regions where fishing is highly geographically distributed.

Therefore, it is necessary to develop alternative routes for catalysis, with the aim of promoting practices that foster sustainable development and the circular economy. The exploration of more efficient, biodegradable or renewable-source catalysts allows for a reduction in environmental impact, in addition to promoting the responsible use of resources. These alternative routes are essential for minimizing waste and reusing industrial byproducts, creating cleaner and more integrated processes, aligned with the principles of green chemistry and the circular economy.

CHAPTER 4

CHAPTER 4 – A STUDY ON BIODIESEL PRODUCTION FROM TILAPIA (*OREOCHROMIS NILOTICUS*) WASTE OIL USING EVERSA® TRANSFORM 2.0 LIQUID LIPASE: THEORETICAL AND EXPERIMENTAL APPROACHES

4.1 Abstract

Fish waste, its by-products found in nature or generated by the processing industry represent a significant portion in obtaining high-value products. Therefore, its disposal has an economic and environmental impact. In this aspect, the objective is to carry out a theoretical and experimental study on the biocatalytic production of ethyl esters through the enzymatic hydroesterification of residual tilapia (*Oreochromis Niloticus*) oil. Hydroesterification is characterized by hydrolysis followed by esterification. Therefore, complete hydrolysis of tilapia oil was performed after preparing a mass solution with a 1:1 ratio (oil/water) and heating it to 40° C. Additionally, 4% of *Thermomyces lanuginosus* lipase (TLL) was incorporated into the mixture and the system was kept under constant stirring for 4 h. Next, the experimental matrix was designed using the Eversa® Transform 2.0 lipase in the esterification step, varying the temperature (25, 40 and 55 °C), the molar ratio between free fatty acids (FFA) and ethanol (1:1, 1:5 and 1:9), the percentage of biocatalyst (1, 5 and 9%) and the reaction time (1, 3 and 5 h) using the Taguchi methodology. The experimental design was applied and the conversion results varied between 19.3 ± 0.38% (1:9, 3 h, 55 ºC and 1% biocatalyst) to 83.8 ± 0.29% (1:5 , 3 h, 25 ºC and 9% biocatalyst). The factors that stood out were the amount of biocatalyst followed by temperature. The ideal reaction levels obtained after statistical treatment were 5 h of reaction at 25 °C in a molar ratio of 1:1 (FFA/ethanol) using 9% of the biocatalyst, indicating a theoretical conversion of 96.73%. After carrying out the reaction with the indicated parameters, a conversion of 89.94 ± 0.09% was noted. It is noted that the experimental conversion value was lower than the theoretical conversion. The bioproduct was properly characterized regarding its density and viscosity properties, obtaining values of 2.47 cSt and 883.6 Kg/m3, respectively. In addition, a docking and molecular dynamics study was conducted, evaluating the stability of Eversa® Transform 2.0 lipase with FFAs, and the coupling of the enzyme's catalytic site with the substrate was observed. Finally, residual tilapia oil proved to be an alternative raw material for the synthesis of bioproducts, and the present study contributes to the development of sustainable biochemical processes.

Keywords: Ethyl esters; Enzymatic hydroesterification; Lipases; Molecular docking; Molecular dynamics.

4.2 Introduction

Feedstocks used for biodiesel synthesis are high-cost vegetable oils (sunflower, soybean, rapeseed, or canola oil), rendering them economically uncompetitive with petroleum diesel (Dias, Amanda M. S. *et al.*, 2022; Garg; Sabouni; Ahmadipour, 2023; Sreeharsha; Dubey; Mohan, 2023). Using edible feedstocks for biofuel synthesis can have adverse effects, especially in developing countries (Oyekunle *et al.*, 2023; Sreeharsha; Dubey; Mohan, 2023; Tibesigwa; Olupot; Kirabira, 2023). Non-edible vegetable oils (such as castor oil, cottonseed oil, neem oil, and rubber tree seed oil), have been explored like alternative feedstocks, including fatty acids derived from algae, and animal fats (Arshad *et al.*, 2023; Nayab *et al.*, 2022; Rambabu *et al.*, 2023). Animal fats are derived from meat processing, and fish oil is one of the byproducts of this process.

Aquatic animal output increased by 4% from 2020 to a new world high of 185 million tons (live weight equivalent) in 2022, with aquaculture contributing USD 296 billion to the total expected first sale value of USD 452 billion (The State of World Fisheries and Aquaculture 2024, 2024). Since more than half of the body weight of fish is wasted during processing, fish oil, which is present in varied amounts in the meat, head, frame, fin, tail, skin, and intestines of fish normally contain 2–30% fat, represents a strong potential for valorization — primarily for human consumption or the production of biodiesel (Coppola *et al.*, 2021; Maksimenko *et al.*, 2024; Mo; Man; Wong, 2018). Exploration of production methods is essential to ensure their proper application.

Esterification or transesterification routes are commonly used in biodiesel synthesis and can be chemically or biologically catalyzed (Al-Saadi; Mathan; He, 2020; Kushwaha *et al.*, 2023; Mandari; Devarai, 2022). The main road used for biodiesel production is transesterification, and there are requirements regarding the moisture content of the feedstock and fatty acids (Chozhavendhan *et al.*, 2020). Chemical compounds with extremely alkaline elements usually perform catalysis (MAS, 2023). However, using the chemical route has disadvantages, such as the generation of polluting waste, high energy consumption and the complexity of the glycerol recovery process (Abomohra *et al.*, 2020).

An alternative is the biological route, which uses enzymes to obtain bioproducts. Hose use on an industrial scale presents advantages in terms of sustainability and efficiency (Cavalcante *et al.*, 2021; Wancura *et al.*, 2023). Enzymes are biological catalysts responsible for promoting the transformation of chemical species into living systems. They can catalyze reactions under mild conditions with a very high degree of substrate specificity, thus reducing the formation of byproducts. (Brena; González-Pombo; Batista-Viera, 2013). In addition, they

are derived from renewable, biodegradable, biocompatible resources; their use reduces the cost of removing metal residues from products (Aboulrous *et al.*, 2022). According to the International Union of Biochemistry (IUB), enzymes can be classified into the following classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.

Lipases, a subclass of hydrolases, are carboxyl esterase enzymes derived from animals, plants, or microorganisms (e.g., bacteria, fungi, and yeast). They are also known as triacylglycerol hydrolases (EC 3.1.1.3) (Almeida *et al.*, 2021; Ismail; Baek, 2020). These enzymes can hydrolyze long-chain acyl glycerol (Ismail; Kashtoh; Baek, 2021), and their relevance is therefore due to their versatility in catalyzing various reactions such as transesterification (Shu *et al.*, 2023), esterification (Zeng; Zhong, 2023), interesterification (Ai *et al.*, 2023), hydrolysis (Culsum *et al.*, 2023), alcoholysis (Dias, Michele do Rocio Gonçalves *et al.*, 2022), acidolysis (Yoshioka *et al.*, 2023), and aminolysis (Mouad *et al.*, 2016).

Eversa Transform 2.0 (ET2) lipase is a genetically modified enzyme industrially derived from *Thermomyces lanuginosus* lipase (TLL), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain (Monteiro *et al.*, 2021). ET2 was designed primarily to produce biodiesel using feedstocks with free fatty acid content, i.e., from low acidity to high acidity oils such as residual oils. ET2 has 269 amino acid residues, a molecular weight corresponding to 31.5 kDa and has an activity of 9100 IU/mL (Acherki; Bouaid; Marchetti, 2022). Enzymes can be used in a variety of sectors (cosmetics, food, biofuels) and their use in processes needs to be optimized to ensure their industrial viability (Gama Cavalcante *et al.*, 2024; Shakilanishi; Shanthi, 2024; Yadav; Biswas; Goyal, 2024).

Thus, parameters such as molar ratio, temperature, percentage of biocatalyst and reaction time influence the yield and efficiency of the process (Ferreira Mota *et al.*, 2022; MAS, 2023) These parameters are relevant, as choosing inadequate conditions compromises the stability of the enzyme, increasing the cost or reducing the conversion of fatty acids into ethyl esters (Ramírez-Verduzco; Hernández-Sánchez, 2024; Riaz *et al.*, 2024; Sales *et al.*, 2022). Therefore, the use of statistical tools, such as the Taguchi method, can be used to determine the ideal combination of parameters at their respective levels, also helping to reduce the number of experiments required and optimizing resources (Karmakar; Dhawane; Halder, 2018; Karmakar; Halder, 2019; Yesilyurt; Cesur, 2022). Therefore, this methodology is not limited to maximizing process efficiency but also proposes a systematic approach to deal with the complexities related to biocatalysis, becoming an economically viable and competitive alternative for industrial biodiesel production (Dey *et al.*, 2024; Singh, Yashvir *et al.*, 2024; Zhu *et al.*, 2024). In addition to the advantages reported regarding the Taguchi methodology, there are computational tools

with interesting functionalities for understanding and expanding on the steps involved in the production process.

Molecular research, docking, and molecular dynamics are some of the pertinent computational methods that are used to describe the interactions between enzymes and substrates to create effective and optimal processes (Huang *et al.*, 2024; Nam *et al.*, 2024; Zhou; Huang, 2024). While molecular dynamics enables the observation of the durability and adaptability of these interactions under simulated settings, docking analysis offers information on the enzyme's binding mechanisms and affinity with substrates (Jin; Wei, 2024; Krishna *et al.*, 2024; Lu *et al.*, 2024). These revelations are essential for clarifying the variables affecting catalytic performance and providing evidence for experimental modifications that optimize biodiesel production.

The current article aims to explore the potential of residual tilapia (*Oreochromis niloticus*) oil in biodiesel production through enzymatic catalysis. To achieve this, we will optimize the synthesis/production process and investigate how different reaction parameters (such as molar ratio, biocatalyst, temperature, and time) affect the outcome. In addition, we will use the Taguchi methodology to analyze the results. Finally, we will apply theoretical chemistry concepts to study the molecular dynamics and coupling during the enzymatic reaction using Eversa® Transform 2.0 lipase as a biocatalyst.

4.3 Methodology

4.3.1 Materials

This study utilized the commercial lipase Eversa® transform 2.0 from *Aspergillus oryzae* and lipase from *Thermomyces lanuginosus*, both of which were procured from Sigma-Aldrich Brasil Ltda, located in Cotia, São Paulo, Brazil. All other chemical reagents used were of analytical grade and were provided by Synth, São Paulo, Brazil and Vetec, São Paulo, Brazil. The experimental design of this study was developed using the advanced Statistica® 10 software, which employs the Taguchi method. The residual tilapia oil used in this research was obtained through collaborative efforts with fish farming companies located in the state of Ceará.

4.3.2 Methods

4.3.2.1 Hydrolysis Step: Conducting enzymatic hydrolysis for the extraction of free fatty acids (FFA)

Initially, the residual tilapia (*Oreochromis niloticus*) oil was hydrolyzed via enzymatic catalysis using *Thermomyces Lanuginosus* lipase. This article followed the

methodology proposed by Carvalho et al. (2021) with some adaptations. Therefore, an oil:water solution of equivalent volumes was prepared, which was kept heated to 40°C, where 0.4% of the biocatalyst was added to the mass of the oil and the system was kept under stirring for 4 hours at a constant temperature (CARVALHO et al., 2021). Then, the solution was transferred to a separating funnel for phase separation. Distilled water previously heated to 60º C was added, and the aqueous phase was discarded. FFAs were washed three times and heated for 10 minutes at a constant temperature of 80º C. During filtration to remove moisture, anhydrous sodium sulfate was used. Finally, the AGLs were stored in an amber bottle (Alexandre *et al.*, 2022).

4.3.2.2 Acidity index

The initial and final (after the hydrolysis step) acidity index (A) was calculated from Equation 1. Aliquots of 0.2 g were removed from the volume of the final reaction mixture and diluted in 5 mL of ethyl alcohol and 3 drops of phenolphthalein were added, then titrated with 0.1 M sodium hydroxide solution.

$$AI = \frac{MM_{NaOH} * M_{NaOH} * f * V_{NaOH}}{m} \quad (1)$$

$MWNaOH$ (g/mol) is the molar mass of NaOH; $MNaOH$ (mol/L) is the molarity of the NaOH solution; f is the correction factor determined by standardizing the NaOH; $VNaOH$ is the volume of NaOH used during the titration (L); and m (g) is the mass of the sample to be studied. The conversion of free fatty acids into esters (Equation 2) was determined considering the initial (AI_i) and final acidity of the sample (AI_F). (Moreira *et al.*, 2020).

$$Conversion\ (\%) = \frac{AI_i - AI_f}{AI_i} x100 \quad (2)$$

4.3.2.3 Esterification Step: Optimization of enzymatic esterification aimed at biodiesel production, utilizing the Taguchi method for variable analysis

To enhance ester conversion efficiency, four parameters were stratified into three levels utilizing a conventional L9 orthogonal matrix based on the Taguchi method. The designation of "L" and "9" denotes the Latin square and the number of experiments, respectively. Table 4.1 delineates the four independent variables (molar ratio, biocatalyst, temperature and time) alongside their corresponding values. It is noteworthy that the reaction volume was employed to determine the biocatalyst percentage after the molar ratio calculation (Esan *et al.*, 2021).

Table 4. 1 — Codified levels of experimental design and independent parameter scope

Levels	Molar ratio (v:v)	Biocatalyst (%)	Temperature (°C)	Time (h)
Level 1 (L1)	1:1	1	25	1
Level 2 (L2)	1:5	5	40	3
Level 3 (L3)	1:9	9	55	5

Source: Author (2024)

Since this study aims to improve the response (fatty acid conversion), the S/N (signal-to-noise) ratio values corresponding to the coatings were determined using the specific characteristics of the "bigger is better" function. The Taguchi method uses the S/N ratio to evaluate quality characteristics and deviations from the desired value. The system performs well when the signal-to-noise (S/N) ratio is used to evaluate the data, as it becomes less sensitive to the causes of fluctuation (Monteiro *et al.*, 2022). For each experiment, the S/N ratio was calculated using Equation 3.

$$\frac{S}{N} = -\log\left(\frac{1}{n}\sum_{i}^{n} = 1\frac{1}{y_i^2}\right) \quad (3)$$

Where y is the fatty acid conversion for the associated sample, i represents the number of replicates, and n corresponds to the number of responses for each given parametric combination of factor values. Equation 4 evaluated the signal-to-noise ratio projected under optimal conditions for the process of obtaining maximum conversion.

$$\frac{S}{N_{predicted}} = \frac{\bar{S}}{N} + \sum_{j}^{n} = 1\left(\frac{S}{N_j} - \frac{\bar{S}}{N}\right) \quad (4)$$

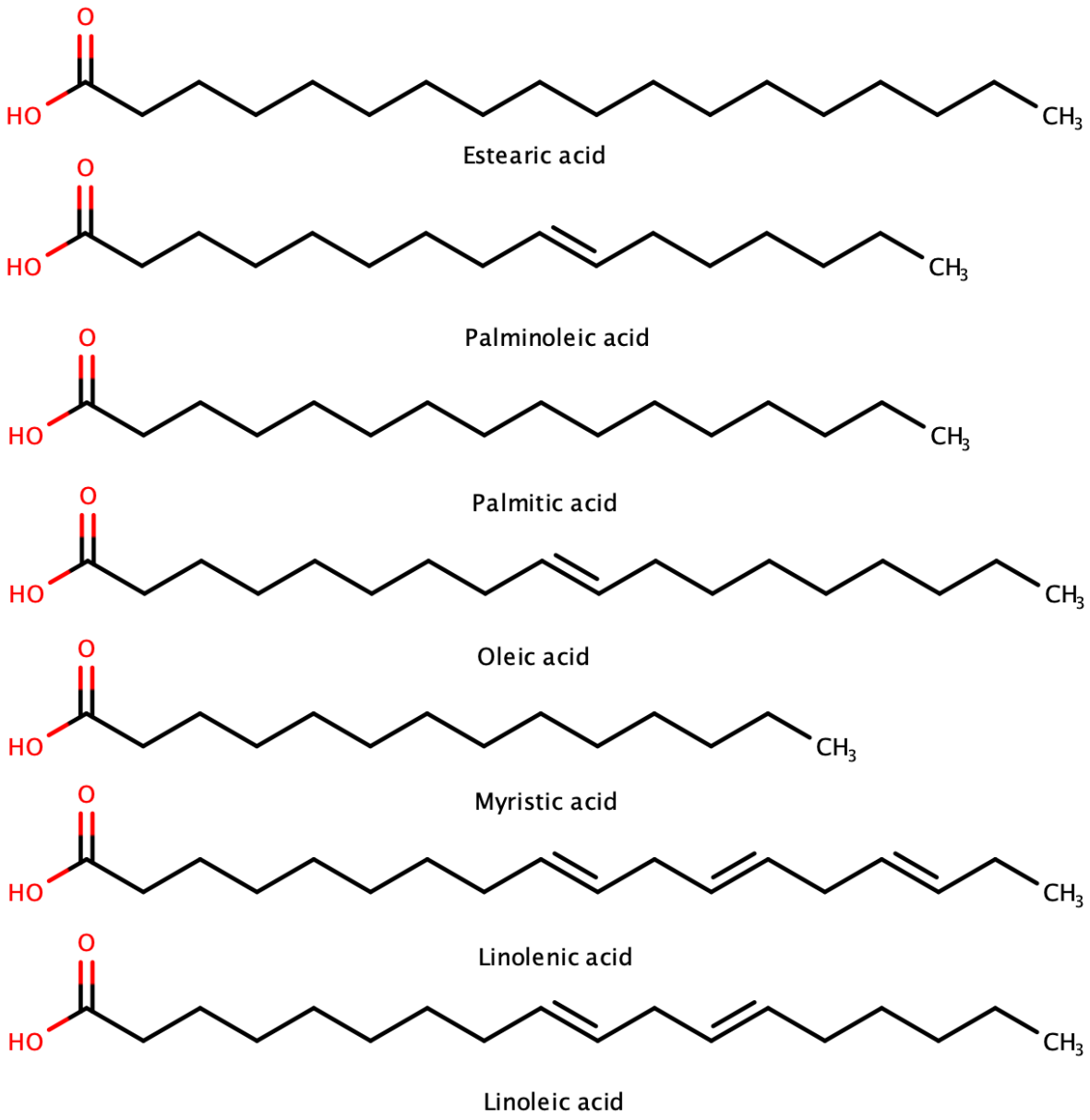
Where $\overline{S}/N$ is the arithmetic mean of all S/N ratios, S/Nj denotes the S/N ratio at the ideal point for each component, and n denotes the number of factors that have a substantial impact on the process.

4.3.2.4 Theoretical study

4.3.2.4.1 Preparation of binders and proteins

The molecules of the lipidic composition of fish oil using Eversa 2.0 were create by Chem3D software (Ahmadi *et al.*, 2005), Figure 4.1, In the auto-optimization settings, the force field has been applied MMFF94S (Wahl *et al.*, 2019), to generate bioactive conformations by minimization of randomly generated conformers, with Steepest Descente algorithm (Petrova; Solov'Ev, 1997), Step per Update 4 (Sutton; Mahmood; White, 2016), pelo software AVOGADRO (Hanwell *et al.*, 2012). The file with ligand was converted to corresponding format (.pdbqt) with addition of ionization and tautometic states at pH 7.4 by using OpenBabel ver. 3.0.0 software (O'Boyle *et al.*, 2011).

Figure 4. 1 – Structures in 2D of the fish oil composition



Source: Author (2024)

The receptor under study was the structure of the lipase Eversa 2.0 that was modeled the following literature procedures (Alexandre *et al.*, 2022; Nobre *et al.*, 2023), whose crystalline structure was obtained by complex X-ray diffraction. To make molecular docking possible, the interfering residues, water molecules, and the synthetic inhibitor were removed. Polar hydrogens were added to the ligands, separately, and the protein. This technique aims to search inside potential virtual ligand databases for a given protein target. The software used was Autodock tools (Morris *et al.*, 2009).

4.3.2.4.2 Molecular docking

Molecular docking was performed by AutoDock Vina (Trott; Olson, 2010) employing 3-ways multithreading, Lamarkian Genetic was performed. For docking of Eversa 2.0 main

protease complex next parameters were used: number grid points in xyz (30 30 30), spacing (0.642), grid center in xyz (-34.282188 24.937188 73.691625). Other parameters were set to default. Input ligands with polar hydrogens were used in .pdbqt format. Between ten and forty molecular docking executions were performed, and number of simulations that were repeated in the same region of the biological receptor. Thus, to validate the performance of the simulation and quantify the quality of the dockings, the RMSD (root mean square deviation) scoring criterion was adopted, which suggests that a successful docking exhibits an RMSD value rmsd ≤ 2.0 Å (Sutherland *et al.*, 2007). The simulation data with the main receptor-ligand interactions were visualized by the Discovery Studio software (Biovia, 2015) e Pymol (DeLano, 2020).

4.3.2.4.3 Statistical analysis

Results are expressed as mean values ± standard error of the mean. After confirming normal distribution and homogeneity of the data, differences between the groups were subjected to analysis of variance (one-way ANOVA) and two-way ANOVA in experiments with antagonists (Fujikoshi, 1993), followed by Tukey's test (Lin; Zhang, 2018). All analyses were performed using GraphPad Prism v.8.0 software. The level of statistical significance was set at 5% ($P < 0.05$).

4.3.2.4.4 Molecular dynamic

Molecular dynamics (MD) simulations were performed with the NAMD program (Phillips *et al.,* 2005). The best conformations obtained in molecular coupling were solved in water in the TIP3P model (Kato *et al.,* 2021), in the CHARMM36 force field and addition of ions to neutralize the total system load. Finally, it was submitted to energy minimization by the Steepest Descent method. The system was then introduced to NVT and NPT balances under conditions described by Langevin (Farago, 2019). The system production simulations were performed with a time of 100 ns. The quality of the structures obtained in MDs was evaluated using the following parameters with NAMD:

- Potential Energy (kcal/mol) (Diez *et al.*, 2014);
- Protein-Ligand Interaction Energy (kcal/mol);
- The mean quadratic deviation of the atomic positions of proteins, binders, and distances between them (RMSD, Å), and the mean quadratic deviation of the atomic positions of proteins, ligands, and distances between them (RMSD, Å);
- Hydrogen bonds were evaluated with Visual Molecular Dynamics (VMD) (Humphrey; Dalke; Schulten, 1996).

- The mean quadratic fluctuation of the minimum distances between proteins and ligands was observed in MD (RMSF, Å) (Arshia *et al.*, 2021). The graphs were generated using the Qtrace program (Lima *et al.*, 2012).

4.3.2.5. Physicochemical characterization of the bioproduct produced

4.3.2.5.1 Kinematic viscosity at 40 ºC

The kinematic viscosity at 40 ºC was determined following the methodology outlined in the ASTM D-70422 standard. The Anton Paar SVM 3000-Stabinger digital viscometer (Austria) was employed for this purpose.

4.3.2.5.2 Density at 20º C

The density at 20 ºC was determined according to the procedure outlined in the ASTM D-7042 standard. The Anton Paar SVM 3000-Stabinger digital viscometer (Austria) was employed for the measurement.

4.3.2.5.3 Gas Chromatography-Mass Spectrometry (GC/MS)

For the determination of ester content (C) of the obtained biodiesel samples, GC/MS analysis was performed based on the methodology described in the EN 14,103 norm (Czech Standards Institute, 2011). Approximately 50 mg of biodiesel sample was added to a vial (2 mL) containing 1 mL of the methyl nonadecanoate solution (10 mg/mL). This mixture was injected (1 µL) into a gas chromatograph-mass spectrometer SHIMADZU QP-2010 ULTRA equipped with a (5%-phenyl)-methylpolysiloxane (DB-5) capillary column (30 m × 0.25 mm × 0.25 µm film thickness) using helium as carrier gas in a splitless mode.

4.3.2.5.4 High-performance liquid chromatography (HPLC)

An aliquot of the reaction medium was extracted at the optimum conversion point and analyzed using high-performance liquid chromatography (HPLC) with a Shimadzu LC-18 column and ultraviolet detector. An isocratic method was employed with a 30% acetonitrile and 70% methanol mixture. A volume of 20 µL was injected per minute for 5 minutes at 40 ºC, with a wavelength of 254 nm selected due to its higher absorption in the spectrum (de Sousa Junior *et al.*, 2023).

4.3.2.5.5 Nuclear Magnetic Resonance (NMR)

Using a Bruker spectrometer, model Advance DRC-300, housed at the Northeastern Center for the Application and Use of Magnetic Resonance at the Federal University of Ceará (CENAUREMN-UFC), one-dimensional spectra of Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) and Carbon (^{13}C NMR) were acquired. The hydrogen frequency was 500 MHz, and

the carbon frequency was 75 MHz. Deuterated chloroform (CDCL3) served as the solvent for sample dissolution, and the samples were analyzed in 5 mm tubes (de Sousa Junior *et al.*, 2023).

4.4 Results and discussion

4.4.1 Hydrolysis Step: Conducting enzymatic hydrolysis for the extraction of free fatty acids (FFA)

The acidity index of the tilapia residual oil increased from 41.56 mg NaOH/g to 94.64 mg NaOH/g following enzymatic hydrolysis, indicating the achievement of the intended objective. This increase signifies the oil's release and elevation of free fatty acids. Santos et al (2010) used tilapia oil to obtain free fatty acids for biodiesel production, however, they opted for chemical hydrolysis (alcoholic saponification with sodium hydroxide followed by hydrolysis with sulfuric acid) assisted by ultrasound (Santos et al., 2010). While Souza et al (2023) investigated the hydrolysis of residual frying oil catalyzed by enzyme blends and obtained satisfactory results (Souza *et al*., 2023).

Therefore, it is important to note the relevance of the issue regarding the hydrolysis of residual oils for the wealth of high-added-value products. It is also important to highlight the challenges faced by each route. Since the chemical route requires subsequent steps for separation and purification, there is an increase in cost concerning time and energy. In contrast, the enzymatic route has advantages in terms of specificity, resulting in the non-generation of unwanted by-products, and does not require subsequent steps related to separation or purification.

4.4.2 Esterification Step: Optimization of enzymatic esterification aimed at biodiesel production, utilizing the Taguchi method for variable analysis

The Taguchi method enabled the optimization of reaction parameters, resulting in a significant reduction in the number of experimental steps in the process. In this way, it was possible to identify the best levels for each parameter, exploring their variations in the experimental design to achieve maximum conversion into esters. Consequently, Table 4.2 presents the conversion values obtained, together with the complete experimental design, which includes parameters, levels and their corresponding values, in addition to the S/N for each experiment analyzed. It is important to emphasize that the reactions were conducted in duplicate, ensuring that the margin of error remained within the desired standards.

Table 4. 2 – L9 orthogonal array of experimental runs accompanied by ethyl ester conversion and signal-to-noise (S/N) ratio

Reaction	Molar ratio	Biocatalyst (% w/w)	Temperature (°C)	Time (h)	Conversion (%)	S/R
1	1:1	1	25	1	61.5±0.05	35.77
2	1:1	5	40	3	49.6±0.17	33.90
3	1:1	9	55	5	70.1±0.02	36.90
4	1:5	1	40	5	48.8±0.74	33.76
5	1:5	5	55	1	50.1±0.72	34.00
6	1:5	9	25	3	83.8±0.29	38.45
7	1:9	1	55	3	19.3±0.38	25.72
8	1:9	5	25	5	74.4±0.54	37.42
9	1:9	9	40	1	75.1±0.41	37.50

Source: Author (2024)

The parameters that most influenced the reaction were identified: the percentage of biocatalyst followed by temperature. This shows the dependence that these factors have, since enzymes can undergo denaturation at high temperatures and consequently have their catalytic activity reduced. (Arcus; Mulholland, 2020). It is observed that the percentage of biocatalysts stood out about the others because as the level increased, the conversion percentages were increased proportionally. Points 6, 8, and 9 presented two of the three highest conversions sharing the highest level of biocatalyst percentage (Table 4.2).

Next, it is noted that the temperature also stood out in relation to the other parameters. In view of this, it is worth mentioning the specific characteristic of the catalytic activity of the lipase used in the temperature range studied. Lipase Eversa® Transform 2.0 has its catalytic activity reduced in reactions conducted at temperatures above 45 ºC, while in experiments carried out at 40 ^{o} C its performance as a biocatalyst is enhanced (Monteiro *et al.*, 2021). Points 6, 8 and 9 presented the three highest conversions of the experimental design and are based on the combination of the highest percentage level of biocatalyst and the temperature range in which the biocatalyst does not suffer/suffered interference by temperature (Table 4.2).

Among the parameters that least influenced the conversion values of ethyl esters were time and molar ratio. It is worth mentioning that their relevance is not discarded, however, some factors were more decisive. Excessive use of alcohol can infer denaturation or inactivation of the enzyme, resulting in reduced yields (Hasnaoui *et al.*, 2025; Koroleva *et al.*, 2024; Sadana,

1988). Point 7 obtained 19.3 ± 0.38%, being the lowest conversion of the experimental matrix and occurred in the presence of excess alcohol and the smallest amount of biocatalyst investigated. The amount of enzymes used is responsible for determining the increase or reduction of process costs (Jegannathan; Nielsen, 2013; Liang *et al.*, 2025).

4.4.2.1 Signal-to-Noise (S/N) Analysis

The Taguchi methodology allows the evaluation and determination of the significance of the investigated parameters, as it provides the S/N ratio values. In this study, the "higher is better" function was applied to identify the relationship values through the conversion values, as they are linked to the S/N ratio. Therefore, Table 4.3 presents the average S/N values for all levels of each parameter at their respective level and the value of their variation (delta). The delta value for each parameter is calculated uniquely and by subtracting the highest S/N value from the lowest value. In this way, the order and significance of the evaluated parameters are classified.

Table 4. 3 – Analysis of signal-to-noise (S/N) ratios for reaction parameters and factor ranking

Factors/Levels	**Molar ratio**	**Biocatalyst**	**Temperature**	**Time**
1	35.53	31.76	37.22	35.76
2	35.41	35.11	35.06	32.70
3	33.55	37.62	32.21	36.03
Delta	1.98	5.86	5.01	3.33
Ranking	4	1	2	3

Source: Author (2024)

The biocatalyst and temperature stood out among the parameters analyzed and obtained deltas of 5.86 and 5.01, respectively (Table 4.3). Therefore, the coherence between the experimental data presented and discussed in Table 3 is highlighted, as they indicated the same significant parameters for the execution of the process. It is noted that increasing the level of biocatalysts resulted in a significant and linear gain, so the variation between 1% (Level 1) and 9% (Level 3) proved to be relevant. It is noteworthy that the temperature variation between 25 ºC (Level 1) and 55 ºC (Level 3) caused a significant decrease in the average S/N values, since, as it is a reaction conducted in the presence of a biological catalyst of Protein origin, raising the temperature can be favorable to the denaturation of the protein and consequently the reduction of its catalytic activity.

Given this, it is worth discussing the dependence between the parameters that stood

out and those that were less significant in the statistical analysis. Therefore, it is possible to point out the relevance of the biocatalyst percentage if it stands out from the increase in the molar proportion. The variation in the molar ratio between 1:1 (Level 1) and 1:9 (Level 3) was decreasing, indicating that excess alcohol is also a factor in the occurrence of denaturation of the enzyme's catalytic triad, therefore Level 1 (1:1) was the most recommended for conducting these reactions using the average S/N results.

4.4.2.2 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) was performed using statistical methods. Therefore, Table 4.4 presents the values obtained and it is highlighted that the p-value is the main highlight, as the literature reports that this value is responsible for determining the significance of the parameters studied.

Table 4. 4 – Analysis of the factors influencing biocatalytic reactions in biodiesel production

Factors	SS	MS	DF	*F value*	*p-value*	Contribution (%)
Molar Ratio	(25.55)	-	(2)	-	-	-
Biocatalyst	1640.37	820.185	2	19.56	0.011	60.39
Temperature	1068.58	534.29	2	12.74	0.023	39.34
Time	7.34	3.67	2	0.09	0.782	0.27
Total	2716.29		6	-	-	100

Source: Author (2024)

To determine the significance of a parameter, the p-value is used, which guarantees up to 95% confidence when less than 0.05. Therefore, the biocatalyst and temperature presented significance in the reliability range, presenting p-values corresponding to 0.011 and 0.023, respectively. The contribution of the biocatalyst exceeded that of the temperature, as it presented 60.39% versus 39.34% (Table 4.4). Given the statistical analysis performed, it was possible to define the levels and values of the parameters under optimized conditions to ensure maximum conversion of esters during the reaction. Thus, the optimum point of the reaction, given the factors investigated, occurs in 5 h (Level 3), at a temperature of 25 ^{o}C (Level 1) with a molar ratio of 1:1 (Level 1) using 9% (Level 3) of the biocatalyst.

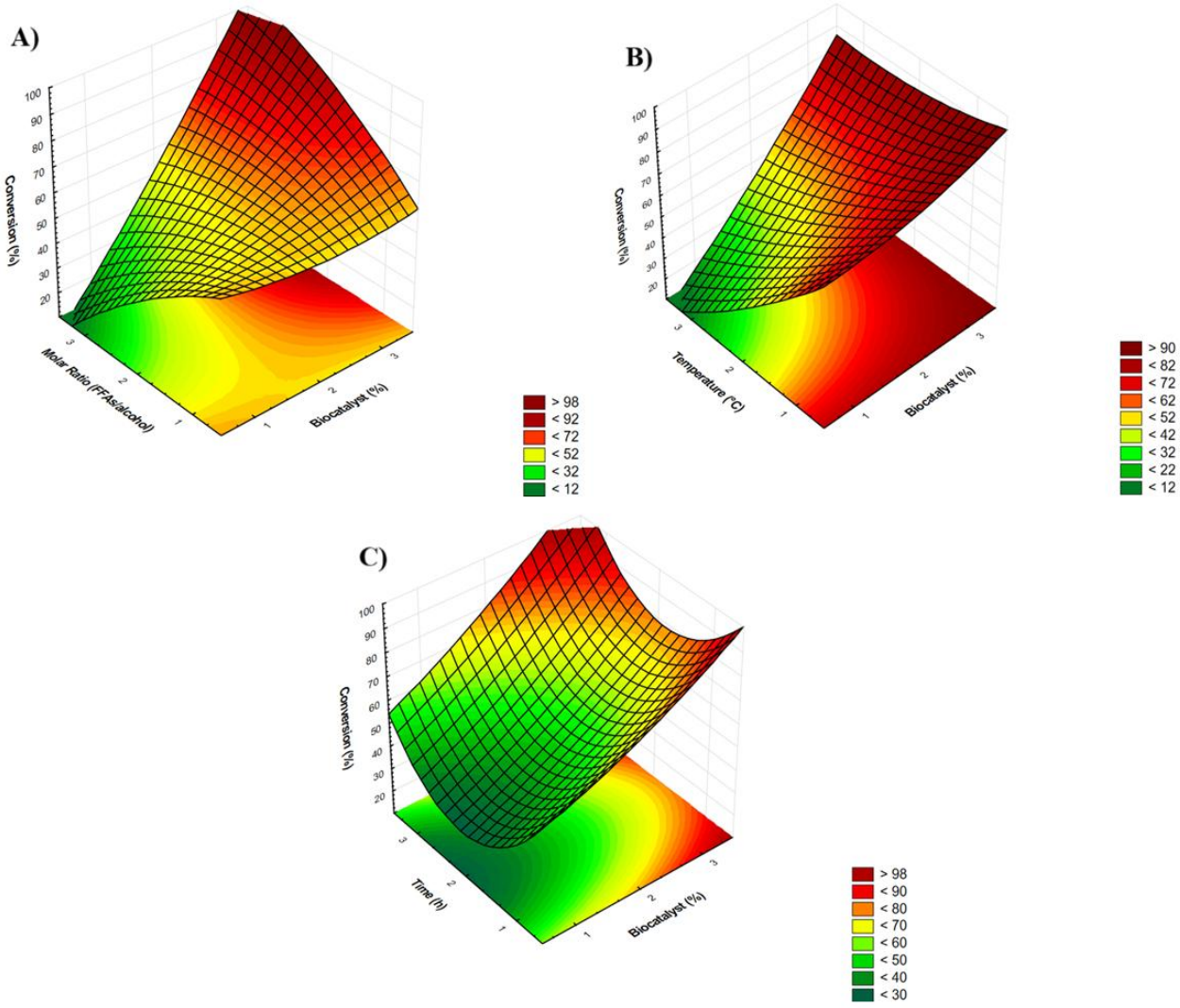
Theoretically, the optimum point would present a conversion equivalent to 96.73%, but after applying this point experimentally, the conversion was 89.94 $\pm$ 0.09%. The methodology satisfactorily fulfilled the purpose of optimization, since a higher conversion was obtained using the lower temperature and the molar ratio of 1:1 in Level 1. Thus, it is observed

that the experimental conversion was lower than the theoretical one.

4.4.3 Conversion Statistical Analysis

After the statistical analysis of the experimental matrix presented, contour surface graphs were prepared that relate the biocatalyst, the parameter with the greatest contribution, about the other parameters studied. Figure 4.2 shows the respective graphs demonstrating the relationship of such combinations and consequently the conversion profile.

Figure 4. 2 — Contour surface plots showing the relationship between the most influential factor and the other parameters. (A) Molar ratio (%) versus Biocatalyst (%). (B) Temperature (ºC) versus Biocatalyst (%). (C) Time versus Biocatalyst (%)



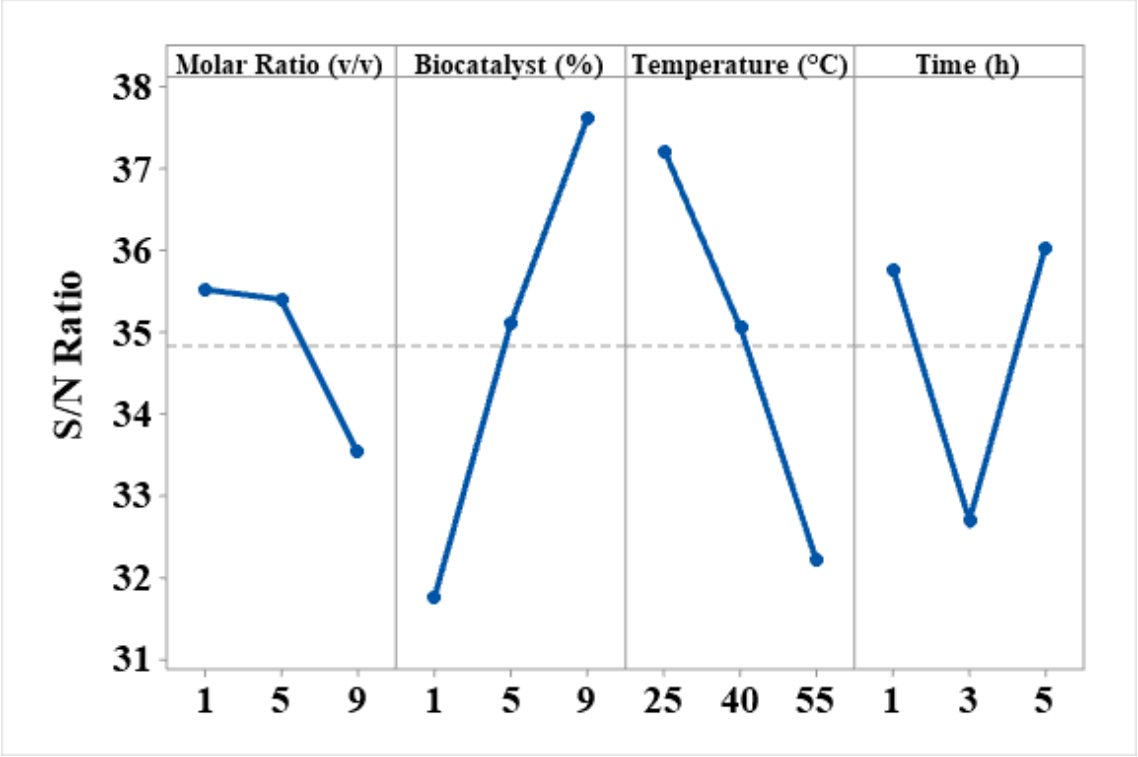
Source: Author (2024)

Figure 4.2 (A) shows the conversion profile corresponding to the dependence between the molar ratio and the biocatalyst, indicating that by increasing the molar ratio to Level 2 (1:5) and the biocatalyst to Level (3), conversions higher than 80% were obtained. Meanwhile, Figure 4.2 (B) demonstrates that by maintaining the temperature at Level 1 (25 º

C) and increasing the percentage of biocatalyst used, conversions in the same range were obtained. The variation between the time and biocatalyst levels did not present linear behavior (Figure 4.2 (C)).

Figure 4.3 allows visualization of the variation in the impact of the analyzed factors at their respective levels investigated. Once again, the relevance of the biocatalyst is observed, being proportional to the increase in level. In contrast, temperature presented greater contributions when used at lower levels. Next, time did not show linear behavior. Finally, the molar ratio was considered in this process as the factor with the lowest contribution to forming ethyl esters.

Figure 4. 3 — Analysis of the effect of investigated reaction parameters on the S/N ratio in enzymatic biodiesel synthesis: influence of molar ratio, biocatalyst concentration, temperature, and reaction time



Source: Author (2024)

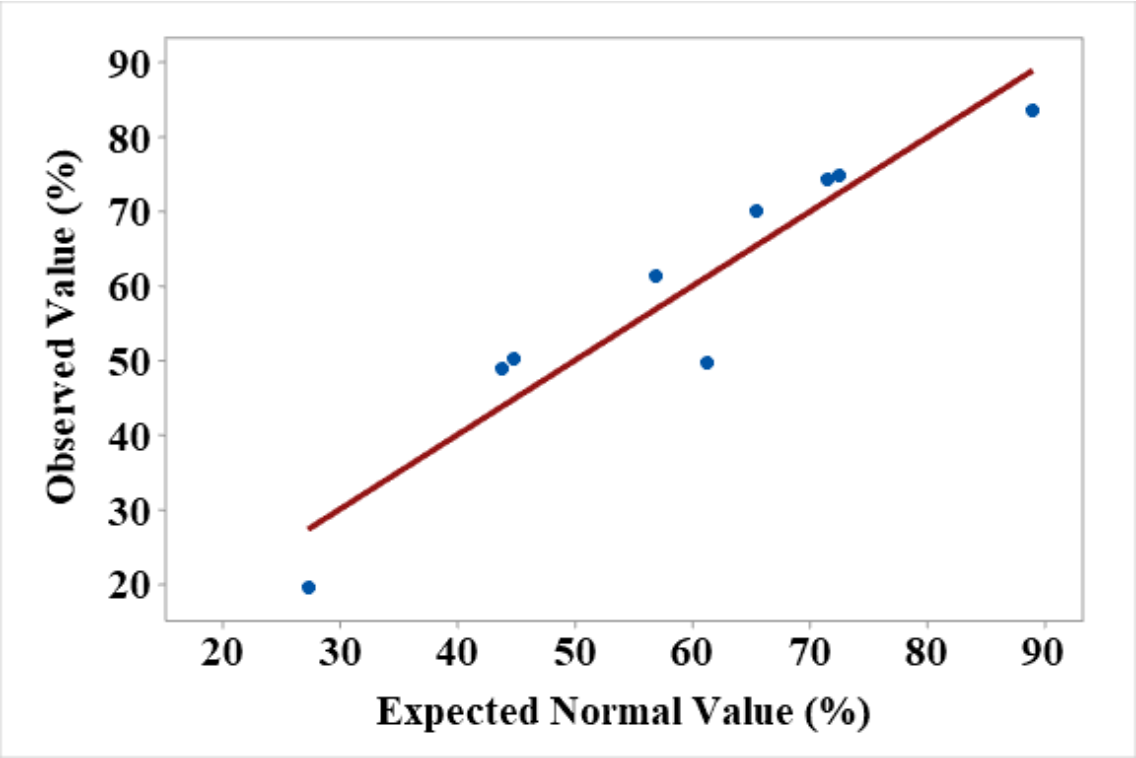
Through the data obtained using the Taguchi methodology, it is possible to determine the regression equation, through the dependent variables and the conversion estimate (Equation 5)

$$\mathbf{Y(\%) = 75{,}0 - 0{,}516}\boldsymbol{A} \mathbf{+ 4{,}134}\boldsymbol{B} \mathbf{- 0{,}890}\boldsymbol{C} \mathbf{+ 0{,}55}\boldsymbol{D} \quad (5)$$

Where Y (%) is the estimated conversion in the esterification reaction studied, A (molar ratio), B (biocatalyst), C (temperature) and D (time). Figure 4.4 is a graph commonly

analyzed to compare the degree of proximity of the experiments performed with reality. Therefore, a smaller gap between the points and the line means that the model is close to reality.

Figure 4. 4 — Regular probability graph obtained as a comparison between theoretical and experimental methods



Source: Author (2024)

4.4.4 Analysis and characterization of the obtained bioproduct

In this section, the results regarding the physical-chemical properties relevant to the commercialization of biodiesel will be presented, as well as the analyses related to nuclear magnetic resonance, high-resolution liquid chromatography and, finally, gas chromatography of the bioproduct obtained.

4.4.4.1 Density and viscosity

Density and kinematic viscosity are relevant properties, as they are quality indicators for performance and compatibility with other fuels and affect the costs related to the storage and handling of biofuel (Bukkarapu; Krishnasamy, 2022; Christopher selvam; Devarajan; Raja, 2025; Mishra; Bukkarapu; Krishnasamy, 2021). Density and viscosity properties were evaluated in comparison with the standard values required by quality regulatory agencies and those responsible for the national circulation of the bioproduct. The kinematic viscosity at 40 ^{o} C and density at 20 ^{o} C were highlighted (Table 4.5).

According to Table 4.5, the kinematic viscosity values at 40 ^{o} C and density at 20 ^{o}

C for the oil obtained in this study are within the "American Society for Testing and Materials" (ASTM) limit. Compared to the values established by the National Petroleum Agency (ANP), only the density remained within the required standard, while the kinematic viscosity is below.

Table 4.5 – Comparative analysis of biodiesel properties based on standard specifications and literature data

Property	ASTM D6751	ANP 920/2023	Author	(Smaisim *et al.*, 2022)	(Martins *et al.*, 2015)
Kinematic viscosity at 40 ºC, cSt	1.9 – 6.0	3.0 – 5.0	2.47	5.14	5.34
Density at 20 ºC, Kg/m³	860 – 900	850 – 900	883.6	798.7	877

Source: Author (2024)

Martins *et al* (2015) were responsible for producing biodiesel using fish oil via the chemical route in the presence of a basic homogeneous catalyst, presenting satisfactory results regarding the final characterization of the product (Martins *et al.*, 2015). It is observed that the bioproduct also remained within the limit established by the ASTM D6751 standard and when compared to ANP 920/2023, only the density remained within the standard. The density of the oil produced in the present study was higher than the product characterized by Martins et al (2015). However, the kinematic viscosity at 40 ºC was twice as low.

Smaisim *et al* (2022) opted for the biological route using immobilized enzymes for biodiesel synthesis and also presented satisfactory results regarding the physical-chemical properties evaluated (Smaisim *et al.*, 2022). The oil produced by the authors presented a value lower than the limit established by the ASTM D6751 standard and the ANP resolution 920/2023. The kinematic viscosity remained within the standard established by the international standard, but when compared with the national standard it remained outside the indicated limit. About the present study, it was observed that the kinematic viscosity obtained by the authors was higher. However, the density was lower.

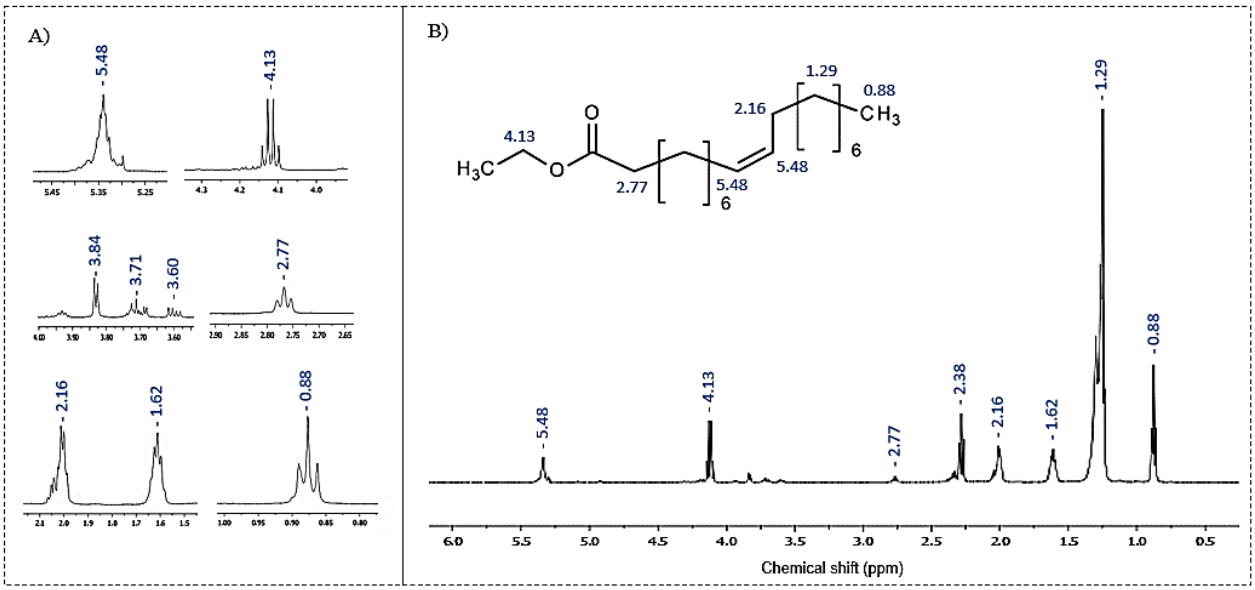
Viscosity tends to increase with chain length and decrease with the presence of double bonds in the chain, which accounts for the lower viscosity observed in fish oil biodiesel, due to its higher content of polyunsaturated fatty acids (PUFA) compared to biodiesel from palm oil and waste frying oil. Additionally, cis double bonds have lower viscosity than trans double bonds, with the latter exhibiting similar viscosity to their corresponding saturated

compounds (de Almeida *et al.*, 2015).

4.4.4.2 Nuclear Magnetic Resonance (NMR)

In the ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the reaction medium, it is possible to observe the signals for the formed esters. Although the spectrum of the reaction mixture presents several overlapping peaks, it is possible to highlight the most important peaks as a criterion for determining the presence of these products in the reaction medium. Figure 4.5 highlights some important chemical shifts of ethyl oleate. Expansions for the highlighted hydrogen atoms in the structure of ethyl oleate have been inserted in Figure 4.5 to identify and assign the signals to their respective chemical shift values and multiplicities.

Figure 4. 5 – ^{1}H Nuclear Magnetic Resonance (NMR) spectrum for the sample of ester mixture with expansions of ethyl oleate as the major product



Source: Author (2024)

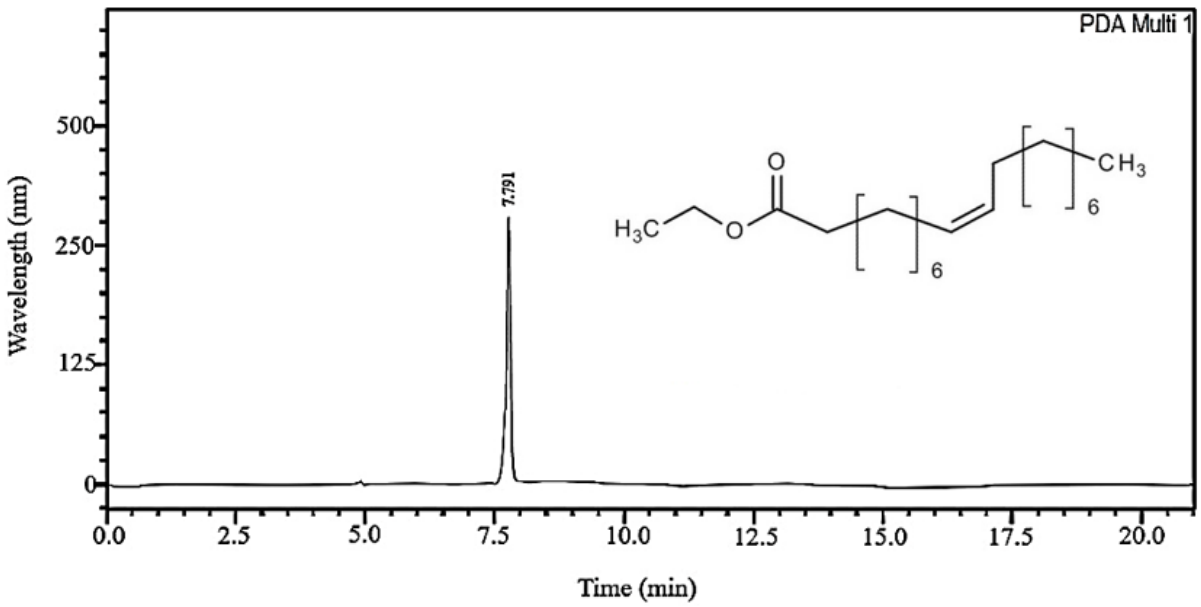
The signal with a chemical shift close to δ 5.48 was attributed to the hydrogen atoms of the sp2 carbon. This chemical profile leads to a signal with multiple characteristics due to its characteristic splitting for alkenes. The signal at δ 4.13 is a quartet, corresponding to the multiplicity and chemical shift attributed to the hydrogen directly bonded to the oxygen of the ester group. The peaks at δ 3.84, δ 3.71, and δ 3.60 were attributed to the more diluted esters in the reaction medium due to their low intensity in the spectrum. The triplet observed at δ 2.77 is characteristic of methylene hydrogens in the α positions to the carbonyl of esters. The signals observed at δ 1.29 and δ 1.62 are related to the methyl and methylene groups common to the esters in the reaction medium. Finally, the signal at δ 0.88 was attributed to the methyl group furthest from the functional group, as it showed greater shielding and lower chemical shift and corresponding multiplicity.

4.4.4.3 High-performance liquid chromatography (HPLC)

The reactional system was analyzed by chromatography to obtain a chromatographic profile of the esterified product, as a qualitative way to confirm the formation of esters, especially ethyl oleate, the major product. The peak displayed in the chromatogram shown in Figure 4.6 corresponds to ethyl oleate obtained via enzymatic reaction. The reverse-phase column interacts more strongly with products of lower polarity, while the polar solvent combination carries higher polarity products. The peaks for each component of the ester mixture had their maximum absorbance observed in the region of 254 nm.

Ethyl oleate has a long carbon chain, giving this molecule low polarity. Thus, the longer the carbon chain of an ester, the greater its interaction with the nonpolar column. The peak was attributed to the major esterified product in the reaction medium, which exhibits the lowest polarity and consequently a strong interaction with the reverse-phase column. Therefore, the highest retention factor is associated with this compound, where other esters of shorter chains and low concentration would have a lower retention factor, showing greater affinity for the polar solvent.

Figure 4. 6 – HPLC chromatogram of ethyl oleate, the major product in the reaction medium for enzymatic esterification



Source: Author (2024)

4.4.5. In silico study

4.4.5.1 Molecular docking

Based on the literature (Almeida *et al.*, 2020) and with some adaptations (Fonseca *et al.*, 2020) this molecular coupling simulation study was used to elucidate the hydrolysis interaction's reaction between the fish oil composition and Eversa lipase.

The docking molecule by Autodock vina made it possible to list their affinity and RMSD energies of the ligands, as shown in Table 4.5.

Table 4. 5 – Molecular docking result with its dock scores.

Compounds	Pubchem	Energy (kcal/mol)	RMSD (Å)
Linoleic acid	CID5280450	-6.1	1.79
Linolenic acid	CID5280934	-5.9	1.74
Palmitoleic acid	CID445638	-5.8	1.66
Palmitic acid	CID985	-5.6	1.80
Oleic acid	CID445639	-5.6	2.00
Stearic acid	CID5281	-5.4	1.51
Myristic acid	CID11005	-5.1	1.38

Source: Author (2024)

Although all allocate in the same region of the protein's active site, which is usually constituted by the catalytic triad (Ser-His-Asp), in the case of for the Eversa 2.0 Ser 153, His 268, and Asp 206 (Alexandre *et al.*, 2022; Nobre *et al.*, 2023).

NAC's are defined as compatible with conformations to the catalytic site attack on the electrophilic carbon of the acyl group (Bruice; Lightstone, 1999). In a typical NAC the distance between the oxygen from the Ser 105 (CALB)/Ser 153 (Eversa) residue and the carbonyl carbon is usually observed to be close to 3 Å in length, and the same atoms, together with the carbonyl oxygen molecule, tend to form an angle of approximately 60°, with a maximum of 90° (Corici *et al.*, 2015).

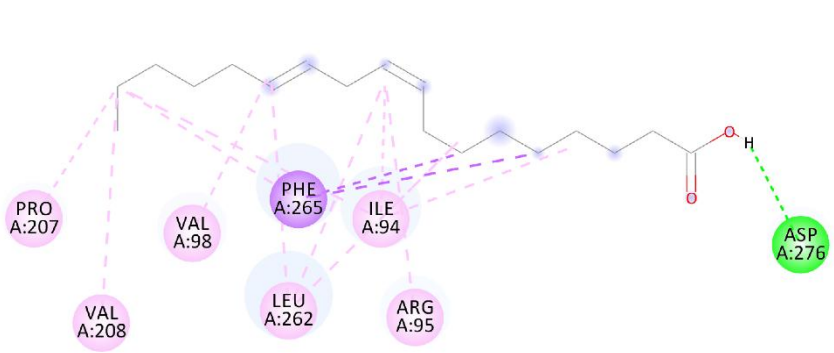
In general, the ligands were close to the catalytic site. However, among the ligands evaluated, Myristic acid (C) showed reaction potential, making an NAC with the residue Ser 153, although its affinity energy was lower than the others. On the other hand, Stearic acid (G) was shown to cause a hydrophobic interaction directly with one of the residues of the catalytic triad, His 268. However, the results suggest that the combination of linoleic acid (A) substrate and lipase proved stable, possibly due to the hydrophobic and hydrophilic interactions in the anchoring study esterification (Bronowska, 2011). In addition, it is possible to observe a direct relationship between the increase in binding affinity and the increase in the carbon chain of the esters. This expansion may be related to increased interactions between ligands and the enzyme's catalytic site (Qin; Zhong; Wang, 2021). The types of residues involved in the interactions between free fatty acids and Eversa® Transform 2.0 are shown in Table 6 and Figure 4.7.

Table 4. 6 – Interactions of ligands with lipase after docking

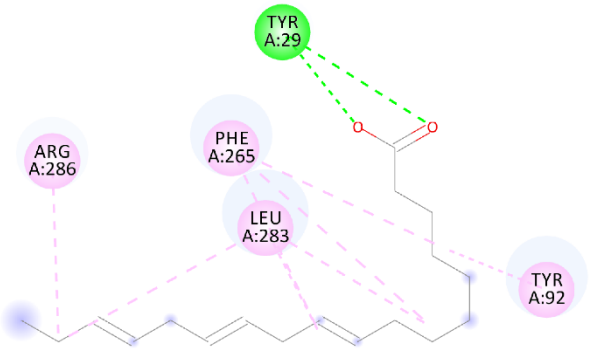
Substrate	Waste Involved	
	Hydrogen Bonds	**Hydrophobic Interactions**
Linoleic acid	Asp276 (2.08 Å)	Phe 265, Pro 207, Val 208, Val 98, Leu 262, Ile 94, Arg 95
Linolenic acid	Tyr 29 (3.30 Å, 2.9 Å)	Tyr 92, Leu 283, Phe 265, Arg 286
Palmitoleic acid	-	Leu 283, Leu 285, Phe 265, Ile 94, Tyr 92
Palmitic acid	Tyr 29 (3.00 Å)	Tyr 92, Phe 265, Val 269, Leu 282, Leu 263, Leu 262
Oleic acid	Tyr 29 (3.01 Å)	Tyr 92, Ile 94, Phe 265, Leu 282, Val 269
Stearic acid	Trp 270 (3.15 Å)	His 268, Ile 94, Tyr 92, Phe 265, Leu 283, Val 269
Myristic acid	Ser 153 (2.95 Å) Tyr 29 (2.94 Å)	Tyr 92, Phe 265, Leu 283, Val 269, Leu 285, Trp 270

Source: Author (2024)

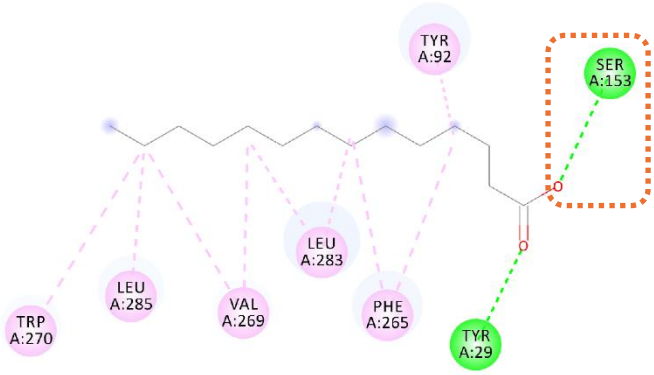
Figure 4. 7 – The active site of Eversa lipase with the catalytic triad Ser 153, His 268, and Asp 206 (A-G)



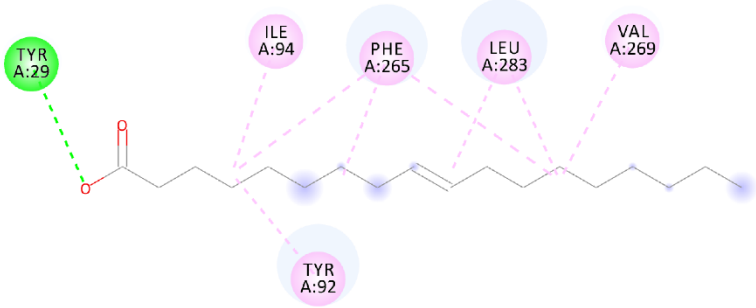
(A) Linoleic acid



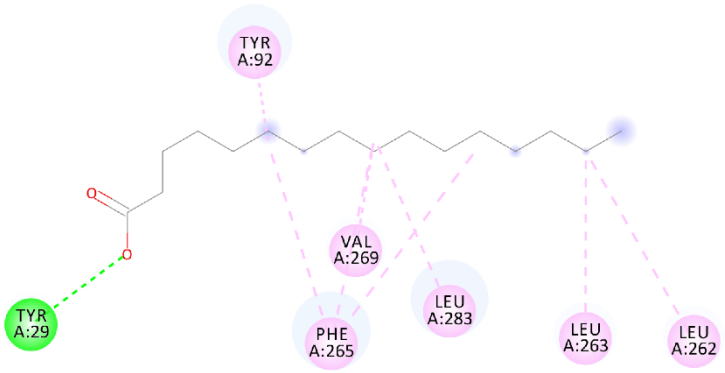
(B) Linolenic acid



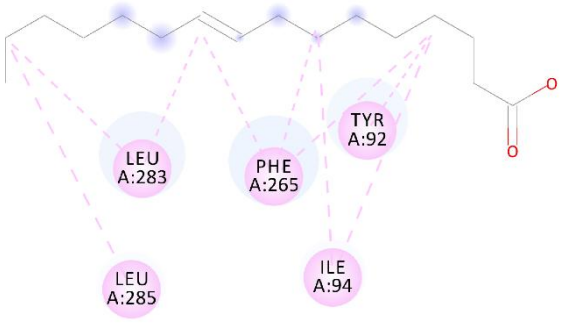
(C) Myristic acid



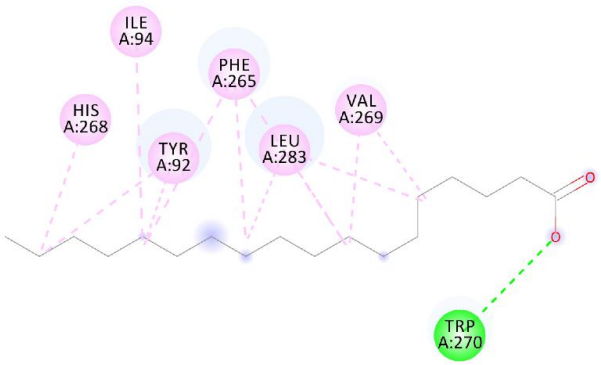
(D) Oleic acid



(E) Palmitic acid



(F) Palmitoleic acid



(G) Stearic acid

Source: Author (2024)

4.4.5.2 Molecular dynamics simulations

Root mean square deviation – RMSD

It was observed that the ligands studied were correctly accommodated in the catalytic site of lipase Eversa® (Guimarães *et al.,* 2021). Thus, through the lipase–ligand complexes (Figure 4.8), simulation studies were carried out to evaluate not only the conformational changes of the enzyme but also its stability after each conformational change. The Root Mean Square Deviation (*RSMD*) of the lipase–ligand complexes were used to evaluate the extent to which conformational changes occurred in the studied molecule during the simulation time. Figure 4.11 shows the *RSMD* behaviors of the complexes studied in the equilibration stage.

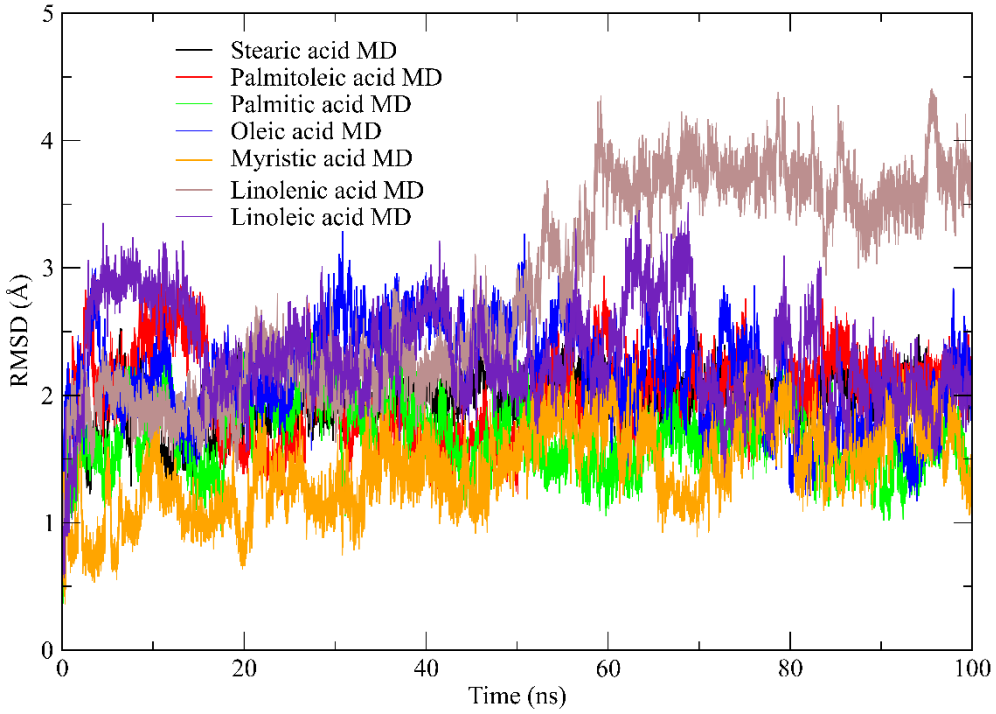
From the equilibrium simulations of the lipase–ligand complexes in the solvent, it was possible to obtain preliminary information on the behavior of the conformations for the dynamics. In this step, it was observed that the *RSMD* stabilization values of the studied ligands oscillated between 1.1 and 4.2 Å in the evaluated time. These low *RSMD* values may be

associated with the movement of ions and solvents in the system during the initial conformation of the complexes. Figure 6 shows the results obtained in the production stage.

As shown in Figure 4.8, Myristic acid and Palmitic acid had, on average, RMSD values below 2.0 Å. On the other hand, Linolenic acid presented values of around 4.0 Å. Thus, the theoretical and experimental results presented in this work indicate that fatty acids from residual oil form stable complexes with the catalytic site of Eversa complexes, which may indicate a viable alternative for future applications.

It is worth noting that the data presented in this work agree with the results obtained by literature, where studies evaluated the affinity and molecular stability of different fatty acids with lipases in sterifications reactions (Qin; Zhong; Wang, 2021). As in the present work, the authors observed that among the complexes studied, octadecanoic acid presented one of the highest RSMD values and therefore one of the lowest stability values when compared to the other complexes.

Figure 4. 8 – Root Mean Square Deviation (*RMSD*) for the initial conformation of the complexes versus the simulation time (nanoseconds) in the production step. Stearic acid (black); Palmitoleic acid (red); Palmitic acid (green); Oleic acid (blue); Myristic acid (orange); Linolenic acid (brown); and Linoleic acid (indigo)



Source: Author (2024).

Hydrogen Bonds

Intermolecular hydrogen bonds, a cornerstone of enzyme-ligand complex stability, have been a long-standing focus of biochemical research. However, in a recent study by Ragunathan et al., (2018), we delve deeper into these bonds, specifically in the context of lipase-ligand complexes, revealing novel insights through the use of molecular dynamics simulations.

The analysis primarily focused on examining the formation of intermolecular hydrogen bonds between the ligands and lipase throughout both equilibration and production phases. Figure 4.9 from the study depicted the fluctuations in hydrogen bond networks during these stages, shedding light on the variations in bond formation.

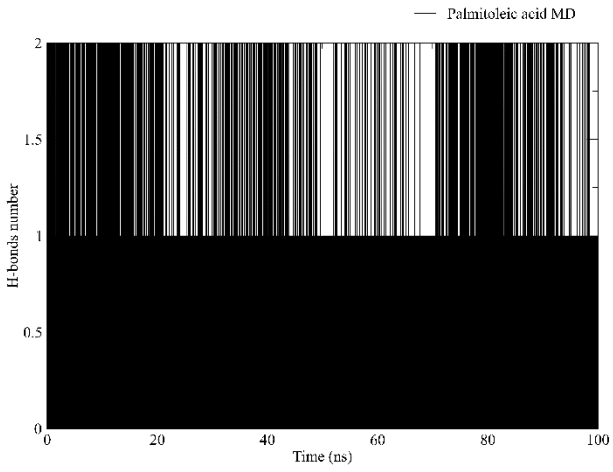
A notable discovery highlighted the dynamic formation of hydrogen bonds, with the bond count fluctuating between 1 and 3 throughout the simulation period. The observation regarding myristic acid and oleic acid was fascinating, as it demonstrated the formation of up to 3 hydrogen bonds during the 100 ns trajectory.

The disruption of hydrogen bonds indicated shifts in stability, often attributed to interactions such as Van der Waals or hydrophobic forces, as emphasized by Qin et al., (2021). Additionally, the analysis revealed a direct correlation between the length of the ligands' carbon chains and the average bond count, reinforcing insights from previous molecular docking investigations (Qu *et al.*, 2022).

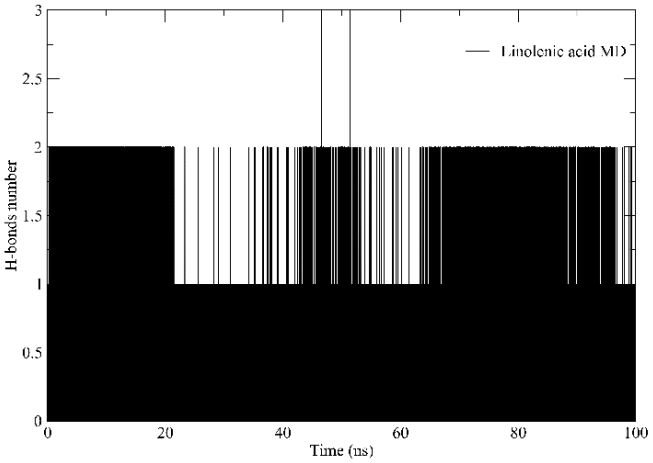
A key revelation from this examination was the differentiation between dynamic (molecular dynamics) and static (molecular docking) processes, as elucidated by Fatma et al., (2021). While molecular docking provides valuable insights into static interactions, molecular dynamics simulations capture the dynamic evolution of these complexes, offering a more comprehensive understanding of their stability.

In summary, our investigation into the dynamic interplay of intermolecular hydrogen bonds in lipase-ligand complexes not only deepens our understanding of protein-ligand recognition processes but also holds significant implications for drug design and enzyme engineering. These findings underscore the practical relevance and impact of our research.

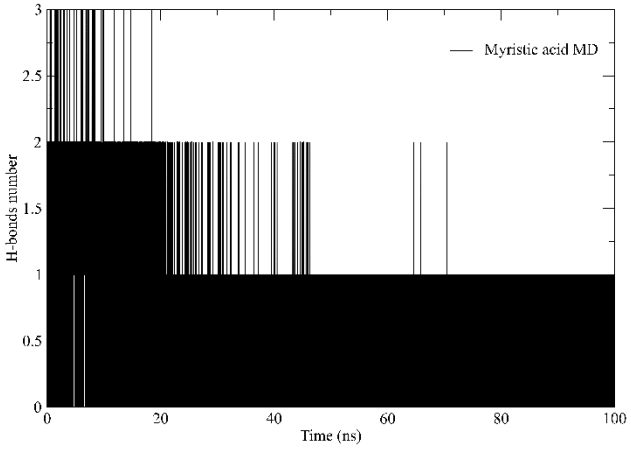
Figure 4. 9 – Hydrogen bonds formed between the protein and the ligand during the two simulation steps



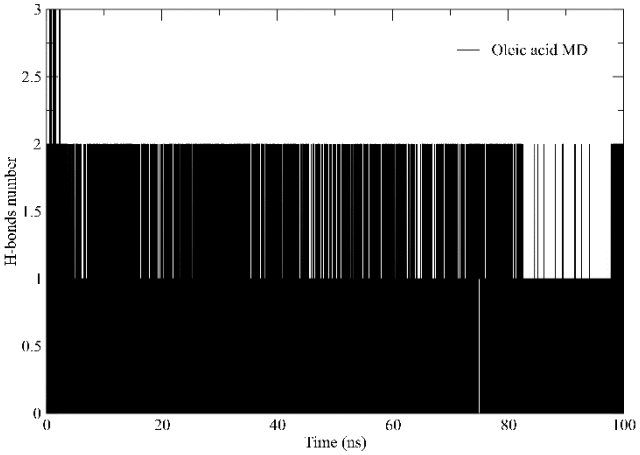
Palmitoleic acid



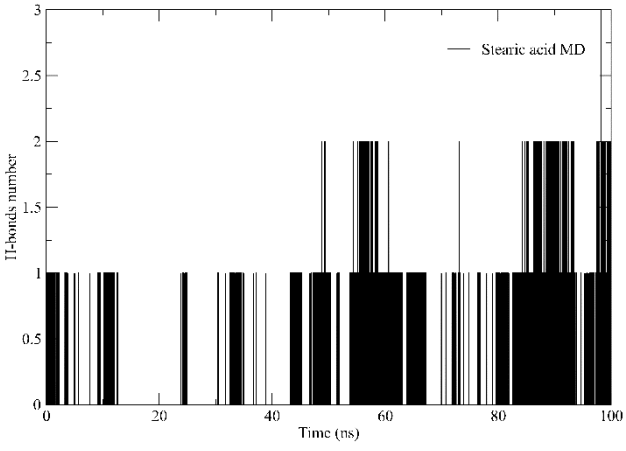
Linolenic acid

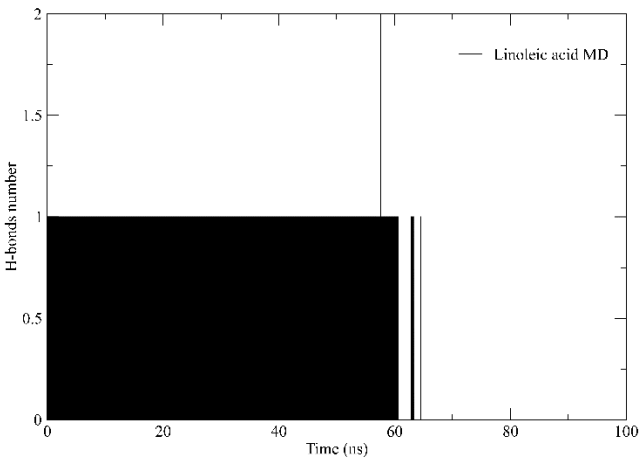


Myristic acid

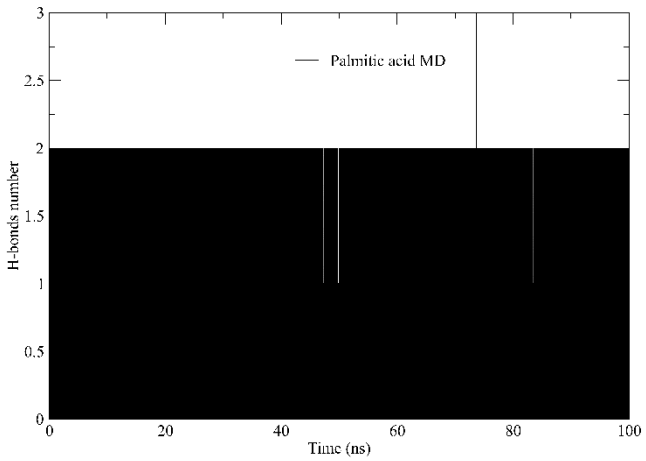


Oleic acid





Stearic a **leic acid**



Palmitic acid

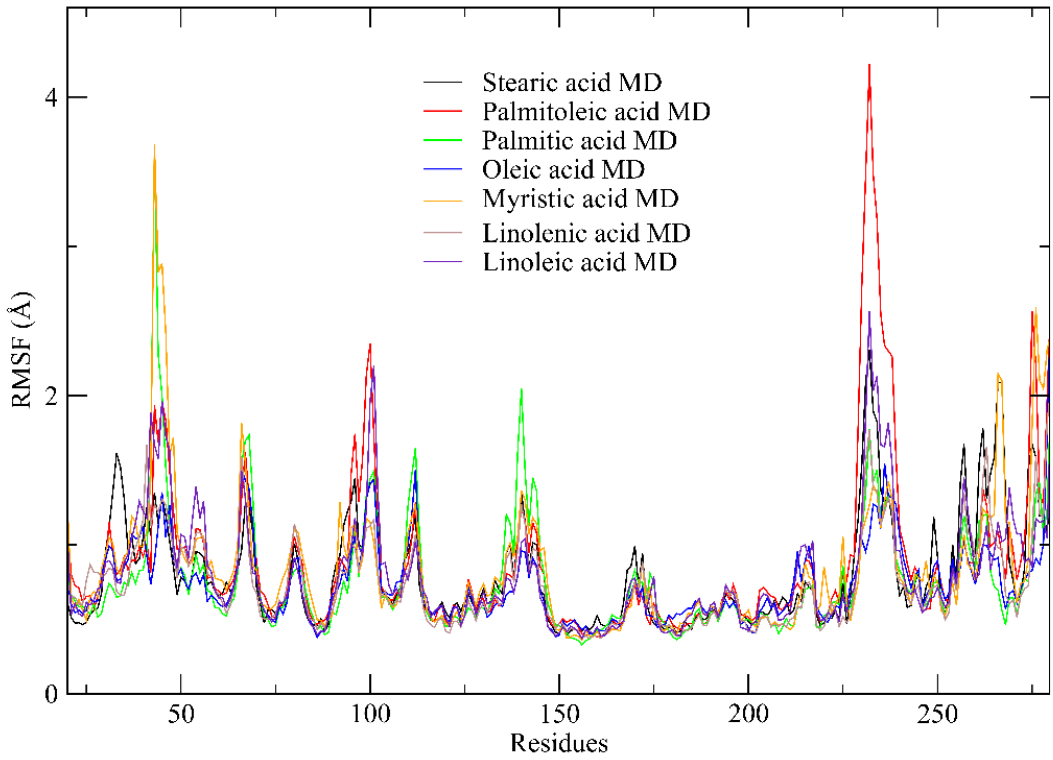
4.4.5.3 RMSF

We conducted an RMSF analysis of the system to delve into the dynamics and stability of each protein residue throughout the 100ns simulation trajectory. Figure 4.10 showcases the primary interactions within the investigated leading complexes, particularly those composed of fish oil. The findings hint at substantial conformational shifts in the compound-Eversa lipase complexes during the simulation period.

Our results unveil consistent fluctuations in the molecular dynamics simulation trajectories across all complexes, showcasing notable correlations with key replication residues, as highlighted in studies by (Qin; Zhong; Wang, 2021) and (Roe; Brooks, 2020). Notably, only the complexes formed between methyl decanoate and Eversa exhibited RMSF values surpassing 2.0 Å for residues Gly 47, Asp 100, Asn 232, and His 268. Additionally, Palmitoleic acid presented RMSF values above 4.2 Å, in the residue Asn 232.

Despite the observed fluctuations, our results indicate satisfactory stability of the structures within an aqueous solution. The conformations derived from MD simulations, complexed with various ligands through docking techniques, offer crucial insights into the binding modes of small molecules across different enzyme folding states, as illuminated by Thirumalai; Lorimer; Hyeon, (2020).

Figure 4. 10 – Root Mean Square Fluctuation (RMSF), concerning the initial confirmation of the ligand-enzyme complex versus the simulation time (ns) in the production simulations step of the MD with fish oil composition/Eversa lipase



Source: Author (2024)

4.4.5.4 SASA calculations

Our research, which combines the powerful computational tool of molecular dynamics (MD) simulations with the insightful metric of Solvent Accessible Surface Area (SASA), offers a unique perspective on protein behavior in solution. Throughout 100 ns, we applied this novel approach to closely monitor the SASA of complexes formed with fish oil compositions, unveiling intricate dynamics and stability patterns within biomolecules (Mazola *et al.*, 2015).

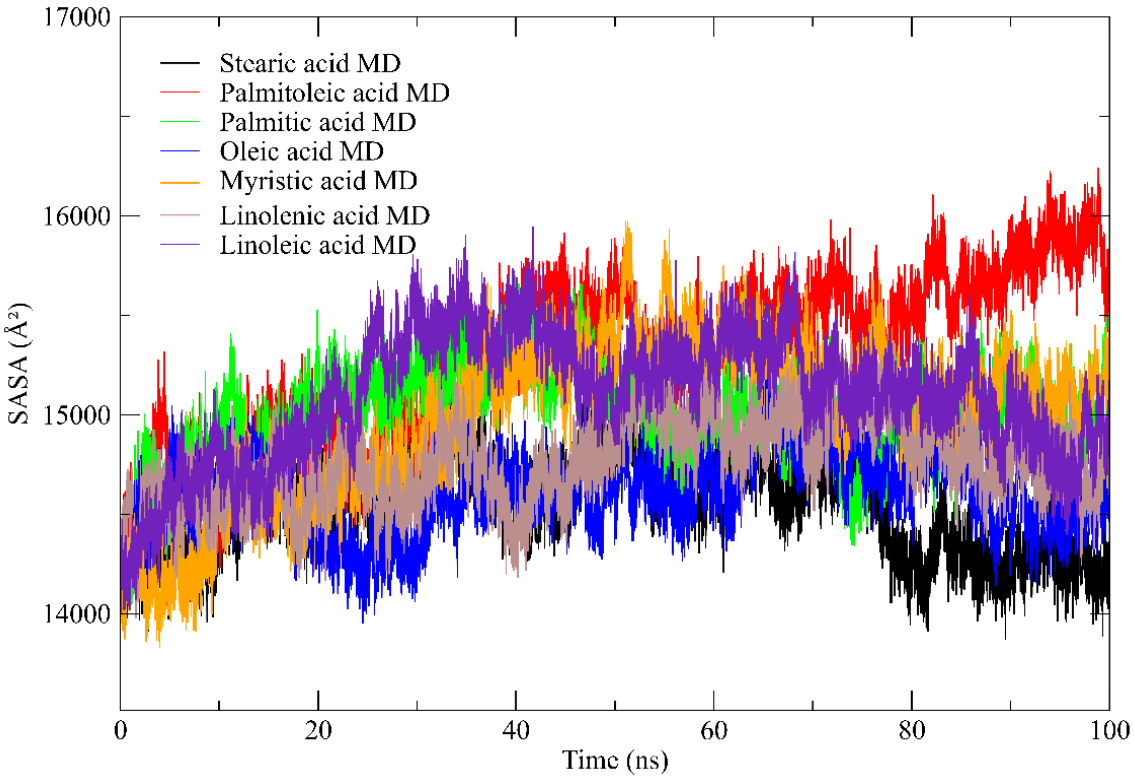
Our SASA analysis yielded intriguing trends. Notably, SASA value for specific compounds, including Pamitoleic acid, exhibited a marked increase during simulation, indicative of structural relaxation. Conversely, the other compounds, showcased a decrease in SASA values, suggesting tension upon complex formation with the enzyme (Durham *et al.*, 2009; Street; Mayo, 1998) (See Figure 4.11).

The negligible impact of ligand binding on SASA values was of particular interest, implying that ligand-protein interactions did not significantly alter solvent accessibility to the protein surface (Marsh & Teichmann, 2011). Following 100 ns of simulation, SASA values stabilized around a constant, suggesting equilibrated systems were sampled. However, intriguingly, complexes with stabilizing monovalent ions exhibited the highest SASA values-higher ion concentrations correlated with smaller areas, potentially indicating surface charge-induced compaction of protein structures.

In conclusion, our SASA analysis during MD simulations has provided crucial insights into the dynamics and stability of fish oil composition complexes. These findings not only deepen our understanding of protein behavior but also hold promise for guiding the design of compounds with enhanced interactions with protein surfaces or the optimization of conditions

for protein complex formation, opening up new avenues for future research and applications.

Figure 4. 11 – Solvent accessible surface area (SASA) of the Eversa lipase as a function of time from the MD simulations. The curves are running averages of the raw data with a window of 100 ns



Source: Author (2024).

4.6 Conclusions

This study focuses on developing technologies to create biodiesel from a variety of raw materials. One promising source is residual tilapia (*Oreochromis Niloticus*) oil, which was found to be suitable for enzymatic hydrolysis with a high acid number. By combining Chemical Engineering and theoretical Chemistry, the researchers were able to analyze the bioprocess development significantly. The Taguchi methodology helped to identify the reaction parameters that were most important in conducting the research. Additionally, the study analyzed the physicochemical properties that are relevant to the commercialization and use of the bioproduct.

In conclusion, this study effectively elucidated the interactions between Eversa Transform® 2.0 lipase and potential substrates pertinent to biodiesel production, encompassing

renewable and eco-friendly sources such as fish oil lipid compositions. Through in silico analysis, we unveiled that myristic acid and Oleic acid bind proximally to the enzyme's active site, exhibiting favorable free energy and establishing specific hydrogen bonds, alkyl, and π-alkyl interactions.

Molecular dynamics simulations underscored the robust stability and minimal root-mean-square deviation (RMSD) values, affirming the suitability of the selected binding postures for unimpeded reaction occurrence. Nevertheless, it is imperative to recognize that while computer simulations serve as valuable initial screening tools, the translation to in vitro and broader applications confronts additional variables and challenges.

These challenges encompass diverse factors such as transport conditions, catalytic activity, enzymatic inhibition, equilibrium conditions, and the physicochemical attributes of the resultant biodiesel. Addressing these complexities demands further comprehensive studies and meticulous considerations, ensuring the effective and sustainable deployment of lipase-mediated biodiesel production processes.

CHAPTER 5

CHAPTER 5 – FINAL CONSIDERATIONS

5.1 Final considerations

This study underscores the critical importance of investigating alternative and sustainable feedstocks for biodiesel production. Utilizing fish waste, which is typically considered a byproduct, not only mitigates environmental impacts but also enhances the circular economy by optimizing the utilization of natural resources. The enzymatic approach presented in this work demonstrates the technical feasibility of implementing more environmentally benign processes in biofuel production, emphasizing the potential for reduced energy consumption and minimized waste generation.

The results indicate the significant potential of biodiesel produced from tilapia waste oil, exhibiting suitable physicochemical properties that render it a viable alternative to conventional diesel. This advancement not only supports the diversification of energy sources but also presents opportunities for the development of new markets, particularly in regions with substantial fish production. Furthermore, this approach could foster local economic growth by integrating sustainable energy solutions with existing industrial byproducts, thus aligning with broader goals of resource efficiency and regional economic resilience.

This study lays the groundwork for future research, suggesting the exploration of additional waste sources and the optimization of enzymatic processes to further enhance the efficiency and sustainability of biodiesel production. Moreover, future investigations should prioritize comprehensive environmental impact assessments and life cycle analyses of the produced biodiesel to rigorously evaluate its long-term viability and scalability. Such studies are essential to ensuring that biodiesel not only meets energy demands but also aligns with global sustainability standards and environmental regulations.

REFERENCES

ABDUL HAKIM SHAAH, Marwan *et al.* A review on non-edible oil as a potential feedstock for biodiesel: physicochemical properties and production technologies. **RSC Advances**, [*s. l.*], v. 11, n. 40, p. 25018–25037, 2021.

ABDULLAH, Muhammad *et al.* Advancements in sustainable production of biofuel by microalgae: Recent insights and future directions. **Environmental Research**, [*s. l.*], v. 262, p. 119902, 2024.

ABOMOHRA, Abd El-Fatah *et al.* Potential of fat, oil and grease (FOG) for biodiesel production: A critical review on the recent progress and future perspectives. **Progress in Energy and Combustion Science**, [*s. l.*], v. 81, p. 100868, 2020.

ABOU-ELYAZED, Ahmed S. *et al.* A green approach for enhancing the hydrophobicity of UiO-66(Zr) catalysts for biodiesel production at 298 K. **RSC Advances**, [*s. l.*], v. 10, n. 68, p. 41283–41295, 2020.

ABOULROUS, Amany A. *et al.* Review of synthesis, characteristics and technical challenges of biodiesel based drilling fluids. **Journal of Cleaner Production**, [*s. l.*], v. 336, p. 130344, 2022.

ABOU-TALEB, Noura Hemdan *et al.* Optimizing the spectrofluorimetric determination of cefdinir through a Taguchi experimental design approach. **Luminescence**, [*s. l.*], v. 31, n. 3, p. 856–864, 2016.

ABREU SILVEIRA, Erick *et al.* Biocatalyst engineering of Thermomyces Lanuginosus lipase adsorbed on hydrophobic supports: Modulation of enzyme properties for ethanolysis of oil in solvent-free systems. **Journal of Biotechnology**, [*s. l.*], v. 289, p. 126–134, 2019.

ACHERKI, Hassan; BOUAID, Abderrahim; MARCHETTI, Jorge Mario. Optimization of the enzymatic butanolysis of <scp>jatropha</scp> oil for biodiesel production using <scp>Eversa</scp>. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 16, n. 1, p. 219–227, 2022.

ADERIBIGBE, Fatai A. *et al.* Heterogeneous Catalysis of Second Generation Oil for Biodiesel Production: A Review. **ChemBioEng Reviews**, [*s. l.*], v. 8, n. 2, p. 78–89, 2021.

ADHITHAN, Balamurugan; SACHDEVA, Gulshan. Analysing the emissions and fuel efficiency of fish-waste biodiesel in a compression ignition engine. **International Journal of Ambient Energy**, [*s. l.*], v. 44, n. 1, p. 1829–1839, 2023.

AGU, P. C. *et al.* Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. **Scientific Reports**, [*s. l.*], v. 13, n. 1, p. 13398, 2023.

AHMADI, Meisam *et al.* Chem3D 15.0 User Guide. **Macromolecules**, [*s. l.*], v. 24, n. 2, p. 1–61, 2005.

AHMED, Rawaz; HUDDERSMAN, Katherine. Review of biodiesel production by the esterification of wastewater containing fats oils and grease (FOGs). **Journal of Industrial**

and Engineering Chemistry, [*s. l.*], v. 110, p. 1–14, 2022.

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**, [*s. l.*], v. 412, p. 135558, 2023.

ALEXANDRE, Jeferson Yves Nunes Holanda *et al.* A Theoretical and Experimental Study for Enzymatic Biodiesel Production from Babassu Oil (Orbignya sp.) Using Eversa Lipase. **Catalysts**, [*s. l.*], v. 12, n. 11, 2022.

ALGAYYIM, Sattar Jabbar Murad *et al.* Influence of natural gas and hydrogen properties on internal combustion engine performance, combustion, and emissions: A review. **Fuel**, [*s. l.*], v. 362, p. 130844, 2024.

ALI, Sajid *et al.* The Recent Advances in the Utility of Microbial Lipases: A Review. **Microorganisms**, [*s. l.*], v. 11, n. 2, p. 510, 2023.

ALI IJAZ MALIK, Muhammad *et al.* A review of major trends, opportunities, and technical challenges in biodiesel production from waste sources. **Energy Conversion and Management: X**, [*s. l.*], v. 23, p. 100675, 2024.

AL-JABIRI, Ali A.; BALLA, Hyder H.; AL-ZUHAIRY, Mudhaffar S. Effect of Biodiesel Produced from Sunflower Oil on Engine Emissions Characteristics. *In*: [*S. l.: s. n.*], 2025. p. 347–358.

ALMEIDA, Lays C. *et al.* Enzymatic transesterification of coconut oil by using immobilized lipase on biochar: An experimental and molecular docking study. **Biotechnology and Applied Biochemistry**, [*s. l.*], p. 1–8, 2020.

ALMEIDA, Francisco Lucas Chaves *et al.* Trends in lipase immobilization: Bibliometric review and patent analysis. **Process Biochemistry**, [*s. l.*], v. 110, p. 37–51, 2021.

AL-SAADI, Ali; MATHAN, Bobby; HE, Yinghe. Esterification and transesterification over SrO–ZnO/Al2O3 as a novel bifunctional catalyst for biodiesel production. **Renewable Energy**, [*s. l.*], v. 158, p. 388–399, 2020.

ALVES, Alanderson Arthu Araújo *et al.* Investigating the thermophysical properties of the 1-Butanol + Biodiesel System: The impact of pressure on volumetric characteristics. **Fuel**, [*s. l.*], v. 371, p. 132076, 2024.

AMBAYE, Teklit Gebregiorgis *et al.* Emerging technologies for biofuel production: A critical review on recent progress, challenges and perspectives. **Journal of Environmental Management**, [*s. l.*], v. 290, p. 112627, 2021.

ANDREO-MARTÍNEZ, Pedro *et al.* Waste animal fats as feedstock for biodiesel production using non-catalytic supercritical alcohol transesterification: A perspective by the PRISMA methodology. **Energy for Sustainable Development**, [*s. l.*], v. 69, p. 150–163, 2022.

ANU PRASANNA, Vankara *et al.* Fish Waste: A Potential Source of Biodiesel. **Fermentation**, [*s. l.*], v. 9, n. 9, p. 861, 2023.

ARANSIOLA, E.F. *et al.* A review of current technology for biodiesel production: State of the art. **Biomass and Bioenergy**, [*s. l.*], v. 61, p. 276–297, 2014.

ARCUS, Vickery L *et al.* Enzyme evolution and the temperature dependence of enzyme catalysis. **Current Opinion in Structural Biology**, [*s. l.*], v. 65, p. 96–101, 2020.

ARCUS, Vickery L.; MULHOLLAND, Adrian J. Temperature, Dynamics, and Enzyme-Catalyzed Reaction Rates. **Annual Review of Biophysics**, [*s. l.*], v. 49, n. 1, p. 163–180, 2020.

ARSHAD, Sumera *et al.* Assessing the potential of green CdO2 nano-catalyst for the synthesis of biodiesel using non-edible seed oil of Malabar Ebony. **Fuel**, [*s. l.*], v. 333, p. 126492, 2023.

ARSHIA, Amir Hossein *et al.* De novo design of novel protease inhibitor candidates in the treatment of SARS-CoV-2 using deep learning, docking, and molecular dynamic simulations. **Computers in Biology and Medicine**, [*s. l.*], v. 139, 2021.

ASSOSSIAÇÃO BRASILEIRA DE PISCICULTURA. **Anuário Peixe BR da Piscicultura**. [*S. l.: s. n.*], 2023.

ATABANI, A.E. *et al.* Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 18, p. 211–245, 2013.

ATADASHI, I.M. *et al.* The effects of catalysts in biodiesel production: A review. **Journal of Industrial and Engineering Chemistry**, [*s. l.*], v. 19, n. 1, p. 14–26, 2013.

ATHIAPPAN, Murugan *et al.* Rhizosphere engineering through pesticides-degrading beneficial bacteria. *In*: DUBEY, Ramesh Chandra; KUMAR, Pankaj (org.). **Rhizosphere Engineering**. 1. ed. [*S. l.*]: Elsevier, 2022. v. 1, p. 239–257.

AZIRI, Sabrina *et al.* Taguchi method for optimization of Cr(VI) removal, isotherm, kinetic and thermodynamic studies. **Nucleosides, Nucleotides & Nucleic Acids**, [*s. l.*], p. 1–25, 2024.

BABADI, Arman Amani *et al.* Emerging technologies for biodiesel production: Processes, challenges, and opportunities. **Biomass and Bioenergy**, [*s. l.*], v. 163, p. 106521, 2022. Disponível em: Acesso em: 2 abr. 2024.

BARELLI, Linda *et al.* Enzymatic Biofuel Cells: A Review on Flow Designs. **Energies**, [*s. l.*], v. 14, n. 4, p. 910, 2021.

BENTI, Natei Ermias *et al.* Biodiesel production in Ethiopia: Current status and future prospects. **Scientific African**, [*s. l.*], v. 19, p. e01531, 2023.

BERMAN, Paula; NIZRI, Shahar; WIESMAN, Zeev. Castor oil biodiesel and its blends as alternative fuel. **Biomass and Bioenergy**, [*s. l.*], v. 35, n. 7, p. 2861–2866, 2011.

BHATTACHARYYA, Subhrajyoti. Transesterification of Yellow Oleander seed oil, its utilization as biodiesel and performance evaluation. **Heliyon**, [*s. l.*], v. 8, n. 4, p. e09250, 2022.

BIOVIA. **Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5**. San Diego: [*s. n.*], 2015.

BORGES, Sandra *et al.* Fish By-Products: A Source of Enzymes to Generate Circular Bioactive Hydrolysates. **Molecules**, [*s. l.*], v. 28, n. 3, p. 1155, 2023.

BRAHMA, Sujata *et al.* Biodiesel production from mixed oils: A sustainable approach towards industrial biofuel production. **Chemical Engineering Journal Advances**, [*s. l.*], v. 10, p. 100284, 2022.

BRANDÃO, Luma M. de S. *et al.* Lipase activation by molecular bioimprinting: The role of interactions between fatty acids and enzyme active site. **Biotechnology Progress**, [*s. l.*], v. 37, n. 1, 2021.

BRENA, Beatriz; GONZÁLEZ-POMBO, Paula; BATISTA-VIERA, Francisco. Immobilization of Enzymes: A Literature Survey. *In*: [*S. l.: s. n.*], 2013. p. 15–31.

BRUICE, Thomas C.; LIGHTSTONE, Felice C. **Ground state and transition state contributions to the rates of intramolecular and enzymatic reactions**. [*S. l.: s. n.*], 1999.

BUESO, Francisco *et al.* Lipase-catalyzed biodiesel production and quality with Jatropha curcas oil: exploring its potential for Central America. **Journal of Biological Engineering**, [*s. l.*], v. 9, n. 1, p. 12, 2015.

BUKKARAPU, Kiran Raj; KRISHNASAMY, Anand. A critical review on available models to predict engine fuel properties of biodiesel. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 155, p. 111925, 2022.

BULLO, Giovanna Totti *et al.* Lipases: market study and potential applications of immobilized derivatives. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], 2024.

CARVALHO, Wagner C.A. *et al.* Eco-friendly production of trimethylolpropane triesters from refined and used soybean cooking oils using an immobilized low-cost lipase (Eversa>® Transform 2.0) as heterogeneous catalyst. **Biomass and Bioenergy**, [*s. l.*], v. 155, p. 106302, 2021. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0961953421003378.

CARVALHO, J. C. *et al.* Growth curve of Nile tilapia from different families of the AquaAmérica variety. **Brazilian Journal of Biology**, [*s. l.*], v. 82, 2022.

CAVALCANTE, Francisco Thálysson Tavares *et al.* Opportunities for improving biodiesel production via lipase catalysis. **Fuel**, [*s. l.*], v. 288, p. 119577, 2021.

CHANAKAEWSOMBOON, Issara *et al.* Biodiesel produced using potassium methoxide homogeneous alkaline catalyst: effects of various factors on soap formation. **Biomass Conversion and Biorefinery**, [*s. l.*], v. 13, n. 10, p. 9237–9247, 2023.

CHAOS-HERNÁNDEZ, D. *et al.* Extraction methods of algae oils for the production of third generation biofuels – A review. **Chemosphere**, [*s. l.*], v. 341, p. 139856, 2023.

CHINTAGUNTA, Anjani Devi *et al.* Biodiesel Production From Lignocellulosic Biomass Using Oleaginous Microbes: Prospects for Integrated Biofuel Production. **Frontiers in Microbiology**, [*s. l.*], v. 12, 2021.

CHOUHAN, A.P. Singh; SARMA, A.K. Modern heterogeneous catalysts for biodiesel production: A comprehensive review. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 15, n. 9, p. 4378–4399, 2011.

CHOZHAVENDHAN, S. *et al.* A review on influencing parameters of biodiesel production and purification processes. **Current Research in Green and Sustainable Chemistry**, [*s. l.*], v. 1–2, p. 1–6, 2020.

CHRISTOPHER SELVAM, D.; DEVARAJAN, Yuvarajan; RAJA, T. Characterization of isopropanol-enhanced Caesalpinia bonduc biodiesel blends: A sustainable strategy for decarbonization. **Results in Engineering**, [*s. l.*], v. 25, p. 103849, 2025.

CHUKWUMA, Ogechukwu Bose *et al.* Lignocellulolytic Enzymes in Biotechnological and Industrial Processes: A Review. **Sustainability**, [*s. l.*], v. 12, n. 18, p. 7282, 2020.

CIEŚLAK, Dominika; KABELKA, Ivo; BARTUZI, Damian. Molecular Dynamics Simulations in Protein–Protein Docking. *In*: [*S. l.: s. n.*], 2024. p. 91–106.

COPPOLA, Daniela *et al.* Fish Waste: From Problem to Valuable Resource. **Marine Drugs**, [*s. l.*], v. 19, n. 2, p. 116, 2021.

CORDERO-RAVELO, Virginia; SCHALLENBERG-RODRIGUEZ, Julieta. Biodiesel production as a solution to waste cooking oil (WCO) disposal. Will any type of WCO do for a transesterification process? A quality assessment. **Journal of Environmental Management**, [*s. l.*], v. 228, p. 117–129, 2018.

CORICI, Livia *et al.* Understanding Potentials and Restrictions of Solvent-Free Enzymatic Polycondensation of Itaconic Acid: An Experimental and Computational Analysis. **Advanced Synthesis and Catalysis**, [*s. l.*], v. 357, n. 8, p. 1763–1774, 2015.

COSTA, Matheus J. *et al.* Enzymatic biodiesel production by hydroesterification using waste cooking oil as feedstock. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 157, p. 108131, 2020.

COSTA, Isabela Oliveira *et al.* Enzyme immobilization technology as a tool to innovate in the production of biofuels: A special review of the Cross-Linked Enzyme Aggregates (CLEAs) strategy. **Enzyme and Microbial Technology**, [*s. l.*], v. 170, p. 110300, 2023.

CRUZ, Jaqueline Matos *et al.* Comparative study of phytotoxicity and genotoxicity of soil contaminated with biodiesel, diesel fuel and petroleum. **Ecotoxicology**, [*s. l.*], v. 28, n. 4, p. 449–456, 2019.

CUBAS, A.L.V. *et al.* Biodiesel production using fatty acids from food industry waste using

corona discharge plasma technology. **Waste Management**, [*s. l.*], v. 47, p. 149–154, 2016.

CULSUM, Neng Tresna Umi *et al.* Fabrication of lipase immobilized-carboxylated cellulose nanocrystals as biocatalyst for enzymatic hydrolysis of palm oil and its kinetical study. **Biocatalysis and Agricultural Biotechnology**, [*s. l.*], v. 54, p. 102915, 2023.

CUNHA, Anildo *et al.* Synthesis and characterization of ethylic biodiesel from animal fat wastes. **Fuel**, [*s. l.*], v. 105, p. 228–234, 2013.

CZECH STANDARDS INSTITUTE. **Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents**. [*S. l.: s. n.*], 2011. Disponível em: www.agilent.com/chem. .

DA SILVA AIRES, Francisco Izaias *et al.* Advanced and prospects in phenol wastewater treatment technologies: unveiling opportunities and trends. **Discover Water**, [*s. l.*], v. 4, n. 1, p. 20, 2024.

DARI, Dayana Nascimento *et al.* An Updated Review of Recent Applications and Perspectives of Hydrogen Production from Biomass by Fermentation: A Comprehensive Analysis. **Biomass**, [*s. l.*], v. 4, n. 1, p. 132–163, 2024.

DE ALMEIDA, Vanessa F. *et al.* Biodiesel production from mixtures of waste fish oil, palm oil and waste frying oil: Optimization of fuel properties. **Fuel Processing Technology**, [*s. l.*], v. 133, p. 152–160, 2015.

DE CASTRO BIZERRA, Viviane *et al.* Exploring the synergy of ionic liquids and lipase in sustainable enzymatic engineering. **Journal of Molecular Liquids**, [*s. l.*], v. 399, p. 124373, 2024a.

DE CASTRO BIZERRA, Viviane *et al.* Opportunities for cleaner leather processing based on protease enzyme: Current evidence from an advanced bibliometric analysis. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 191, p. 114162, 2024b.

DE JESUS, Sérgio S.; FILHO, Rubens Maciel. Recent advances in lipid extraction using green solvents. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 133, p. 110289, 2020.

DE SOUSA, Isamayra Germano *et al.* Renewable processes of synthesis of biolubricants catalyzed by lipases. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 11, n. 1, p. 109006, 2023.

DE SOUSA JUNIOR, Paulo Gonçalves *et al.* Optimization and theoretical analysis of lipase-catalyzed enzymatic esterification of glycerol for efficient glycerides synthesis. **Biochemical Engineering Journal**, [*s. l.*], v. 198, p. 109033, 2023.

DELANO, W L. **The PyMOL Molecular Graphics System, Version 2.3**. [*S. l.: s. n.*], 2020.

DEMIRBAS, A.; KARSLIOGLU, S. Biodiesel Production Facilities from Vegetable Oils and Animal Fats. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 29, n. 2, p. 133–141, 2007.

DEY, Suman *et al.* Study on performance-emission trade-off and multi-objective optimization of diesel-ethanol-palm biodiesel in a single cylinder CI engine: a Taguchi-fuzzy approach. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 46, n. 1, p. 8498–8518, 2024.

DIAS, Amanda M.S. *et al.* Are Environmental Impact Assessments effectively addressing the biodiversity issues in Brazil?. **Environmental Impact Assessment Review**, [*s. l.*], v. 95, p. 106801, 2022.

DIAS, Michele do Rocio Gonçalves *et al.* Biocatalytic asymmetric synthesis of secondary allylic alcohols using <scp> *Burkholderia cepacia* </scp> lipase immobilized on multiwalled carbon nanotubes. **Chirality**, [*s. l.*], v. 34, n. 7, p. 1008–1018, 2022.

DIEZ, Mikel *et al.* Insights into mechanism kinematics for protein motion simulation. **BMC Bioinformatics**, [*s. l.*], v. 15, n. 1, 2014.

DING, Li-Na *et al.* Advances in plant GDSL lipases: from sequences to functional mechanisms. **Acta Physiologiae Plantarum**, [*s. l.*], v. 41, n. 9, p. 151, 2019.

DOMINGUES, Otávio *et al.* Evaluation of Candida rugosa Lipase Immobilized on Magnetic Nanoparticles in Enzymatic/Chemical Hydroesterification for Biodiesel Production. **Applied Biochemistry and Biotechnology**, [*s. l.*], v. 194, n. 11, p. 5419–5442, 2022.

DOS SANTOS, Letícia Karen *et al.* Production of biodiesel from crude palm oil by a sequential hydrolysis/esterification process using subcritical water. **Renewable Energy**, [*s. l.*], v. 130, p. 633–640, 2019.

DURHAM, Elizabeth *et al.* Solvent accessible surface area approximations for rapid and accurate protein structure prediction. **Journal of Molecular Modeling**, [*s. l.*], v. 15, n. 9, p. 1093–1108, 2009.

ESAN, Akintomiwa O. *et al.* Synthesis of biodiesel from waste palm fatty acid distillate (PFAD) and dimethyl carbonate (DMC) via Taguchi optimisation method. **Biomass and Bioenergy**, [*s. l.*], v. 154, p. 106262, 2021.

ESMI, Fahimeh; BORUGADDA, Venu Babu; DALAI, Ajay K. Heteropoly acids as supported solid acid catalysts for sustainable biodiesel production using vegetable oils: A review. **Catalysis Today**, [*s. l.*], v. 404, p. 19–34, 2022.

EZEORBA, Timothy Prince Chidike *et al.* Recent advances in biotechnological valorization of agro-food wastes (AFW): Optimizing integrated approaches for sustainable biorefinery and circular bioeconomy. **Bioresource Technology Reports**, [*s. l.*], v. 26, p. 101823, 2024.

FAO. **Food and Agriculture Organization of the United Nations**. [*S. l.*], 2018. Disponível em: https://openknowledge.fao.org/server/api/core/bitstreams/9b83c384-975b-49f7-9710-745d2cc201a0/content. Acesso em: 2 jul. 2024.

FARAGO, Oded. Langevin thermostat for robust configurational and kinetic sampling. **Physica A: Statistical Mechanics and its Applications**, [*s. l.*], v. 534, n. 122210, 2019.

FAROUK, Sabah Mohamed *et al.* Recent advances in transesterification for sustainable biodiesel production, challenges, and prospects: a comprehensive review. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 9, p. 12722–12747, 2024.

FARUQUE, Mohammed O.; RAZZAK, Shaikh A.; HOSSAIN, Mohammad M. Application of Heterogeneous Catalysts for Biodiesel Production from Microalgal Oil—A Review. **Catalysts**, [*s. l.*], v. 10, n. 9, p. 1025, 2020.

FATMA, Tehsin *et al.* Computational assessment of Botrytis cinerea lipase for biofuel production. **Catalysts**, [*s. l.*], v. 11, n. 11, 2021.

FAYYAZI, E. *et al.* An ultrasound-assisted system for the optimization of biodiesel production from chicken fat oil using a genetic algorithm and response surface methodology. **Ultrasonics Sonochemistry**, [*s. l.*], v. 26, p. 312–320, 2015.

FERNANDEZ-LAFUENTE, Roberto. Lipase from Thermomyces lanuginosus: Uses and prospects as an industrial biocatalyst. **Journal of Molecular Catalysis B: Enzymatic**, [*s. l.*], v. 62, n. 3–4, p. 197–212, 2010.

FERREIRA MOTA, Gabrielly *et al.* Biodiesel production from microalgae using lipase-based catalysts: Current challenges and prospects. **Algal Research**, [*s. l.*], v. 62, p. 102616, 2022.

FILHO, Danielle Gonçalves; SILVA, Amanda Gonçalves; GUIDINI, Carla Zanella. Lipases: sources, immobilization methods, and industrial applications. **Applied Microbiology and Biotechnology**, [*s. l.*], v. 103, n. 18, p. 7399–7423, 2019.

FILIPE, Hugo A. L.; LOURA, Luís M. S. Molecular Dynamics Simulations: Advances and Applications. **Molecules**, [*s. l.*], v. 27, n. 7, p. 2105, 2022.

FONSECA, Aluísio Marques da *et al.* Study of the Inhibition Potential of Remdesivir Derivatives on Mpro of SARS-COV-2. **International Journal of Research - GRANTHAALAYAH**, [*s. l.*], v. 8, n. 11, p. 164–174, 2020.

FREIJE GARCÍA, Fabricio; GARCÍA LIÑARES, Guadalupe. Use of Lipases as a Sustainable and Efficient Method for the Synthesis and Degradation of Polymers. **Journal of Polymers and the Environment**, [*s. l.*], v. 32, n. 5, p. 2484–2516, 2024.

FUJIKOSHI, Yasunori. Two-way ANOVA models with unbalanced data. **Discrete Mathematics**, [*s. l.*], v. 116, n. 1–3, p. 315–334, 1993.

GAMA CAVALCANTE, Antônio Luthierre *et al.* Advancements in enzyme immobilization on magnetic nanomaterials: toward sustainable industrial applications. **RSC Advances**, [*s. l.*], v. 14, n. 25, p. 17946–17988, 2024.

GARCIA-SILVERA, E. E. *et al.* Enzymatic Biodiesel Production: Challenges and Opportunities. *In*: ESPINOZA-ANDALUZ, Mayken *et al.* (org.). **Congress On Research, Development and Innovation in Renewable Energies**. [*S. l.*]: Springer, Cham, 2023. p. 19–37. Disponível em: https://link.springer.com/chapter/10.1007/978-3-031-26813-7_2. Acesso em: 26 abr. 2025.

GARG, Renuka; SABOUNI, Rana; AHMADIPOUR, Mohsen. From waste to fuel: Challenging aspects in sustainable biodiesel production from lignocellulosic biomass feedstocks and role of metal organic framework as innovative heterogeneous catalysts. **Industrial Crops and Products**, [*s. l.*], v. 206, p. 117554, 2023.

GHAREHGHANI, Ayatallah; MIRSALIM, Mostafa; HOSSEINI, Reza. Effects of waste fish oil biodiesel on diesel engine combustion characteristics and emission. **Renewable Energy**, [*s. l.*], v. 101, p. 930–936, 2017.

GHESTI, Grace F. *et al.* Towards a sustainable waste-to-energy pathway to pequi biomass residues: Biochar, syngas, and biodiesel analysis. **Waste Management**, [*s. l.*], v. 143, p. 144–156, 2022.

GONÇALVES, Tiago dos S. *et al.* Challenges for energy guidelines in crop-based liquid biofuels development in Brazil. **Next Sustainability**, [*s. l.*], v. 2, p. 100002, 2023.

GRANJO, José F.O.; DUARTE, Belmiro P.D.; OLIVEIRA, Nuno M.C. Kinetic Models for the Homogeneous Alkaline and Acid Catalysis in Biodiesel Production. **Computer Aided Chemical Engineering**, [*s. l.*], v. 27, n. C, p. 483–488, 2009. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1570794609703013. Acesso em: 26 abr. 2025.

GUEDES JÚNIOR, José G.E. *et al.* Design of a sustainable process for enzymatic production of ethylene glycol diesters via hydroesterification of used soybean cooking oil. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 10, n. 1, p. 107062, 2022.

GUIMARÃES, José Renato *et al.* Immobilization of Eversa® Transform via CLEA Technology Converts It in a Suitable Biocatalyst for Biolubricant Production Using Waste Cooking Oil. **Molecules**, [*s. l.*], v. 26, n. 1, p. 193, 2021.

GUO, Yingying *et al.* Recent developments in solid acid catalysts for biodiesel production. **Molecular Catalysis**, [*s. l.*], v. 547, p. 113362, 2023.

GUPTA, Vartika; PAL SINGH, Kishan. The impact of heterogeneous catalyst on biodiesel production; a review. **Materials Today: Proceedings**, [*s. l.*], v. 78, p. 364–371, 2023.

HAJJARI, Masoumeh *et al.* A review on the prospects of sustainable biodiesel production: A global scenario with an emphasis on waste-oil biodiesel utilization. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 72, p. 445–464, 2017.

HAMZA, Muhammad *et al.* A review on the waste biomass derived catalysts for biodiesel production. **Environmental Technology & Innovation**, [*s. l.*], v. 21, p. 101200, 2021.

HANWELL, Marcus D *et al.* Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. **Journal of Cheminformatics**, [*s. l.*], v. 4, n. 1, p. 17, 2012.

HASNAOUI, Ismail *et al.* Preparation and Biochemical Characterization of Penicillium crustosum Thom P22 Lipase Immobilization Using Adsorption, Encapsulation, and Adsorption–Encapsulation Approaches. **Molecules**, [*s. l.*], v. 30, n. 3, p. 434, 2025.

HASSAN, Qusay *et al.* The renewable energy role in the global energy Transformations. **Renewable Energy Focus**, [*s. l.*], v. 48, p. 100545, 2024.

HOLECHEK, Jerry L. *et al.* A Global Assessment: Can Renewable Energy Replace Fossil Fuels by 2050?. **Sustainability**, [*s. l.*], v. 14, n. 8, p. 4792, 2022.

HOSSEINZADEH-BANDBAFHA, Homa *et al.* Managing the hazardous waste cooking oil by conversion into bioenergy through the application of waste-derived green catalysts: A review. **Journal of Hazardous Materials**, [*s. l.*], v. 424, p. 127636, 2022.

HUANG, Zhaolin *et al.* Application of molecular dynamics simulation in the field of food enzymes: improving the thermal-stability and catalytic ability. **Critical Reviews in Food Science and Nutrition**, [*s. l.*], v. 64, n. 31, p. 11396–11408, 2024.

HUMPHREY, William; DALKE, Andrew; SCHULTEN, Klaus. VMD: Visual molecular dynamics. **Journal of Molecular Graphics**, [*s. l.*], v. 14, n. 1, p. 33–38, 1996.

ISHAK, Siti nor Hasmah *et al.* Expanding the horizon of biodiesel production via enzyme engineering. **International Journal of Green Energy**, [*s. l.*], v. 21, n. 14, p. 3367–3390, 2024.

ISLAM RONY, Zahidul *et al.* Alternative fuels to reduce greenhouse gas emissions from marine transport and promote UN sustainable development goals. **Fuel**, [*s. l.*], v. 338, p. 127220, 2023.

ISMAEEL, Hiba Kh. *et al.* Strategies for biodiesel production with the role of reactor technologies: A comprehensive review. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 200, p. 109767, 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**, [*s. l.*], v. 163, p. 1624–1639, 2020.

ISMAIL, Abdallah R.; KASHTOH, Hamdy; BAEK, Kwang-Hyun. Temperature-resistant and solvent-tolerant lipases as industrial biocatalysts: Biotechnological approaches and applications. **International Journal of Biological Macromolecules**, [*s. l.*], v. 187, p. 127–142, 2021.

ISSARIYAKUL, Titipong; DALAI, Ajay K. Biodiesel from vegetable oils. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 31, p. 446–471, 2014.

JAEGER, K; EGGERT, T. Enantioselective biocatalysis optimized by directed evolution. **Current Opinion in Biotechnology**, [*s. l.*], v. 15, n. 4, p. 305–313, 2004.

JAIN, Akshay *et al.* Theoretical potential estimation and multi-objective optimization of Water Hyacinth (Eichhornia Crassipes) biodiesel powered diesel engine at variable injection timings. **Renewable Energy**, [*s. l.*], v. 206, p. 514–530, 2023.

JAISWAL, Krishna Kumar *et al.* Valorization of fish processing industry waste for biodiesel

production: Opportunities, challenges, and technological perspectives. **Renewable Energy**, [*s. l.*], v. 220, p. 119601, 2024.

JAYABAL, Ravikumar. Advancements in catalysts, process intensification, and feedstock utilization for sustainable biodiesel production. **Results in Engineering**, [*s. l.*], v. 24, p. 103668, 2024.

JEGANNATHAN, Kenthorai Raman; NIELSEN, Per Henning. Environmental assessment of enzyme use in industrial production – a literature review. **Journal of Cleaner Production**, [*s. l.*], v. 42, p. 228–240, 2013.

JIN, Zihan; WEI, Zihao. Molecular simulation for food protein–ligand interactions: A comprehensive review on principles, current applications, and emerging trends. **Comprehensive Reviews in Food Science and Food Safety**, [*s. l.*], v. 23, n. 1, 2024.

K., Agnieszka. Thermodynamics of Ligand-Protein Interactions: Implications for Molecular Design. *In*: THERMODYNAMICS - INTERACTION STUDIES - SOLIDS, LIQUIDS AND GASES. [*S. l.: s. n.*], 2011.

KAIRYS, Visvaldas *et al.* Recent advances in computational and experimental protein-ligand affinity determination techniques. **Expert Opinion on Drug Discovery**, [*s. l.*], v. 19, n. 6, p. 649–670, 2024.

KALITA, Pinaki *et al.* Biodiesel as renewable biofuel produced via enzyme-based catalyzed transesterification. **Energy Nexus**, [*s. l.*], v. 6, p. 100087, 2022.

KAMAL PASHA, Mustafa *et al.* Comparative study of a two-step enzymatic process and conventional chemical methods for biodiesel production: Economic and environmental perspectives. **Chemical Engineering Journal**, [*s. l.*], v. 489, p. 151254, 2024.

KANT BHATIA, Shashi *et al.* An overview on advancements in biobased transesterification methods for biodiesel production: Oil resources, extraction, biocatalysts, and process intensification technologies. **Fuel**, [*s. l.*], v. 285, p. 119117, 2021.

KARIA, Mahejbin *et al.* Marine-Derived Lipases for Enhancing Enrichment of Very-Long-Chain Polyunsaturated Fatty Acids with Reference to Omega-3 Fatty Acids. **Marine Drugs**, [*s. l.*], v. 22, n. 7, p. 301, 2024.

KARIMI, Kimia *et al.* Biodiesel production from <scp> *Nannochloropsis* </scp> microalgal biomass-derived oil: An experimental and theoretical study using <scp>the RSM-CCD</scp> approach. **The Canadian Journal of Chemical Engineering**, [*s. l.*], v. 101, n. 10, p. 5600–5610, 2023.

KARMAKAR, Bisheswar; DHAWANE, Sumit H.; HALDER, Gopinath. Optimization of biodiesel production from castor oil by Taguchi design. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 6, n. 2, p. 2684–2695, 2018.

KARMAKAR, Bisheswar; HALDER, Gopinath. Progress and future of biodiesel synthesis: Advancements in oil extraction and conversion technologies. **Energy Conversion and Management**, [*s. l.*], v. 182, p. 307–339, 2019.

KATO, Koichi *et al.* Molecular dynamics simulations for the protein–ligand complex structures obtained by computational docking studies using implicit or explicit solvents. **Chemical Physics Letters**, [*s. l.*], v. 781, n. 139022, 2021.

KATSIMPOURAS, Constantinos; STEPHANOPOULOS, Gregory. Enzymes in biotechnology: Critical platform technologies for bioprocess development. **Current Opinion in Biotechnology**, [*s. l.*], v. 69, p. 91–102, 2021.

KAYA, Cenk; KÖKKÜLÜNK, Görkem. Biodiesel as alternative additive fuel for diesel engines: An experimental and theoretical investigation on emissions and performance characteristics. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 45, n. 4, p. 10741–10763, 2023.

KETZER, Felipe *et al.* Kinetic and thermodynamic study of enzymatic hydroesterification mechanism to fatty acid methyl esters synthesis. **Bioresource Technology**, [*s. l.*], v. 356, p. 127335, 2022.

KHAN, Zarmeena *et al.* Current developments in esterification reaction: A review on process and parameters. **Journal of Industrial and Engineering Chemistry**, [*s. l.*], v. 103, p. 80–101, 2021.

KOCH, Elisabeth; KAMPSCHULTE, Nadja; SCHEBB, Nils Helge. Comprehensive Analysis of Fatty Acid and Oxylipin Patterns in n3-PUFA Supplements. **Journal of Agricultural and Food Chemistry**, [*s. l.*], v. 70, n. 13, p. 3979–3988, 2022.

KOROLEVA, Victoria *et al.* Thermal Inactivation, Denaturation and Aggregation Processes of Papain-Like Proteases. **Chemistry & Biodiversity**, [*s. l.*], v. 21, n. 8, 2024.

KOSURU, Sai Mani Yogesh *et al.* A review on the biodiesel production: Selection of catalyst, Pre-treatment, Post treatment methods. **Green Technologies and Sustainability**, [*s. l.*], v. 2, n. 1, p. 100061, 2024.

KRISHNA, Naveen Banchallihundi *et al.* Computational studies on the catalytic potential of the double active site for enzyme engineering. **Scientific Reports**, [*s. l.*], v. 14, n. 1, p. 17892, 2024.

KUSHWAHA, Tribhuvan *et al.* Esterification of oleic acid to biodiesel using biowaste-based solid acid catalyst under microwave irradiation. **Environmental Progress & Sustainable Energy**, [*s. l.*], v. 42, n. 6, 2023.

LI, Ning *et al.* A review on mixture design methods for geopolymer concrete. **Composites Part B: Engineering**, [*s. l.*], v. 178, p. 107490, 2019.

LI, Chenlin *et al.* Impact of feedstock quality and variation on biochemical and thermochemical conversion. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 65, p. 525–536, 2016.

LIANG, Qinlang *et al.* Impact of Feeding Frequency on Growth Performance and Antioxidant Capacity of Litopenaeus vannamei in Recirculating Aquaculture Systems. **Animals**, [*s. l.*], v.

15, n. 2, p. 192, 2025.

LIMA, Anderson H. *et al.* Molecular Modeling of T. rangeli, T. brucei gambiense, and T. evansi Sialidases in Complex with the DANA Inhibitor. **Chemical Biology and Drug Design**, [*s. l.*], v. 80, n. 1, p. 114–120, 2012.

LIN, Cherng-Yuan; LI, Rong-Ji. Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish. **Fuel Processing Technology**, [*s. l.*], v. 90, n. 1, p. 130–136, 2009.

LIN, Chih Kai; ZHANG, Jinming. Detecting Nonadditivity in Single-Facet Generalizability Theory Applications: Tukey's Test. **Journal of Educational Measurement**, [*s. l.*], v. 55, n. 1, p. 78–89, 2018.

LINDER, Michel; FANNI, Jacques; PARMENTIER, Michel. Proteolytic Extraction of Salmon Oil and PUFA Concentration by Lipases. **Marine Biotechnology**, [*s. l.*], v. 7, n. 1, p. 70–76, 2005.

LÓPEZ, J.Mendieta *et al.* A theoretical and experimental study of liquid-liquid equilibrium to refine raw glycerol obtained as a byproduct on the biodiesel production. **Chemical Engineering Journal Advances**, [*s. l.*], v. 10, p. 100257, 2022.

LÓPEZ-YERENA, Anallely *et al.* Waste from Persea schiedeana Fruits as Potential Alternative for Biodiesel Production. **Plants**, [*s. l.*], v. 11, n. 3, p. 252, 2022.

LU, Jiaxin *et al.* Microbial Enzymes for the Biodegradation of Polylactic Acid (PLA): Molecular Docking Simulation and Biodegradation Pathway Proposal. **Environmental Reviews**, [*s. l.*], 2024.

MA, Yingqun *et al.* Biodiesels from microbial oils: Opportunity and challenges. **Bioresource Technology**, [*s. l.*], v. 263, p. 631–641, 2018.

MA, Xinxiu *et al.* Dynamics of Bacterial Composition and Association with Quality Formation and Biogenic Amines Accumulation during Fish Sauce Spontaneous Fermentation. **Applied and Environmental Microbiology**, [*s. l.*], v. 88, n. 13, 2022.

MAHAPATRA, Sangita *et al.* Biofuels and their sources of production: A review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. **Energy Nexus**, [*s. l.*], v. 4, p. 100036, 2021.

MAHDAVI, Mohmmad; ABEDINI, Ebrahim; DARABI, Amir hosein. Biodiesel synthesis from oleic acid by nano-catalyst (ZrO_2/Al_2O_3) under high voltage conditions. **RSC Advances**, [*s. l.*], v. 5, n. 68, p. 55027–55032, 2015.

MAHDI, Hilman Ibnu *et al.* A comprehensive review on nanocatalysts and nanobiocatalysts for biodiesel production in Indonesia, Malaysia, Brazil and USA. **Chemosphere**, [*s. l.*], v. 319, p. 138003, 2023.

MAHESHWARI, Pranjal *et al.* A review on latest trends in cleaner biodiesel production: Role of feedstock, production methods, and catalysts. **Journal of Cleaner Production**, [*s. l.*], v.

355, p. 131588, 2022.

MAKSIMENKO, Anastasiia *et al.* Exploring Sustainable Aquafeed Alternatives with a Specific Focus on the Ensilaging Technology of Fish Waste. **Fermentation**, [*s. l.*], v. 10, n. 5, p. 258, 2024.

MALEKI, B.; ASHRAF TALESH, S.S.; MANSOURI, M. Comparison of catalysts types performance in the generation of sustainable biodiesel via transesterification of various oil sources: a review study. **Materials Today Sustainability**, [*s. l.*], v. 18, p. 100157, 2022.

MANDARI, Venkatesh; DEVARAI, Santhosh Kumar. Biodiesel Production Using Homogeneous, Heterogeneous, and Enzyme Catalysts via Transesterification and Esterification Reactions: a Critical Review. **BioEnergy Research**, [*s. l.*], v. 15, n. 2, p. 935–961, 2022.

MANUBOLU, Manjunath *et al.* Enzymes as direct decontaminating agents—mycotoxins. *In*: ENZYMES IN HUMAN AND ANIMAL NUTRITION. [*S. l.*]: Elsevier, 2018. p. 313–330.

MAROA, Semakula; INAMBAO, Freddie. A review of sustainable biodiesel production using biomass derived heterogeneous catalysts. **Engineering in Life Sciences**, [*s. l.*], v. 21, n. 12, p. 790–824, 2021.

MARTINS, Gislaine Iastiaque *et al.* Physical and chemical properties of fish oil biodiesel produced in Brazil. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 42, p. 154–157, 2015.

MARTI-QUIJAL, Francisco J *et al.* Fermentation in fish and by-products processing: an overview of current research and future prospects. **Current Opinion in Food Science**, [*s. l.*], v. 31, p. 9–16, 2020.

MAS, Rios. Production, Characterization, and Exergy Analysis of the Soybean Biodiesel Produced by Laboratory Scale. **Petroleum & Petrochemical Engineering Journal**, [*s. l.*], v. 7, n. 1, p. 1–9, 2023.

MAZOLA, Yuliet *et al.* A comparative molecular dynamics study of thermophilic and mesophilic β-fructosidase enzymes. **Journal of Molecular Modeling**, [*s. l.*], v. 21, n. 228, p. 1–11, 2015.

MBATIA, Betty *et al.* Enzymatic oil extraction and positional analysis of ω-3 fatty acids in Nile perch and salmon heads. **Process Biochemistry**, [*s. l.*], v. 45, n. 5, p. 815–819, 2010.

MELO, Rafael Leandro Fernandes *et al.* A comprehensive review on enzyme-based biosensors: Advanced analysis and emerging applications in nanomaterial-enzyme linkage. **International Journal of Biological Macromolecules**, [*s. l.*], v. 264, p. 130817, 2024a.

MELO, Rafael Leandro Fernandes *et al.* Enhancing biocatalyst performance through immobilization of lipase (Eversa® Transform 2.0) on hybrid amine-epoxy core-shell magnetic nanoparticles. **International Journal of Biological Macromolecules**, [*s. l.*], v. 264, p. 130730, 2024b.

MESBAH, Noha M. Industrial Biotechnology Based on Enzymes From Extreme Environments. **Frontiers in Bioengineering and Biotechnology**, [*s. l.*], v. 10, 2022.

MISHRA, Shashank; BUKKARAPU, Kiran Raj; KRISHNASAMY, Anand. A composition based approach to predict density, viscosity and surface tension of biodiesel fuels. **Fuel**, [*s. l.*], v. 285, p. 119056, 2021.

MIYURANGA, K. A. Viraj *et al.* Biodiesel Production through the Transesterification of Waste Cooking Oil over Typical Heterogeneous Base or Acid Catalysts. **Catalysts**, [*s. l.*], v. 13, n. 3, p. 546, 2023.

MIZIK, Tamás; GYARMATI, Gábor. Economic and Sustainability of Biodiesel Production—A Systematic Literature Review. **Clean Technologies**, [*s. l.*], v. 3, n. 1, p. 19–36, 2021.

MO, Wing Yin; MAN, Yu Bon; WONG, Ming Hung. Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. **Science of The Total Environment**, [*s. l.*], v. 613–614, p. 635–643, 2018.

MODENUTTI, Carlos P. *et al.* The Structural Biology of Galectin-Ligand Recognition: Current Advances in Modeling Tools, Protein Engineering, and Inhibitor Design. **Frontiers in Chemistry**, [*s. l.*], v. 7, 2019.

MOFIJUR, M. *et al.* Effect of nanocatalysts on the transesterification reaction of first, second and third generation biodiesel sources- A mini-review. **Chemosphere**, [*s. l.*], v. 270, p. 128642, 2021.

MOFIJUR, M. *et al.* Recent Development in the Production of Third Generation Biodiesel from Microalgae. **Energy Procedia**, [*s. l.*], v. 156, p. 53–58, 2019.

MONIKA; BANGA, Sangita; PATHAK, Vinayak V. Biodiesel production from waste cooking oil: A comprehensive review on the application of heterogenous catalysts. **Energy Nexus**, [*s. l.*], v. 10, p. 100209, 2023.

MONTEIRO, Rodolpho R.C. *et al.* Improvement of enzymatic activity and stability of lipase A from Candida antartica onto halloysite nanotubes with Taguchi method for optimized immobilization. **Applied Clay Science**, [*s. l.*], v. 228, p. 106634, 2022.

MONTEIRO, Rodolpho R.C. *et al.* Liquid lipase preparations designed for industrial production of biodiesel. Is it really an optimal solution?. **Renewable Energy**, [*s. l.*], v. 164, p. 1566–1587, 2021.

MOREIRA, Katerine S. *et al.* Optimization of the Production of Enzymatic Biodiesel from Residual Babassu Oil (Orbignya sp.) via RSM. **Catalysts**, [*s. l.*], v. 10, n. 4, p. 414, 2020.

MORRIS, Garrett M. *et al.* Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. **Journal of Computational Chemistry**, [*s. l.*], v. 30, n. 16, p. 2785–2791, 2009.

MORRIS, Connor J.; CORTE, Dennis Della. Using molecular docking and molecular dynamics to investigate protein-ligand interactions. **Modern Physics Letters B**, [*s. l.*], v. 35,

n. 08, p. 2130002, 2021.

MOUAD, Ana Maria *et al.* Aminolysis of linoleic and salicylic acid derivatives with Candida antarctica lipase B: A solvent-free process to obtain amphiphilic amides for cosmetic application. **Journal of Molecular Catalysis B: Enzymatic**, [*s. l.*], v. 126, p. 64–68, 2016.

MUKHTAR, Ahmad *et al.* Current status and challenges in the heterogeneous catalysis for biodiesel production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 157, p. 112012, 2022.

MUSA, Idris Atadashi. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. **Egyptian Journal of Petroleum**, [*s. l.*], v. 25, n. 1, p. 21–31, 2016.

NAM, Kwangho *et al.* Perspectives on Computational Enzyme Modeling: From Mechanisms to Design and Drug Development. **ACS Omega**, [*s. l.*], 2024.

NAMBIRAJ, M.; SURESH KUMAR, K. Impact of essential oils from agro waste as antioxidants on biodiesel long-term storage stability for CI engines: Fresh and aged evaluations. **Process Safety and Environmental Protection**, [*s. l.*], v. 186, p. 1214–1228, 2024.

NAQVI, Ahmad Abu Turab *et al.* Advancements in Docking and Molecular Dynamics Simulations Towards Ligand-receptor Interactions and Structure-function Relationships. **Current Topics in Medicinal Chemistry**, [*s. l.*], v. 18, n. 20, p. 1755–1768, 2018.

NAYAB, Rida *et al.* Sustainable biodiesel production via catalytic and non-catalytic transesterification of feedstock materials – A review. **Fuel**, [*s. l.*], v. 328, p. 125254, 2022.

NEAG, Emilia *et al.* Optimization of Lipid Extraction from Spirulina spp. by Ultrasound Application and Mechanical Stirring Using the Taguchi Method of Experimental Design. **Molecules**, [*s. l.*], v. 27, n. 20, p. 6794, 2022.

NEUPANE, Dhurba. Biofuels from Renewable Sources, a Potential Option for Biodiesel Production. **Bioengineering**, [*s. l.*], v. 10, n. 1, p. 29, 2022.

NOBRE, Millena Mara Rabelo *et al.* Ester Production Using the Lipid Composition of Coffee Ground Oil (Coffea arabica): A Theoretical Study of Eversa® Transform 2.0 Lipase as an Enzymatic Biocatalyst. **Compounds 2023, Vol. 3, Pages 411-429**, [*s. l.*], v. 3, n. 3, p. 411–429, 2023.

O'BOYLE, Noel M. *et al.* Open Babel: An Open chemical toolbox. **Journal of Cheminformatics**, [*s. l.*], v. 3, n. 10, p. 1–14, 2011.

OKECHUKWU, Onukwuli Dominic *et al.* Improving heterogeneous catalysis for biodiesel production process. **Cleaner Chemical Engineering**, [*s. l.*], v. 3, p. 100038, 2022.

OREGE, Joshua Iseoluwa *et al.* Recent advances in heterogeneous catalysis for green biodiesel production by transesterification. **Energy Conversion and Management**, [*s. l.*], v. 258, p. 115406, 2022.

ORMOND, Nathan *et al.* Production of Sustainable Liquid Fuels. **Energies**, [*s. l.*], v. 17, n. 14, p. 3506, 2024.

ORTEGA-REQUENA, Salvadora *et al.* Esters in the Food and Cosmetic Industries: An Overview of the Reactors Used in Their Biocatalytic Synthesis. **Materials**, [*s. l.*], v. 17, n. 1, p. 268, 2024.

OYEKUNLE, Daniel T. *et al.* Heterogeneous catalytic transesterification for biodiesel production: Feedstock properties, catalysts and process parameters. **Process Safety and Environmental Protection**, [*s. l.*], v. 177, p. 844–867, 2023.

P. RAMOS, Luiz *et al.* Biodiesel: Raw Materials, Production Technologies and Fuel Properties. **Revista Virtual de Química**, [*s. l.*], v. 9, n. 1, p. 317–369, 2017.

PARIDA, Soumya *et al.* Production of biodiesel from waste fish fat through ultrasound-assisted transesterification using petro-diesel as cosolvent and optimization of process parameters using response surface methodology. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 17, p. 25524–25537, 2024.

PASHA, Mustafa Kamal *et al.* Biodiesel production with enzymatic technology: progress and perspectives. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 15, n. 5, p. 1526–1548, 2021.

PAUL, Pandeeti Emmanuel Vijay; SANGEETHA, Veeraiah; DEEPIKA, Routhu Gyana. Emerging Trends in the Industrial Production of Chemical Products by Microorganisms. *In*: BUDDOLLA, Viswanath (org.). **Recent Developments in Applied Microbiology and Biochemistry**. 1. ed. [*S. l.*]: Elsevier, 2019. v. 1, p. 107–125.

PEER, Mohamed Shameer *et al.* Experimental evaluation on oxidation stability of biodiesel/diesel blends with alcohol addition by rancimat instrument and FTIR spectroscopy. **Journal of Mechanical Science and Technology**, [*s. l.*], v. 31, n. 1, p. 455–463, 2017.

PERUMAL, Govindhan *et al.* A systematic review on the production of biodiesel using various feedstocks and effective catalysts. **Petroleum Science and Technology**, [*s. l.*], p. 1–17, 2024.

PETROVA, Svetlana S.; SOLOV'EV, Alexander D. The Origin of the Method of Steepest Descent. **Historia Mathematica**, [*s. l.*], v. 24, n. 4, p. 361–375, 1997.

PHILLIPS, James C. *et al.* Scalable molecular dynamics with NAMD. **Journal of Computational Chemistry**, [*s. l.*], v. 26, n. 16, p. 1781–1802, 2005.

POPPE, Jakeline Kathiele *et al.* Transesterification of Waste Frying Oil and Soybean Oil by Combi-lipases Under Ultrasound-Assisted Reactions. **Applied Biochemistry and Biotechnology**, [*s. l.*], v. 186, n. 3, p. 576–589, 2018.

POURZOLFAGHAR, Hamed *et al.* A review of the enzymatic hydroesterification process for biodiesel production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 61, p. 245–257, 2016.

PRETO, Fernando; ZHANG, Frank; WANG, Jinsheng. A study on using fish oil as an alternative fuel for conventional combustors. **Fuel**, [*s. l.*], v. 87, n. 10–11, p. 2258–2268, 2008.

PUDTIKAJORN, Khamtorn; BENJAKUL, Soottawat. Simple Wet Rendering Method for Extraction of Prime Quality Oil from Skipjack Tuna Eyeballs. **European Journal of Lipid Science and Technology**, [*s. l.*], v. 122, n. 8, 2020.

PULLEN, James; SAEED, Khizer. An overview of biodiesel oxidation stability. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 16, n. 8, p. 5924–5950, 2012.

QIAN, Jingya *et al.* Ultrasound-Assisted Enzymatic Protein Hydrolysis in Food Processing: Mechanism and Parameters. **Foods**, [*s. l.*], v. 12, n. 21, p. 4027, 2023.

QIN, Xiaoli; ZHONG, Jinfeng; WANG, Yonghua. A mutant T1 lipase homology modeling, and its molecular docking and molecular dynamics simulation with fatty acids. **Journal of Biotechnology**, [*s. l.*], v. 337, n. February, p. 24–34, 2021.

QU, Pingping *et al.* Improved thermostability of Thermomyces lanuginosus lipase by molecular dynamics simulation and in silico mutation prediction and its application in biodiesel production. **Fuel**, [*s. l.*], v. 327, 2022.

QUISPE, César A.G.; CORONADO, Christian J.R.; CARVALHO JR., João A. Glycerol: Production, consumption, prices, characterization and new trends in combustion. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 27, p. 475–493, 2013.

RAGUNATHAN, Adhithya; MALATHI, Kullappan; ANBARASU, Anand. MurB as a target in an alternative approach to tackle the Vibrio cholerae resistance using molecular docking and simulation study. **Journal of Cellular Biochemistry**, [*s. l.*], v. 119, n. 2, p. 1726–1732, 2018.

RAI, Amit Kumar *et al.* Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. **Enzyme and Microbial Technology**, [*s. l.*], v. 46, n. 1, p. 9–13, 2010.

RAMBABU, K. *et al.* Enhancing biodiesel production from waste date seed oil through ultrasonic-assisted optimization: A sustainable approach using non-edible feedstocks. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 194, p. 109601, 2023.

RAMÍREZ-VERDUZCO, Luis Felipe; HERNÁNDEZ-SÁNCHEZ, Mirna Jimena. Group contribution method for predicting viscosity of alkyl esters and biodiesel. **Fuel**, [*s. l.*], v. 357, p. 129666, 2024.

RAMOS, Marta *et al.* Biodiesel Production Processes and Sustainable Raw Materials. **Energies**, [*s. l.*], v. 12, n. 23, p. 4408, 2019.

RATHORE, Dheeraj *et al.* Bioengineering to Accelerate Biodiesel Production for a Sustainable Biorefinery. **Bioengineering**, [*s. l.*], v. 9, n. 11, p. 618, 2022.

RIAZ, Iqra *et al.* A review on catalysts of biodiesel (methyl esters) production. **Catalysis**

Reviews, [*s. l.*], v. 66, n. 4, p. 1084–1136, 2024.

RIGOGLIUSO, Salvatrice *et al.* Recovery of Bioactive Compounds from Marine Organisms: Focus on the Future Perspectives for Pharmacological, Biomedical and Regenerative Medicine Applications of Marine Collagen. **Molecules**, [*s. l.*], v. 28, n. 3, p. 1152, 2023.

RIZWANUL FATTAH, I. M. *et al.* State of the Art of Catalysts for Biodiesel Production. **Frontiers in Energy Research**, [*s. l.*], v. 8, 2020.

ROBALO, Silvino Sasso *et al.* Review: Enzymatic hydrolysates of fish by-products: technological advantages and bioactive properties. **Ciência Rural**, [*s. l.*], v. 54, n. 8, 2024.

ROE, Daniel R.; BROOKS, Bernard R. A protocol for preparing explicitly solvated systems for stable molecular dynamics simulations. **Journal of Chemical Physics**, [*s. l.*], v. 153, n. 5, p. 1–9, 2020.

ROY, Dalim Kumer; ABEDIN, Mohammad Zoynal. Potentiality of biodiesel and bioethanol production from feedstock in Bangladesh: A review. **Heliyon**, [*s. l.*], v. 8, n. 11, p. e11213, 2022.

RYU, Young-Jin *et al.* Development of Carbon-Based Solid Acid Catalysts Using a Lipid-Extracted Alga, Dunaliella tertiolecta, for Esterification. **Journal of Microbiology and Biotechnology**, [*s. l.*], v. 28, n. 5, p. 732–738, 2018.

SADANA, Ajit. Enzyme deactivation. **Biotechnology Advances**, [*s. l.*], v. 6, n. 3, p. 349-IN2, 1988.

SAINI, Ramesh Kumar *et al.* Advances in Lipid Extraction Methods—A Review. **International Journal of Molecular Sciences**, [*s. l.*], v. 22, n. 24, p. 13643, 2021.

SALAHELDEEN, Mohammed *et al.* Current State and Perspectives on Transesterification of Triglycerides for Biodiesel Production. **Catalysts**, [*s. l.*], v. 11, n. 9, p. 1121, 2021.

SALES, Misael B. *et al.* Sustainable Feedstocks and Challenges in Biodiesel Production: An Advanced Bibliometric Analysis. **Bioengineering**, [*s. l.*], v. 9, n. 10, p. 539, 2022.

SÁNCHEZ-SOLÍS, Alejandra *et al.* Biodiesel Production Processes with Yeast: A Sustainable Approach. **Energies**, [*s. l.*], v. 17, n. 2, p. 302, 2024.

ŠÁNEK, Lubomír *et al.* Pilot-scale production of biodiesel from waste fats and oils using tetramethylammonium hydroxide. **Waste Management**, [*s. l.*], v. 48, p. 630–637, 2016.

SANTOS, Antonio Lucas R. *et al.* Study of molecular arrangement and density estimation of soybean oil biodiesel-diesel blends employing molecular dynamic simulation. **Fuel**, [*s. l.*], v. 377, p. 132760, 2024.

SARVE, Antaram N.; VARMA, Mahesh N.; SONAWANE, Shriram S. Ultrasound assisted two-stage biodiesel synthesis from non-edible Schleichera triguga oil using heterogeneous catalyst: Kinetics and thermodynamic analysis. **Ultrasonics Sonochemistry**, [*s. l.*], v. 29, p. 288–298, 2016.

SEMWAL, Surbhi *et al.* Biodiesel production using heterogeneous catalysts. **Bioresource Technology**, [*s. l.*], v. 102, n. 3, p. 2151–2161, 2011.

SHAKILANISHI, S.; SHANTHI, C. An overview on preparation of enzymes for industrial use. **Biocatalysis and Biotransformation**, [*s. l.*], v. 42, n. 4, p. 485–496, 2024.

SHAMEER, P. Mohamed; RAMESH, K. Green technology and performance consequences of an eco-friendly substance on a 4-stroke diesel engine at standard injection timing and compression ratio. **Journal of Mechanical Science and Technology**, [*s. l.*], v. 31, n. 3, p. 1497–1507, 2017.

SHARMA, Binod; SHRESTHA, Ashish. Petroleum dependence in developing countries with an emphasis on Nepal and potential keys. **Energy Strategy Reviews**, [*s. l.*], v. 45, p. 101053, 2023.

SHU, Liwen *et al.* Transesterification of phosphatidylcholine with DHA-rich algal oil using immobilized Candida antarctica lipase B to produce DHA-phosphatidylcholine. **Enzyme and Microbial Technology**, [*s. l.*], v. 169, p. 110266, 2023.

SINGH, Yashvir *et al.* Biodiesel production and exploring properties of Datura stramonium L. oil with its optimization using combined approaches—Taguchi, grey relational analysis, and response surface methodology. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 16, p. 23802–23821, 2024.

SINGH, Narender *et al.* Progress and facts on biodiesel generations, production methods, influencing factors, and reactors: A comprehensive review from 2000 to 2023. **Energy Conversion and Management**, [*s. l.*], v. 302, p. 118157, 2024.

SINGH, Sakshi; BANI BAKER, Qanita; SINGH, Dev Bukhsh. Molecular docking and molecular dynamics simulation. *In*: SINGH, Dev Bukhsh; PATHAK, Rajesh Kumar (org.). **Bioinformatics: Methods and Applications**. 1. ed. [*S. l.*]: Elsevier, 2022. p. 291–304.

SINGH, Bhim; JANA, Asim Kumar. Agri-residues and agro-industrial waste substrates bioconversion by fungal cultures to biocatalyst lipase for green chemistry: A review. **Journal of Environmental Management**, [*s. l.*], v. 348, p. 119219, 2023.

SMAISIM, Ghassan F. *et al.* Synthesis of biodiesel from fish processing waste by nano magnetic catalyst and its thermodynamic analysis. **Case Studies in Thermal Engineering**, [*s. l.*], v. 35, p. 102115, 2022.

SREEHARSHA, Rachapudi Venkata; DUBEY, Namrata; MOHAN, S Venkata. Orienting biodiesel production towards sustainability and circularity by tailoring the feedstock and processes. **Journal of Cleaner Production**, [*s. l.*], v. 414, p. 137526, 2023.

SREEPRASANTH, P.S. *et al.* Hydrophobic, solid acid catalysts for production of biofuels and lubricants. **Applied Catalysis A: General**, [*s. l.*], v. 314, n. 2, p. 148–159, 2006.

STREET, Arthur G.; MAYO, Stephen L. Pairwise calculation of protein solvent-accessible surface areas. **Folding and Design**, [*s. l.*], v. 3, n. 4, p. 253–258, 1998.

SU, Jung-Jeng; CHOU, Yu-Chun. Biodiesel Production by Acid Methanolysis of Slaughterhouse Sludge Cake. **Animals**, [*s. l.*], v. 9, n. 12, p. 1029, 2019.

SUHARA, Ade *et al.* Biodiesel Sustainability: Review of Progress and Challenges of Biodiesel as Sustainable Biofuel. **Clean Technologies**, [*s. l.*], v. 6, n. 3, p. 886–906, 2024.

SUNDARAM, Thanigaivel *et al.* Advancements in biosurfactant production using agro-industrial waste for industrial and environmental applications. **Frontiers in Microbiology**, [*s. l.*], v. 15, 2024.

SUTHERLAND, Jeffrey J. *et al.* Lessons in molecular recognition. 2. Assessing and improving cross-docking accuracy. **Journal of Chemical Information and Modeling**, [*s. l.*], v. 47, n. 6, p. 2293–2302, 2007.

SUTTON, Richard S.; MAHMOOD, A. Rupam; WHITE, Martha. An emphatic approach to the problem of off-policy temporal-difference learning. **Journal of Machine Learning Research**, [*s. l.*], v. 17, p. 1–29, 2016.

TAHERI-GARAVAND, Amin *et al.* Application of artificial neural networks for the prediction of performance and exhaust emissions in IC engine using biodiesel-diesel blends containing quantum dot based on carbon doped. **Energy Conversion and Management: X**, [*s. l.*], v. 16, p. 100304, 2022.

TAMRAT, Samuel *et al.* Emission and performance analysis of diesel engine running with CeO2 nanoparticle additive blended into castor oil biodiesel as a substitute fuel. **Scientific Reports**, [*s. l.*], v. 14, n. 1, p. 7634, 2024.

TARIQ, Muhammad; ALI, Saqib; KHALID, Nasir. Activity of homogeneous and heterogeneous catalysts, spectroscopic and chromatographic characterization of biodiesel: A review. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 16, n. 8, p. 6303–6316, 2012.

THAMARAI KANNAN, B. *et al.* Use of waste fish oil biodiesel blended with aluminium oxide nanoparticle in IC engines: an experimental on performance, combustion and emission study. **Scientific Reports**, [*s. l.*], v. 12, n. 1, p. 12930, 2022.

THANIGAIVEL, Sundaram *et al.* Sustainability and carbon neutralization trends in microalgae bioenergy production from wastewater treatment: A review. **Bioresource Technology**, [*s. l.*], v. 364, p. 128057, 2022.

THE STATE OF WORLD FISHERIES AND AQUACULTURE 2024. [*S. l.*]: FAO, 2024.

THIRUMALAI, D.; LORIMER, George H.; HYEON, Changbong. Iterative annealing mechanism explains the functions of the GroEL and RNA chaperones. **Protein Science**, [*s. l.*], v. 29, n. 2, p. 360–377, 2020.

TIAN, Li *et al.* Marine microorganisms: natural factories for polyunsaturated fatty acid production. **Blue Biotechnology**, [*s. l.*], v. 1, n. 1, p. 15, 2024.

TIBESIGWA, Timothy; OLUPOT, Peter Wilberforce; KIRABIRA, John Baptist. Assessment

of the techno-economic viability of B10 synthesis from second-generation biodiesel feedstocks in Uganda. **International Journal of Sustainable Energy**, [*s. l.*], v. 42, n. 1, p. 351–373, 2023.

TOGUN, Hussein *et al.* A review on recent advances on improving fuel economy and performance of a fuel cell hybrid electric vehicle. **International Journal of Hydrogen Energy**, [*s. l.*], v. 89, p. 22–47, 2024.

TORRES-VALENZUELA, Laura Sofía; BALLESTEROS-GÓMEZ, Ana; RUBIO, Soledad. Green Solvents for the Extraction of High Added-Value Compounds from Agri-food Waste. **Food Engineering Reviews**, [*s. l.*], v. 12, n. 1, p. 83–100, 2020.

TROTT, Oleg; OLSON, Arthur J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. **Journal of Computational Chemistry**, [*s. l.*], v. 31, n. 2, p. 455–461, 2010.

TUBINO, Matthieu; JUNIOR, José Geraldo Rocha; BAUERFELDT, Glauco Favilla. Biodiesel synthesis with alkaline catalysts: A new refractometric monitoring and kinetic study. **Fuel**, [*s. l.*], v. 125, p. 164–172, 2014.

ULLAH, Zahoor *et al.* Unveiling Biodiesel Production: Exploring Reaction Protocols, Catalysts, and Influential Factors. **ChemBioEng Reviews**, [*s. l.*], v. 11, n. 6, 2024.

UNUGUL, Tuba *et al.* Environmentally friendly processes from coffee wastes to trimethylolpropane esters to be considered biolubricants. **Journal of the Air & Waste Management Association**, [*s. l.*], v. 70, n. 11, p. 1198–1215, 2020.

VALENTI, Wagner C. *et al.* Aquaculture in Brazil: past, present and future. **Aquaculture Reports**, [*s. l.*], v. 19, p. 100611, 2021.

VERMA, Puneet; SHARMA, M.P. Review of process parameters for biodiesel production from different feedstocks. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 62, p. 1063–1071, 2016.

VICTORINO DA SILVA AMATTO, Isabela *et al.* Enzyme engineering and its industrial applications. **Biotechnology and Applied Biochemistry**, [*s. l.*], v. 69, n. 2, p. 389–409, 2022.

VIDAL-LIMON, Abraham; AGUILAR-TOALÁ, José E.; LICEAGA, Andrea M. Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides. **Journal of Agricultural and Food Chemistry**, [*s. l.*], v. 70, n. 4, p. 934–943, 2022.

WAHL, Joel *et al.* Accuracy evaluation and addition of improved dihedral parameters for the MMFF94s. **Journal of Cheminformatics**, [*s. l.*], v. 11, n. 1, p. 1–10, 2019.

WANCURA, João H.C. *et al.* Demystifying the enzymatic biodiesel: How lipases are contributing to its technological advances. **Renewable Energy**, [*s. l.*], v. 216, p. 119085, 2023.

WANG, Hong hai *et al.* Application of Lipase B from *Candida antarctica* in the

Pharmaceutical Industry. **Industrial & Engineering Chemistry Research**, [*s. l.*], v. 62, n. 39, p. 15733–15751, 2023.

WANG, Baohua *et al.* Enabling Catalysts for Biodiesel Production via Transesterification. **Catalysts**, [*s. l.*], v. 13, n. 4, p. 740, 2023.

WANG, Lu *et al.* Synthesis, catalysts and enhancement technologies of biodiesel from oil feedstock – A review. **Science of The Total Environment**, [*s. l.*], v. 904, p. 166982, 2023.

WELDESLASE, Mebrhit Gebreyohanes *et al.* Maximizing biodiesel production from waste cooking oil with lime-based zinc-doped CaO using response surface methodology. **Scientific Reports**, [*s. l.*], v. 13, n. 1, p. 4430, 2023.

XIA, Shuaibo *et al.* Lipase-catalyzed production of biodiesel: a critical review on feedstock, enzyme carrier and process factors. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 18, n. 1, p. 291–309, 2024.

XIE, Dongming. Integrating Cellular and Bioprocess Engineering in the Non-Conventional Yeast Yarrowia lipolytica for Biodiesel Production: A Review. **Frontiers in Bioengineering and Biotechnology**, [*s. l.*], v. 5, 2017.

XU, Chao *et al.* Experimental and theoretical evidence of enhanced catalytic performance of lipase B from Candida antarctica acquired by the chemical modification with amino acid ionic liquids. **Molecular Catalysis**, [*s. l.*], v. 501, p. 111355, 2021.

YADAV, Vishwanath; BISWAS, Shreya; GOYAL, Arun. Enzymes of Industrial Significance and Their Applications. *In*: VERMA, Pradeep (org.). **Industrial Microbiology and Biotechnology**. 1. ed. Singapore: Springer Singapore, 2024. p. 277–307.

YAHYAEE, R.; GHOBADIAN, B.; NAJAFI, G. Waste fish oil biodiesel as a source of renewable fuel in Iran. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 17, p. 312–319, 2013.

YAN, Wenxin *et al.* Advanced strategies in high-throughput droplet screening for enzyme engineering. **Biosensors and Bioelectronics**, [*s. l.*], v. 248, p. 115972, 2024.

YESILYURT, Murat Kadir; CESUR, Cüneyt. A statistical optimization attempt by applying the Taguchi technique for the optimum transesterification process parameters in the production of biodiesel from Papaver somniferum L. seed oil. **Fuel**, [*s. l.*], v. 329, p. 125406, 2022.

YOSHIOKA, Taichi *et al.* Accelerated acidolysis for triglyceride modification using commercial lipases activated by a hydration–aggregation pretreatment. **Process Biochemistry**, [*s. l.*], v. 132, p. 130–139, 2023.

YUE, Mengmeng *et al.* Recent advances of biodiesel production enhanced by external field via heterogeneous catalytic transesterification system. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 205, p. 109997, 2024.

YUSUF, Basiru O.; OLADEPO, Sulayman A.; GANIYU, Saheed A. Efficient and Sustainable

Biodiesel Production via Transesterification: Catalysts and Operating Conditions. **Catalysts**, [*s. l.*], v. 14, n. 9, p. 581, 2024.

ZENG, Can; ZHONG, Nanjing. Encapsulation of lipases on coordination polymers and their catalytic performance in glycerolysis and esterification. **Grain & Oil Science and Technology**, [*s. l.*], v. 6, n. 3, p. 113–119, 2023.

ZHANG, Yiqi *et al.* Fish Scale Valorization by Hydrothermal Pretreatment Followed by Enzymatic Hydrolysis for Gelatin Hydrolysate Production. **Molecules**, [*s. l.*], v. 24, n. 16, p. 2998, 2019.

ZHANG, Zhuangzhuang *et al.* Lewis Acid-Base Site-Assisted In Situ Transesterification Catalysis to Produce Biodiesel. **Catalysts**, [*s. l.*], v. 14, n. 10, p. 731, 2024.

ZHANG, Yi; NARLIKAR, Geeta J.; KUTATELADZE, Tatiana G. Enzymatic Reactions inside Biological Condensates. **Journal of Molecular Biology**, [*s. l.*], v. 433, n. 12, p. 166624, 2021.

ZHAO, Zhen *et al.* A review of biophysical strategies to investigate protein-ligand binding: What have we employed?. **International Journal of Biological Macromolecules**, [*s. l.*], v. 276, p. 133973, 2024.

ZHAO, Jie *et al.* Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. **Asian-Australasian Journal of Animal Sciences**, [*s. l.*], v. 32, n. 6, p. 783–791, 2019.

ZHAO, Junxin *et al.* Production, purification, properties and current perspectives for modification and application of microbial lipases. **Preparative Biochemistry & Biotechnology**, [*s. l.*], v. 54, n. 8, p. 1001–1016, 2024.

ZHOU, Jiahui; HUANG, Meilan. Navigating the landscape of enzyme design: from molecular simulations to machine learning. **Chemical Society Reviews**, [*s. l.*], v. 53, n. 16, p. 8202–8239, 2024.

ZHU, Jishen *et al.* Taguchi method for optimizing the cation exchange resin catalyzed esterification of oleic acid with ethanol and adsorption kinetics study. **Process Safety and Environmental Protection**, [*s. l.*], v. 182, p. 989–998, 2024.

ZULQARNAIN *et al.* Overview of Feedstocks for Sustainable Biodiesel Production and Implementation of the Biodiesel Program in Pakistan. **ACS Omega**, [*s. l.*], v. 6, n. 29, p. 19099–19114, 2021.

ABDUL HAKIM SHAAH, Marwan *et al.* A review on non-edible oil as a potential feedstock for biodiesel: physicochemical properties and production technologies. **RSC Advances**, [*s. l.*], v. 11, n. 40, p. 25018–25037, 2021.

ABDULLAH, Muhammad *et al.* Advancements in sustainable production of biofuel by microalgae: Recent insights and future directions. **Environmental Research**, [*s. l.*], v. 262, p. 119902, 2024.

ABOMOHRA, Abd El-Fatah *et al.* Potential of fat, oil and grease (FOG) for biodiesel production: A critical review on the recent progress and future perspectives. **Progress in Energy and Combustion Science**, [*s. l.*], v. 81, p. 100868, 2020.

ABOU-ELYAZED, Ahmed S. *et al.* A green approach for enhancing the hydrophobicity of UiO-66(Zr) catalysts for biodiesel production at 298 K. **RSC Advances**, [*s. l.*], v. 10, n. 68, p. 41283–41295, 2020.

ABOULROUS, Amany A. *et al.* Review of synthesis, characteristics and technical challenges of biodiesel based drilling fluids. **Journal of Cleaner Production**, [*s. l.*], v. 336, p. 130344, 2022.

ABOU-TALEB, Noura Hemdan *et al.* Optimizing the spectrofluorimetric determination of cefdinir through a Taguchi experimental design approach. **Luminescence**, [*s. l.*], v. 31, n. 3, p. 856–864, 2016.

ABREU SILVEIRA, Erick *et al.* Biocatalyst engineering of Thermomyces Lanuginosus lipase adsorbed on hydrophobic supports: Modulation of enzyme properties for ethanolysis of oil in solvent-free systems. **Journal of Biotechnology**, [*s. l.*], v. 289, p. 126–134, 2019.

ACHERKI, Hassan; BOUAID, Abderrahim; MARCHETTI, Jorge Mario. Optimization of the enzymatic butanolysis of <scp>jatropha</scp> oil for biodiesel production using <scp>Eversa</scp>. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 16, n. 1, p. 219–227, 2022.

ADERIBIGBE, Fatai A. *et al.* Heterogeneous Catalysis of Second Generation Oil for Biodiesel Production: A Review. **ChemBioEng Reviews**, [*s. l.*], v. 8, n. 2, p. 78–89, 2021.

ADHITHAN, Balamurugan; SACHDEVA, Gulshan. Analysing the emissions and fuel efficiency of fish-waste biodiesel in a compression ignition engine. **International Journal of Ambient Energy**, [*s. l.*], v. 44, n. 1, p. 1829–1839, 2023.

AGU, P. C. *et al.* Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. **Scientific Reports**, [*s. l.*], v. 13, n. 1, p. 13398, 2023.

AHMADI, Meisam *et al.* Chem3D 15.0 User Guide. **Macromolecules**, [*s. l.*], v. 24, n. 2, p. 1–61, 2005.

AHMED, Rawaz; HUDDERSMAN, Katherine. Review of biodiesel production by the esterification of wastewater containing fats oils and grease (FOGs). **Journal of Industrial and Engineering Chemistry**, [*s. l.*], v. 110, p. 1–14, 2022.

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**, [*s. l.*], v. 412, p. 135558, 2023.

ALEXANDRE, Jeferson Yves Nunes Holanda *et al.* A Theoretical and Experimental Study for Enzymatic Biodiesel Production from Babassu Oil (Orbignya sp.) Using Eversa Lipase. **Catalysts**, [*s. l.*], v. 12, n. 11, 2022.

ALGAYYIM, Sattar Jabbar Murad *et al.* Influence of natural gas and hydrogen properties on internal combustion engine performance, combustion, and emissions: A review. **Fuel**, [*s. l.*], v. 362, p. 130844, 2024.

ALI, Sajid *et al.* The Recent Advances in the Utility of Microbial Lipases: A Review. **Microorganisms**, [*s. l.*], v. 11, n. 2, p. 510, 2023.

ALI IJAZ MALIK, Muhammad *et al.* A review of major trends, opportunities, and technical challenges in biodiesel production from waste sources. **Energy Conversion and Management: X**, [*s. l.*], v. 23, p. 100675, 2024.

AL-JABIRI, Ali A.; BALLA, Hyder H.; AL-ZUHAIRY, Mudhaffar S. Effect of Biodiesel Produced from Sunflower Oil on Engine Emissions Characteristics. *In*: [*S. l.: s. n.*], 2025. p. 347–358.

ALMEIDA, Lays C. *et al.* Enzymatic transesterification of coconut oil by using immobilized lipase on biochar: An experimental and molecular docking study. **Biotechnology and Applied Biochemistry**, [*s. l.*], p. 1–8, 2020.

ALMEIDA, Francisco Lucas Chaves *et al.* Trends in lipase immobilization: Bibliometric review and patent analysis. **Process Biochemistry**, [*s. l.*], v. 110, p. 37–51, 2021.

AL-SAADI, Ali; MATHAN, Bobby; HE, Yinghe. Esterification and transesterification over SrO–ZnO/Al2O3 as a novel bifunctional catalyst for biodiesel production. **Renewable Energy**, [*s. l.*], v. 158, p. 388–399, 2020.

ALVES, Alanderson Arthu Araújo *et al.* Investigating the thermophysical properties of the 1-Butanol + Biodiesel System: The impact of pressure on volumetric characteristics. **Fuel**, [*s. l.*], v. 371, p. 132076, 2024.

AMBAYE, Teklit Gebregiorgis *et al.* Emerging technologies for biofuel production: A critical review on recent progress, challenges and perspectives. **Journal of Environmental Management**, [*s. l.*], v. 290, p. 112627, 2021.

ANDREO-MARTÍNEZ, Pedro *et al.* Waste animal fats as feedstock for biodiesel production using non-catalytic supercritical alcohol transesterification: A perspective by the PRISMA methodology. **Energy for Sustainable Development**, [*s. l.*], v. 69, p. 150–163, 2022.

ANU PRASANNA, Vankara *et al.* Fish Waste: A Potential Source of Biodiesel. **Fermentation**, [*s. l.*], v. 9, n. 9, p. 861, 2023.

ARANSIOLA, E.F. *et al.* A review of current technology for biodiesel production: State of the art. **Biomass and Bioenergy**, [*s. l.*], v. 61, p. 276–297, 2014.

ARCUS, Vickery L *et al.* Enzyme evolution and the temperature dependence of enzyme catalysis. **Current Opinion in Structural Biology**, [*s. l.*], v. 65, p. 96–101, 2020.

ARCUS, Vickery L.; MULHOLLAND, Adrian J. Temperature, Dynamics, and Enzyme-Catalyzed Reaction Rates. **Annual Review of Biophysics**, [*s. l.*], v. 49, n. 1, p. 163–180,

2020.

ARSHAD, Sumera *et al.* Assessing the potential of green CdO2 nano-catalyst for the synthesis of biodiesel using non-edible seed oil of Malabar Ebony. **Fuel**, [*s. l.*], v. 333, p. 126492, 2023.

ARSHIA, Amir Hossein *et al.* De novo design of novel protease inhibitor candidates in the treatment of SARS-CoV-2 using deep learning, docking, and molecular dynamic simulations. **Computers in Biology and Medicine**, [*s. l.*], v. 139, 2021.

ASSOSSIAÇÃO BRASILEIRA DE PISCICULTURA. **Anuário Peixe BR da Piscicultura**. [*S. l.: s. n.*], 2023.

ATABANI, A.E. *et al.* Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 18, p. 211–245, 2013.

ATADASHI, I.M. *et al.* The effects of catalysts in biodiesel production: A review. **Journal of Industrial and Engineering Chemistry**, [*s. l.*], v. 19, n. 1, p. 14–26, 2013.

ATHIAPPAN, Murugan *et al.* Rhizosphere engineering through pesticides-degrading beneficial bacteria. *In*: DUBEY, Ramesh Chandra; KUMAR, Pankaj (org.). **Rhizosphere Engineering**. 1. ed. [*S. l.*]: Elsevier, 2022. v. 1, p. 239–257.

AZIRI, Sabrina *et al.* Taguchi method for optimization of Cr(VI) removal, isotherm, kinetic and thermodynamic studies. **Nucleosides, Nucleotides & Nucleic Acids**, [*s. l.*], p. 1–25, 2024.

BABADI, Arman Amani *et al.* Emerging technologies for biodiesel production: Processes, challenges, and opportunities. **Biomass and Bioenergy**, [*s. l.*], v. 163, p. 106521, 2022. Disponível em: Acesso em: 2 abr. 2024.

BARELLI, Linda *et al.* Enzymatic Biofuel Cells: A Review on Flow Designs. **Energies**, [*s. l.*], v. 14, n. 4, p. 910, 2021.

BENTI, Natei Ermias *et al.* Biodiesel production in Ethiopia: Current status and future prospects. **Scientific African**, [*s. l.*], v. 19, p. e01531, 2023.

BERMAN, Paula; NIZRI, Shahar; WIESMAN, Zeev. Castor oil biodiesel and its blends as alternative fuel. **Biomass and Bioenergy**, [*s. l.*], v. 35, n. 7, p. 2861–2866, 2011.

BHATTACHARYYA, Subhrajyoti. Transesterification of Yellow Oleander seed oil, its utilization as biodiesel and performance evaluation. **Heliyon**, [*s. l.*], v. 8, n. 4, p. e09250, 2022.

BIOVIA. **Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5**. San Diego: [*s. n.*], 2015.

BORGES, Sandra *et al.* Fish By-Products: A Source of Enzymes to Generate Circular Bioactive Hydrolysates. **Molecules**, [*s. l.*], v. 28, n. 3, p. 1155, 2023.

BRAHMA, Sujata *et al.* Biodiesel production from mixed oils: A sustainable approach towards industrial biofuel production. **Chemical Engineering Journal Advances**, [*s. l.*], v. 10, p. 100284, 2022.

BRANDÃO, Luma M. de S. *et al.* Lipase activation by molecular bioimprinting: The role of interactions between fatty acids and enzyme active site. **Biotechnology Progress**, [*s. l.*], v. 37, n. 1, 2021.

BRENA, Beatriz; GONZÁLEZ-POMBO, Paula; BATISTA-VIERA, Francisco. Immobilization of Enzymes: A Literature Survey. *In*: [*S. l.: s. n.*], 2013. p. 15–31.

BRUICE, Thomas C.; LIGHTSTONE, Felice C. **Ground state and transition state contributions to the rates of intramolecular and enzymatic reactions**. [*S. l.: s. n.*], 1999.

BUESO, Francisco *et al.* Lipase-catalyzed biodiesel production and quality with Jatropha curcas oil: exploring its potential for Central America. **Journal of Biological Engineering**, [*s. l.*], v. 9, n. 1, p. 12, 2015.

BUKKARAPU, Kiran Raj; KRISHNASAMY, Anand. A critical review on available models to predict engine fuel properties of biodiesel. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 155, p. 111925, 2022.

BULLO, Giovanna Totti *et al.* Lipases: market study and potential applications of immobilized derivatives. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], 2024.

CARVALHO, Wagner C.A. *et al.* Eco-friendly production of trimethylolpropane triesters from refined and used soybean cooking oils using an immobilized low-cost lipase (Eversa>® Transform 2.0) as heterogeneous catalyst. **Biomass and Bioenergy**, [*s. l.*], v. 155, p. 106302, 2021. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0961953421003378.

CARVALHO, J. C. *et al.* Growth curve of Nile tilapia from different families of the AquaAmérica variety. **Brazilian Journal of Biology**, [*s. l.*], v. 82, 2022.

CAVALCANTE, Francisco Thálysson Tavares *et al.* Opportunities for improving biodiesel production via lipase catalysis. **Fuel**, [*s. l.*], v. 288, p. 119577, 2021.

CHANAKAEWSOMBOON, Issara *et al.* Biodiesel produced using potassium methoxide homogeneous alkaline catalyst: effects of various factors on soap formation. **Biomass Conversion and Biorefinery**, [*s. l.*], v. 13, n. 10, p. 9237–9247, 2023.

CHAOS-HERNÁNDEZ, D. *et al.* Extraction methods of algae oils for the production of third generation biofuels – A review. **Chemosphere**, [*s. l.*], v. 341, p. 139856, 2023.

CHINTAGUNTA, Anjani Devi *et al.* Biodiesel Production From Lignocellulosic Biomass Using Oleaginous Microbes: Prospects for Integrated Biofuel Production. **Frontiers in Microbiology**, [*s. l.*], v. 12, 2021.

CHOUHAN, A.P. Singh; SARMA, A.K. Modern heterogeneous catalysts for biodiesel production: A comprehensive review. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v.

15, n. 9, p. 4378–4399, 2011.

CHOZHAVENDHAN, S. *et al.* A review on influencing parameters of biodiesel production and purification processes. **Current Research in Green and Sustainable Chemistry**, [*s. l.*], v. 1–2, p. 1–6, 2020.

CHRISTOPHER SELVAM, D.; DEVARAJAN, Yuvarajan; RAJA, T. Characterization of isopropanol-enhanced Caesalpinia bonduc biodiesel blends: A sustainable strategy for decarbonization. **Results in Engineering**, [*s. l.*], v. 25, p. 103849, 2025.

CHUKWUMA, Ogechukwu Bose *et al.* Lignocellulolytic Enzymes in Biotechnological and Industrial Processes: A Review. **Sustainability**, [*s. l.*], v. 12, n. 18, p. 7282, 2020.

CIEŚLAK, Dominika; KABELKA, Ivo; BARTUZI, Damian. Molecular Dynamics Simulations in Protein–Protein Docking. *In*: [*S. l.: s. n.*], 2024. p. 91–106.

COPPOLA, Daniela *et al.* Fish Waste: From Problem to Valuable Resource. **Marine Drugs**, [*s. l.*], v. 19, n. 2, p. 116, 2021.

CORDERO-RAVELO, Virginia; SCHALLENBERG-RODRIGUEZ, Julieta. Biodiesel production as a solution to waste cooking oil (WCO) disposal. Will any type of WCO do for a transesterification process? A quality assessment. **Journal of Environmental Management**, [*s. l.*], v. 228, p. 117–129, 2018.

CORICI, Livia *et al.* Understanding Potentials and Restrictions of Solvent-Free Enzymatic Polycondensation of Itaconic Acid: An Experimental and Computational Analysis. **Advanced Synthesis and Catalysis**, [*s. l.*], v. 357, n. 8, p. 1763–1774, 2015.

COSTA, Matheus J. *et al.* Enzymatic biodiesel production by hydroesterification using waste cooking oil as feedstock. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 157, p. 108131, 2020.

COSTA, Isabela Oliveira *et al.* Enzyme immobilization technology as a tool to innovate in the production of biofuels: A special review of the Cross-Linked Enzyme Aggregates (CLEAs) strategy. **Enzyme and Microbial Technology**, [*s. l.*], v. 170, p. 110300, 2023.

CRUZ, Jaqueline Matos *et al.* Comparative study of phytotoxicity and genotoxicity of soil contaminated with biodiesel, diesel fuel and petroleum. **Ecotoxicology**, [*s. l.*], v. 28, n. 4, p. 449–456, 2019.

CUBAS, A.L.V. *et al.* Biodiesel production using fatty acids from food industry waste using corona discharge plasma technology. **Waste Management**, [*s. l.*], v. 47, p. 149–154, 2016.

CULSUM, Neng Tresna Umi *et al.* Fabrication of lipase immobilized-carboxylated cellulose nanocrystals as biocatalyst for enzymatic hydrolysis of palm oil and its kinetical study. **Biocatalysis and Agricultural Biotechnology**, [*s. l.*], v. 54, p. 102915, 2023.

CUNHA, Anildo *et al.* Synthesis and characterization of ethylic biodiesel from animal fat wastes. **Fuel**, [*s. l.*], v. 105, p. 228–234, 2013.

CZECH STANDARDS INSTITUTE. **Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents**. [*S. l.: s. n.*], 2011. Disponível em: www.agilent.com/chem. .

DA SILVA AIRES, Francisco Izaias *et al.* Advanced and prospects in phenol wastewater treatment technologies: unveiling opportunities and trends. **Discover Water**, [*s. l.*], v. 4, n. 1, p. 20, 2024.

DARI, Dayana Nascimento *et al.* An Updated Review of Recent Applications and Perspectives of Hydrogen Production from Biomass by Fermentation: A Comprehensive Analysis. **Biomass**, [*s. l.*], v. 4, n. 1, p. 132–163, 2024.

DE ALMEIDA, Vanessa F. *et al.* Biodiesel production from mixtures of waste fish oil, palm oil and waste frying oil: Optimization of fuel properties. **Fuel Processing Technology**, [*s. l.*], v. 133, p. 152–160, 2015.

DE CASTRO BIZERRA, Viviane *et al.* Exploring the synergy of ionic liquids and lipase in sustainable enzymatic engineering. **Journal of Molecular Liquids**, [*s. l.*], v. 399, p. 124373, 2024a.

DE CASTRO BIZERRA, Viviane *et al.* Opportunities for cleaner leather processing based on protease enzyme: Current evidence from an advanced bibliometric analysis. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 191, p. 114162, 2024b.

DE JESUS, Sérgio S.; FILHO, Rubens Maciel. Recent advances in lipid extraction using green solvents. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 133, p. 110289, 2020.

DE SOUSA, Isamayra Germano *et al.* Renewable processes of synthesis of biolubricants catalyzed by lipases. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 11, n. 1, p. 109006, 2023.

DE SOUSA JUNIOR, Paulo Gonçalves *et al.* Optimization and theoretical analysis of lipase-catalyzed enzymatic esterification of glycerol for efficient glycerides synthesis. **Biochemical Engineering Journal**, [*s. l.*], v. 198, p. 109033, 2023.

DELANO, W L. **The PyMOL Molecular Graphics System, Version 2.3**. [*S. l.: s. n.*], 2020.

DEMIRBAS, A.; KARSLIOGLU, S. Biodiesel Production Facilities from Vegetable Oils and Animal Fats. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 29, n. 2, p. 133–141, 2007.

DEY, Suman *et al.* Study on performance-emission trade-off and multi-objective optimization of diesel-ethanol-palm biodiesel in a single cylinder CI engine: a Taguchi-fuzzy approach. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 46, n. 1, p. 8498–8518, 2024.

DIAS, Amanda M.S. *et al.* Are Environmental Impact Assessments effectively addressing the biodiversity issues in Brazil?. **Environmental Impact Assessment Review**, [*s. l.*], v. 95, p. 106801, 2022.

DIAS, Michele do Rocio Gonçalves *et al.* Biocatalytic asymmetric synthesis of secondary allylic alcohols using <scp> *Burkholderia cepacia* </scp> lipase immobilized on multiwalled carbon nanotubes. **Chirality**, [*s. l.*], v. 34, n. 7, p. 1008–1018, 2022.

DIEZ, Mikel *et al.* Insights into mechanism kinematics for protein motion simulation. **BMC Bioinformatics**, [*s. l.*], v. 15, n. 1, 2014.

DING, Li-Na *et al.* Advances in plant GDSL lipases: from sequences to functional mechanisms. **Acta Physiologiae Plantarum**, [*s. l.*], v. 41, n. 9, p. 151, 2019.

DOMINGUES, Otávio *et al.* Evaluation of Candida rugosa Lipase Immobilized on Magnetic Nanoparticles in Enzymatic/Chemical Hydroesterification for Biodiesel Production. **Applied Biochemistry and Biotechnology**, [*s. l.*], v. 194, n. 11, p. 5419–5442, 2022.

DOS SANTOS, Letícia Karen *et al.* Production of biodiesel from crude palm oil by a sequential hydrolysis/esterification process using subcritical water. **Renewable Energy**, [*s. l.*], v. 130, p. 633–640, 2019.

DURHAM, Elizabeth *et al.* Solvent accessible surface area approximations for rapid and accurate protein structure prediction. **Journal of Molecular Modeling**, [*s. l.*], v. 15, n. 9, p. 1093–1108, 2009.

ESAN, Akintomiwa O. *et al.* Synthesis of biodiesel from waste palm fatty acid distillate (PFAD) and dimethyl carbonate (DMC) via Taguchi optimisation method. **Biomass and Bioenergy**, [*s. l.*], v. 154, p. 106262, 2021.

ESMI, Fahimeh; BORUGADDA, Venu Babu; DALAI, Ajay K. Heteropoly acids as supported solid acid catalysts for sustainable biodiesel production using vegetable oils: A review. **Catalysis Today**, [*s. l.*], v. 404, p. 19–34, 2022.

EZEORBA, Timothy Prince Chidike *et al.* Recent advances in biotechnological valorization of agro-food wastes (AFW): Optimizing integrated approaches for sustainable biorefinery and circular bioeconomy. **Bioresource Technology Reports**, [*s. l.*], v. 26, p. 101823, 2024.

FAO. **Food and Agriculture Organization of the United Nations**. [*S. l.*], 2018. Disponível em: https://openknowledge.fao.org/server/api/core/bitstreams/9b83c384-975b-49f7-9710-745d2cc201a0/content. Acesso em: 2 jul. 2024.

FARAGO, Oded. Langevin thermostat for robust configurational and kinetic sampling. **Physica A: Statistical Mechanics and its Applications**, [*s. l.*], v. 534, n. 122210, 2019.

FAROUK, Sabah Mohamed *et al.* Recent advances in transesterification for sustainable biodiesel production, challenges, and prospects: a comprehensive review. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 9, p. 12722–12747, 2024.

FARUQUE, Mohammed O.; RAZZAK, Shaikh A.; HOSSAIN, Mohammad M. Application of Heterogeneous Catalysts for Biodiesel Production from Microalgal Oil—A Review. **Catalysts**, [*s. l.*], v. 10, n. 9, p. 1025, 2020.

FATMA, Tehsin *et al.* Computational assessment of Botrytis cinerea lipase for biofuel production. **Catalysts**, [*s. l.*], v. 11, n. 11, 2021.

FAYYAZI, E. *et al.* An ultrasound-assisted system for the optimization of biodiesel production from chicken fat oil using a genetic algorithm and response surface methodology. **Ultrasonics Sonochemistry**, [*s. l.*], v. 26, p. 312–320, 2015.

FERNANDEZ-LAFUENTE, Roberto. Lipase from Thermomyces lanuginosus: Uses and prospects as an industrial biocatalyst. **Journal of Molecular Catalysis B: Enzymatic**, [*s. l.*], v. 62, n. 3–4, p. 197–212, 2010.

FERREIRA MOTA, Gabrielly *et al.* Biodiesel production from microalgae using lipase-based catalysts: Current challenges and prospects. **Algal Research**, [*s. l.*], v. 62, p. 102616, 2022.

FILHO, Danielle Gonçalves; SILVA, Amanda Gonçalves; GUIDINI, Carla Zanella. Lipases: sources, immobilization methods, and industrial applications. **Applied Microbiology and Biotechnology**, [*s. l.*], v. 103, n. 18, p. 7399–7423, 2019.

FILIPE, Hugo A. L.; LOURA, Luís M. S. Molecular Dynamics Simulations: Advances and Applications. **Molecules**, [*s. l.*], v. 27, n. 7, p. 2105, 2022.

FONSECA, Aluísio Marques da *et al.* Study of the Inhibition Potential of Remdesivir Derivatives on Mpro of SARS-COV-2. **International Journal of Research - GRANTHAALAYAH**, [*s. l.*], v. 8, n. 11, p. 164–174, 2020.

FREIJE GARCÍA, Fabricio; GARCÍA LIÑARES, Guadalupe. Use of Lipases as a Sustainable and Efficient Method for the Synthesis and Degradation of Polymers. **Journal of Polymers and the Environment**, [*s. l.*], v. 32, n. 5, p. 2484–2516, 2024.

FUJIKOSHI, Yasunori. Two-way ANOVA models with unbalanced data. **Discrete Mathematics**, [*s. l.*], v. 116, n. 1–3, p. 315–334, 1993.

GAMA CAVALCANTE, Antônio Luthierre *et al.* Advancements in enzyme immobilization on magnetic nanomaterials: toward sustainable industrial applications. **RSC Advances**, [*s. l.*], v. 14, n. 25, p. 17946–17988, 2024.

GARCIA-SILVERA, E. E. *et al.* Enzymatic Biodiesel Production: Challenges and Opportunities. *In*: ESPINOZA-ANDALUZ, Mayken *et al.* (org.). **Congress On Research, Development and Innovation in Renewable Energies**. [*S. l.*]: Springer, Cham, 2023. p. 19–37. Disponível em: https://link.springer.com/chapter/10.1007/978-3-031-26813-7_2. Acesso em: 26 abr. 2025.

GARG, Renuka; SABOUNI, Rana; AHMADIPOUR, Mohsen. From waste to fuel: Challenging aspects in sustainable biodiesel production from lignocellulosic biomass feedstocks and role of metal organic framework as innovative heterogeneous catalysts. **Industrial Crops and Products**, [*s. l.*], v. 206, p. 117554, 2023.

GHAREHGHANI, Ayatallah; MIRSALIM, Mostafa; HOSSEINI, Reza. Effects of waste fish oil biodiesel on diesel engine combustion characteristics and emission. **Renewable Energy**, [*s. l.*], v. 101, p. 930–936, 2017.

GHESTI, Grace F. *et al.* Towards a sustainable waste-to-energy pathway to pequi biomass residues: Biochar, syngas, and biodiesel analysis. **Waste Management**, [*s. l.*], v. 143, p. 144–156, 2022.

GONÇALVES, Tiago dos S. *et al.* Challenges for energy guidelines in crop-based liquid biofuels development in Brazil. **Next Sustainability**, [*s. l.*], v. 2, p. 100002, 2023.

GRANJO, José F.O.; DUARTE, Belmiro P.D.; OLIVEIRA, Nuno M.C. Kinetic Models for the Homogeneous Alkaline and Acid Catalysis in Biodiesel Production. **Computer Aided Chemical Engineering**, [*s. l.*], v. 27, n. C, p. 483–488, 2009. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1570794609703013. Acesso em: 26 abr. 2025.

GUEDES JÚNIOR, José G.E. *et al.* Design of a sustainable process for enzymatic production of ethylene glycol diesters via hydroesterification of used soybean cooking oil. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 10, n. 1, p. 107062, 2022.

GUIMARÃES, José Renato *et al.* Immobilization of Eversa® Transform via CLEA Technology Converts It in a Suitable Biocatalyst for Biolubricant Production Using Waste Cooking Oil. **Molecules**, [*s. l.*], v. 26, n. 1, p. 193, 2021.

GUO, Yingying *et al.* Recent developments in solid acid catalysts for biodiesel production. **Molecular Catalysis**, [*s. l.*], v. 547, p. 113362, 2023.

GUPTA, Vartika; PAL SINGH, Kishan. The impact of heterogeneous catalyst on biodiesel production; a review. **Materials Today: Proceedings**, [*s. l.*], v. 78, p. 364–371, 2023.

HAJJARI, Masoumeh *et al.* A review on the prospects of sustainable biodiesel production: A global scenario with an emphasis on waste-oil biodiesel utilization. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 72, p. 445–464, 2017.

HAMZA, Muhammad *et al.* A review on the waste biomass derived catalysts for biodiesel production. **Environmental Technology & Innovation**, [*s. l.*], v. 21, p. 101200, 2021.

HANWELL, Marcus D *et al.* Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. **Journal of Cheminformatics**, [*s. l.*], v. 4, n. 1, p. 17, 2012.

HASNAOUI, Ismail *et al.* Preparation and Biochemical Characterization of Penicillium crustosum Thom P22 Lipase Immobilization Using Adsorption, Encapsulation, and Adsorption–Encapsulation Approaches. **Molecules**, [*s. l.*], v. 30, n. 3, p. 434, 2025.

HASSAN, Qusay *et al.* The renewable energy role in the global energy Transformations. **Renewable Energy Focus**, [*s. l.*], v. 48, p. 100545, 2024.

HOLECHEK, Jerry L. *et al.* A Global Assessment: Can Renewable Energy Replace Fossil Fuels by 2050?. **Sustainability**, [*s. l.*], v. 14, n. 8, p. 4792, 2022.

HOSSEINZADEH-BANDBAFHA, Homa *et al.* Managing the hazardous waste cooking oil by conversion into bioenergy through the application of waste-derived green catalysts: A

review. **Journal of Hazardous Materials**, [*s. l.*], v. 424, p. 127636, 2022.

HUANG, Zhaolin *et al.* Application of molecular dynamics simulation in the field of food enzymes: improving the thermal-stability and catalytic ability. **Critical Reviews in Food Science and Nutrition**, [*s. l.*], v. 64, n. 31, p. 11396–11408, 2024.

HUMPHREY, William; DALKE, Andrew; SCHULTEN, Klaus. VMD: Visual molecular dynamics. **Journal of Molecular Graphics**, [*s. l.*], v. 14, n. 1, p. 33–38, 1996.

ISHAK, Siti nor Hasmah *et al.* Expanding the horizon of biodiesel production via enzyme engineering. **International Journal of Green Energy**, [*s. l.*], v. 21, n. 14, p. 3367–3390, 2024.

ISLAM RONY, Zahidul *et al.* Alternative fuels to reduce greenhouse gas emissions from marine transport and promote UN sustainable development goals. **Fuel**, [*s. l.*], v. 338, p. 127220, 2023.

ISMAEEL, Hiba Kh. *et al.* Strategies for biodiesel production with the role of reactor technologies: A comprehensive review. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 200, p. 109767, 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**, [*s. l.*], v. 163, p. 1624–1639, 2020.

ISMAIL, Abdallah R.; KASHTOH, Hamdy; BAEK, Kwang-Hyun. Temperature-resistant and solvent-tolerant lipases as industrial biocatalysts: Biotechnological approaches and applications. **International Journal of Biological Macromolecules**, [*s. l.*], v. 187, p. 127–142, 2021.

ISSARIYAKUL, Titipong; DALAI, Ajay K. Biodiesel from vegetable oils. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 31, p. 446–471, 2014.

JAEGER, K; EGGERT, T. Enantioselective biocatalysis optimized by directed evolution. **Current Opinion in Biotechnology**, [*s. l.*], v. 15, n. 4, p. 305–313, 2004.

JAIN, Akshay *et al.* Theoretical potential estimation and multi-objective optimization of Water Hyacinth (Eichhornia Crassipes) biodiesel powered diesel engine at variable injection timings. **Renewable Energy**, [*s. l.*], v. 206, p. 514–530, 2023.

JAISWAL, Krishna Kumar *et al.* Valorization of fish processing industry waste for biodiesel production: Opportunities, challenges, and technological perspectives. **Renewable Energy**, [*s. l.*], v. 220, p. 119601, 2024.

JAYABAL, Ravikumar. Advancements in catalysts, process intensification, and feedstock utilization for sustainable biodiesel production. **Results in Engineering**, [*s. l.*], v. 24, p. 103668, 2024.

JEGANNATHAN, Kenthorai Raman; NIELSEN, Per Henning. Environmental assessment of enzyme use in industrial production – a literature review. **Journal of Cleaner Production**, [*s.*

l.], v. 42, p. 228–240, 2013.

JIN, Zihan; WEI, Zihao. Molecular simulation for food protein–ligand interactions: A comprehensive review on principles, current applications, and emerging trends. **Comprehensive Reviews in Food Science and Food Safety**, [*s. l.*], v. 23, n. 1, 2024.

K., Agnieszka. Thermodynamics of Ligand-Protein Interactions: Implications for Molecular Design. *In*: THERMODYNAMICS - INTERACTION STUDIES - SOLIDS, LIQUIDS AND GASES. [*S. l.: s. n.*], 2011.

KAIRYS, Visvaldas *et al.* Recent advances in computational and experimental protein-ligand affinity determination techniques. **Expert Opinion on Drug Discovery**, [*s. l.*], v. 19, n. 6, p. 649–670, 2024.

KALITA, Pinaki *et al.* Biodiesel as renewable biofuel produced via enzyme-based catalyzed transesterification. **Energy Nexus**, [*s. l.*], v. 6, p. 100087, 2022.

KAMAL PASHA, Mustafa *et al.* Comparative study of a two-step enzymatic process and conventional chemical methods for biodiesel production: Economic and environmental perspectives. **Chemical Engineering Journal**, [*s. l.*], v. 489, p. 151254, 2024.

KANT BHATIA, Shashi *et al.* An overview on advancements in biobased transesterification methods for biodiesel production: Oil resources, extraction, biocatalysts, and process intensification technologies. **Fuel**, [*s. l.*], v. 285, p. 119117, 2021.

KARIA, Mahejbin *et al.* Marine-Derived Lipases for Enhancing Enrichment of Very-Long-Chain Polyunsaturated Fatty Acids with Reference to Omega-3 Fatty Acids. **Marine Drugs**, [*s. l.*], v. 22, n. 7, p. 301, 2024.

KARIMI, Kimia *et al.* Biodiesel production from <scp> *Nannochloropsis* </scp> microalgal biomass-derived oil: An experimental and theoretical study using <scp>the RSM-CCD</scp> approach. **The Canadian Journal of Chemical Engineering**, [*s. l.*], v. 101, n. 10, p. 5600–5610, 2023.

KARMAKAR, Bisheswar; DHAWANE, Sumit H.; HALDER, Gopinath. Optimization of biodiesel production from castor oil by Taguchi design. **Journal of Environmental Chemical Engineering**, [*s. l.*], v. 6, n. 2, p. 2684–2695, 2018.

KARMAKAR, Bisheswar; HALDER, Gopinath. Progress and future of biodiesel synthesis: Advancements in oil extraction and conversion technologies. **Energy Conversion and Management**, [*s. l.*], v. 182, p. 307–339, 2019.

KATO, Koichi *et al.* Molecular dynamics simulations for the protein–ligand complex structures obtained by computational docking studies using implicit or explicit solvents. **Chemical Physics Letters**, [*s. l.*], v. 781, n. 139022, 2021.

KATSIMPOURAS, Constantinos; STEPHANOPOULOS, Gregory. Enzymes in biotechnology: Critical platform technologies for bioprocess development. **Current Opinion in Biotechnology**, [*s. l.*], v. 69, p. 91–102, 2021.

KAYA, Cenk; KÖKKÜLÜNK, Görkem. Biodiesel as alternative additive fuel for diesel engines: An experimental and theoretical investigation on emissions and performance characteristics. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, [*s. l.*], v. 45, n. 4, p. 10741–10763, 2023.

KETZER, Felipe *et al.* Kinetic and thermodynamic study of enzymatic hydroesterification mechanism to fatty acid methyl esters synthesis. **Bioresource Technology**, [*s. l.*], v. 356, p. 127335, 2022.

KHAN, Zarmeena *et al.* Current developments in esterification reaction: A review on process and parameters. **Journal of Industrial and Engineering Chemistry**, [*s. l.*], v. 103, p. 80–101, 2021.

KOCH, Elisabeth; KAMPSCHULTE, Nadja; SCHEBB, Nils Helge. Comprehensive Analysis of Fatty Acid and Oxylipin Patterns in n3-PUFA Supplements. **Journal of Agricultural and Food Chemistry**, [*s. l.*], v. 70, n. 13, p. 3979–3988, 2022.

KOROLEVA, Victoria *et al.* Thermal Inactivation, Denaturation and Aggregation Processes of Papain-Like Proteases. **Chemistry & Biodiversity**, [*s. l.*], v. 21, n. 8, 2024.

KOSURU, Sai Mani Yogesh *et al.* A review on the biodiesel production: Selection of catalyst, Pre-treatment, Post treatment methods. **Green Technologies and Sustainability**, [*s. l.*], v. 2, n. 1, p. 100061, 2024.

KRISHNA, Naveen Banchallihundi *et al.* Computational studies on the catalytic potential of the double active site for enzyme engineering. **Scientific Reports**, [*s. l.*], v. 14, n. 1, p. 17892, 2024.

KUSHWAHA, Tribhuvan *et al.* Esterification of oleic acid to biodiesel using biowaste-based solid acid catalyst under microwave irradiation. **Environmental Progress & Sustainable Energy**, [*s. l.*], v. 42, n. 6, 2023.

LI, Ning *et al.* A review on mixture design methods for geopolymer concrete. **Composites Part B: Engineering**, [*s. l.*], v. 178, p. 107490, 2019.

LI, Chenlin *et al.* Impact of feedstock quality and variation on biochemical and thermochemical conversion. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 65, p. 525–536, 2016.

LIANG, Qinlang *et al.* Impact of Feeding Frequency on Growth Performance and Antioxidant Capacity of Litopenaeus vannamei in Recirculating Aquaculture Systems. **Animals**, [*s. l.*], v. 15, n. 2, p. 192, 2025.

LIMA, Anderson H. *et al.* Molecular Modeling of T. rangeli, T. brucei gambiense, and T. evansi Sialidases in Complex with the DANA Inhibitor. **Chemical Biology and Drug Design**, [*s. l.*], v. 80, n. 1, p. 114–120, 2012.

LIN, Cherng-Yuan; LI, Rong-Ji. Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish. **Fuel Processing Technology**, [*s. l.*], v. 90, n. 1, p. 130–136, 2009.

LIN, Chih Kai; ZHANG, Jinming. Detecting Nonadditivity in Single-Facet Generalizability Theory Applications: Tukey's Test. **Journal of Educational Measurement**, [*s. l.*], v. 55, n. 1, p. 78–89, 2018.

LINDER, Michel; FANNI, Jacques; PARMENTIER, Michel. Proteolytic Extraction of Salmon Oil and PUFA Concentration by Lipases. **Marine Biotechnology**, [*s. l.*], v. 7, n. 1, p. 70–76, 2005.

LÓPEZ, J.Mendieta *et al.* A theoretical and experimental study of liquid-liquid equilibrium to refine raw glycerol obtained as a byproduct on the biodiesel production. **Chemical Engineering Journal Advances**, [*s. l.*], v. 10, p. 100257, 2022.

LÓPEZ-YERENA, Anallely *et al.* Waste from Persea schiedeana Fruits as Potential Alternative for Biodiesel Production. **Plants**, [*s. l.*], v. 11, n. 3, p. 252, 2022.

LU, Jiaxin *et al.* Microbial Enzymes for the Biodegradation of Polylactic Acid (PLA): Molecular Docking Simulation and Biodegradation Pathway Proposal. **Environmental Reviews**, [*s. l.*], 2024.

MA, Yingqun *et al.* Biodiesels from microbial oils: Opportunity and challenges. **Bioresource Technology**, [*s. l.*], v. 263, p. 631–641, 2018.

MA, Xinxiu *et al.* Dynamics of Bacterial Composition and Association with Quality Formation and Biogenic Amines Accumulation during Fish Sauce Spontaneous Fermentation. **Applied and Environmental Microbiology**, [*s. l.*], v. 88, n. 13, 2022.

MAHAPATRA, Sangita *et al.* Biofuels and their sources of production: A review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. **Energy Nexus**, [*s. l.*], v. 4, p. 100036, 2021.

MAHDAVI, Mohmmad; ABEDINI, Ebrahim; DARABI, Amir hosein. Biodiesel synthesis from oleic acid by nano-catalyst (ZrO_2/Al_2O_3) under high voltage conditions. **RSC Advances**, [*s. l.*], v. 5, n. 68, p. 55027–55032, 2015.

MAHDI, Hilman Ibnu *et al.* A comprehensive review on nanocatalysts and nanobiocatalysts for biodiesel production in Indonesia, Malaysia, Brazil and USA. **Chemosphere**, [*s. l.*], v. 319, p. 138003, 2023.

MAHESHWARI, Pranjal *et al.* A review on latest trends in cleaner biodiesel production: Role of feedstock, production methods, and catalysts. **Journal of Cleaner Production**, [*s. l.*], v. 355, p. 131588, 2022.

MAKSIMENKO, Anastasiia *et al.* Exploring Sustainable Aquafeed Alternatives with a Specific Focus on the Ensilaging Technology of Fish Waste. **Fermentation**, [*s. l.*], v. 10, n. 5, p. 258, 2024.

MALEKI, B.; ASHRAF TALESH, S.S.; MANSOURI, M. Comparison of catalysts types performance in the generation of sustainable biodiesel via transesterification of various oil sources: a review study. **Materials Today Sustainability**, [*s. l.*], v. 18, p. 100157, 2022.

MANDARI, Venkatesh; DEVARAI, Santhosh Kumar. Biodiesel Production Using Homogeneous, Heterogeneous, and Enzyme Catalysts via Transesterification and Esterification Reactions: a Critical Review. **BioEnergy Research**, [*s. l.*], v. 15, n. 2, p. 935–961, 2022.

MANUBOLU, Manjunath *et al.* Enzymes as direct decontaminating agents—mycotoxins. *In*: ENZYMES IN HUMAN AND ANIMAL NUTRITION. [*S. l.*]: Elsevier, 2018. p. 313–330.

MAROA, Semakula; INAMBAO, Freddie. A review of sustainable biodiesel production using biomass derived heterogeneous catalysts. **Engineering in Life Sciences**, [*s. l.*], v. 21, n. 12, p. 790–824, 2021.

MARTINS, Gislaine Iastiaque *et al.* Physical and chemical properties of fish oil biodiesel produced in Brazil. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 42, p. 154–157, 2015.

MARTI-QUIJAL, Francisco J *et al.* Fermentation in fish and by-products processing: an overview of current research and future prospects. **Current Opinion in Food Science**, [*s. l.*], v. 31, p. 9–16, 2020.

MAS, Rios. Production, Characterization, and Exergy Analysis of the Soybean Biodiesel Produced by Laboratory Scale. **Petroleum & Petrochemical Engineering Journal**, [*s. l.*], v. 7, n. 1, p. 1–9, 2023.

MAZOLA, Yuliet *et al.* A comparative molecular dynamics study of thermophilic and mesophilic β-fructosidase enzymes. **Journal of Molecular Modeling**, [*s. l.*], v. 21, n. 228, p. 1–11, 2015.

MBATIA, Betty *et al.* Enzymatic oil extraction and positional analysis of ω-3 fatty acids in Nile perch and salmon heads. **Process Biochemistry**, [*s. l.*], v. 45, n. 5, p. 815–819, 2010.

MELO, Rafael Leandro Fernandes *et al.* A comprehensive review on enzyme-based biosensors: Advanced analysis and emerging applications in nanomaterial-enzyme linkage. **International Journal of Biological Macromolecules**, [*s. l.*], v. 264, p. 130817, 2024a.

MELO, Rafael Leandro Fernandes *et al.* Enhancing biocatalyst performance through immobilization of lipase (Eversa® Transform 2.0) on hybrid amine-epoxy core-shell magnetic nanoparticles. **International Journal of Biological Macromolecules**, [*s. l.*], v. 264, p. 130730, 2024b.

MESBAH, Noha M. Industrial Biotechnology Based on Enzymes From Extreme Environments. **Frontiers in Bioengineering and Biotechnology**, [*s. l.*], v. 10, 2022.

MISHRA, Shashank; BUKKARAPU, Kiran Raj; KRISHNASAMY, Anand. A composition based approach to predict density, viscosity and surface tension of biodiesel fuels. **Fuel**, [*s. l.*], v. 285, p. 119056, 2021.

MIYURANGA, K. A. Viraj *et al.* Biodiesel Production through the Transesterification of Waste Cooking Oil over Typical Heterogeneous Base or Acid Catalysts. **Catalysts**, [*s. l.*], v.

13, n. 3, p. 546, 2023.

MIZIK, Tamás; GYARMATI, Gábor. Economic and Sustainability of Biodiesel Production—A Systematic Literature Review. **Clean Technologies**, [*s. l.*], v. 3, n. 1, p. 19–36, 2021.

MO, Wing Yin; MAN, Yu Bon; WONG, Ming Hung. Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. **Science of The Total Environment**, [*s. l.*], v. 613–614, p. 635–643, 2018.

MODENUTTI, Carlos P. *et al.* The Structural Biology of Galectin-Ligand Recognition: Current Advances in Modeling Tools, Protein Engineering, and Inhibitor Design. **Frontiers in Chemistry**, [*s. l.*], v. 7, 2019.

MOFIJUR, M. *et al.* Effect of nanocatalysts on the transesterification reaction of first, second and third generation biodiesel sources- A mini-review. **Chemosphere**, [*s. l.*], v. 270, p. 128642, 2021.

MOFIJUR, M. *et al.* Recent Development in the Production of Third Generation Biodiesel from Microalgae. **Energy Procedia**, [*s. l.*], v. 156, p. 53–58, 2019.

MONIKA; BANGA, Sangita; PATHAK, Vinayak V. Biodiesel production from waste cooking oil: A comprehensive review on the application of heterogenous catalysts. **Energy Nexus**, [*s. l.*], v. 10, p. 100209, 2023.

MONTEIRO, Rodolpho R.C. *et al.* Improvement of enzymatic activity and stability of lipase A from Candida antartica onto halloysite nanotubes with Taguchi method for optimized immobilization. **Applied Clay Science**, [*s. l.*], v. 228, p. 106634, 2022.

MONTEIRO, Rodolpho R.C. *et al.* Liquid lipase preparations designed for industrial production of biodiesel. Is it really an optimal solution?. **Renewable Energy**, [*s. l.*], v. 164, p. 1566–1587, 2021.

MOREIRA, Katerine S. *et al.* Optimization of the Production of Enzymatic Biodiesel from Residual Babassu Oil (Orbignya sp.) via RSM. **Catalysts**, [*s. l.*], v. 10, n. 4, p. 414, 2020.

MORRIS, Garrett M. *et al.* Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. **Journal of Computational Chemistry**, [*s. l.*], v. 30, n. 16, p. 2785–2791, 2009.

MORRIS, Connor J.; CORTE, Dennis Della. Using molecular docking and molecular dynamics to investigate protein-ligand interactions. **Modern Physics Letters B**, [*s. l.*], v. 35, n. 08, p. 2130002, 2021.

MOUAD, Ana Maria *et al.* Aminolysis of linoleic and salicylic acid derivatives with Candida antarctica lipase B: A solvent-free process to obtain amphiphilic amides for cosmetic application. **Journal of Molecular Catalysis B: Enzymatic**, [*s. l.*], v. 126, p. 64–68, 2016.

MUKHTAR, Ahmad *et al.* Current status and challenges in the heterogeneous catalysis for biodiesel production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 157, p. 112012, 2022.

MUSA, Idris Atadashi. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. **Egyptian Journal of Petroleum**, [*s. l.*], v. 25, n. 1, p. 21–31, 2016.

NAM, Kwangho *et al.* Perspectives on Computational Enzyme Modeling: From Mechanisms to Design and Drug Development. **ACS Omega**, [*s. l.*], 2024.

NAMBIRAJ, M.; SURESH KUMAR, K. Impact of essential oils from agro waste as antioxidants on biodiesel long-term storage stability for CI engines: Fresh and aged evaluations. **Process Safety and Environmental Protection**, [*s. l.*], v. 186, p. 1214–1228, 2024.

NAQVI, Ahmad Abu Turab *et al.* Advancements in Docking and Molecular Dynamics Simulations Towards Ligand-receptor Interactions and Structure-function Relationships. **Current Topics in Medicinal Chemistry**, [*s. l.*], v. 18, n. 20, p. 1755–1768, 2018.

NAYAB, Rida *et al.* Sustainable biodiesel production via catalytic and non-catalytic transesterification of feedstock materials – A review. **Fuel**, [*s. l.*], v. 328, p. 125254, 2022.

NEAG, Emilia *et al.* Optimization of Lipid Extraction from Spirulina spp. by Ultrasound Application and Mechanical Stirring Using the Taguchi Method of Experimental Design. **Molecules**, [*s. l.*], v. 27, n. 20, p. 6794, 2022.

NEUPANE, Dhurba. Biofuels from Renewable Sources, a Potential Option for Biodiesel Production. **Bioengineering**, [*s. l.*], v. 10, n. 1, p. 29, 2022.

NOBRE, Millena Mara Rabelo *et al.* Ester Production Using the Lipid Composition of Coffee Ground Oil (Coffea arabica): A Theoretical Study of Eversa® Transform 2.0 Lipase as an Enzymatic Biocatalyst. **Compounds 2023, Vol. 3, Pages 411-429**, [*s. l.*], v. 3, n. 3, p. 411–429, 2023.

O'BOYLE, Noel M. *et al.* Open Babel: An Open chemical toolbox. **Journal of Cheminformatics**, [*s. l.*], v. 3, n. 10, p. 1–14, 2011.

OKECHUKWU, Onukwuli Dominic *et al.* Improving heterogeneous catalysis for biodiesel production process. **Cleaner Chemical Engineering**, [*s. l.*], v. 3, p. 100038, 2022.

OREGE, Joshua Iseoluwa *et al.* Recent advances in heterogeneous catalysis for green biodiesel production by transesterification. **Energy Conversion and Management**, [*s. l.*], v. 258, p. 115406, 2022.

ORMOND, Nathan *et al.* Production of Sustainable Liquid Fuels. **Energies**, [*s. l.*], v. 17, n. 14, p. 3506, 2024.

ORTEGA-REQUENA, Salvadora *et al.* Esters in the Food and Cosmetic Industries: An Overview of the Reactors Used in Their Biocatalytic Synthesis. **Materials**, [*s. l.*], v. 17, n. 1, p. 268, 2024.

OYEKUNLE, Daniel T. *et al.* Heterogeneous catalytic transesterification for biodiesel

production: Feedstock properties, catalysts and process parameters. **Process Safety and Environmental Protection**, [*s. l.*], v. 177, p. 844–867, 2023.

P. RAMOS, Luiz *et al.* Biodiesel: Raw Materials, Production Technologies and Fuel Properties. **Revista Virtual de Química**, [*s. l.*], v. 9, n. 1, p. 317–369, 2017.

PARIDA, Soumya *et al.* Production of biodiesel from waste fish fat through ultrasound-assisted transesterification using petro-diesel as cosolvent and optimization of process parameters using response surface methodology. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 17, p. 25524–25537, 2024.

PASHA, Mustafa Kamal *et al.* Biodiesel production with enzymatic technology: progress and perspectives. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 15, n. 5, p. 1526–1548, 2021.

PAUL, Pandeeti Emmanuel Vijay; SANGEETHA, Veeraiah; DEEPIKA, Routhu Gyana. Emerging Trends in the Industrial Production of Chemical Products by Microorganisms. *In*: BUDDOLLA, Viswanath (org.). **Recent Developments in Applied Microbiology and Biochemistry**. 1. ed. [*S. l.*]: Elsevier, 2019. v. 1, p. 107–125.

PEER, Mohamed Shameer *et al.* Experimental evaluation on oxidation stability of biodiesel/diesel blends with alcohol addition by rancimat instrument and FTIR spectroscopy. **Journal of Mechanical Science and Technology**, [*s. l.*], v. 31, n. 1, p. 455–463, 2017.

PERUMAL, Govindhan *et al.* A systematic review on the production of biodiesel using various feedstocks and effective catalysts. **Petroleum Science and Technology**, [*s. l.*], p. 1–17, 2024.

PETROVA, Svetlana S.; SOLOV'EV, Alexander D. The Origin of the Method of Steepest Descent. **Historia Mathematica**, [*s. l.*], v. 24, n. 4, p. 361–375, 1997.

PHILLIPS, James C. *et al.* Scalable molecular dynamics with NAMD. **Journal of Computational Chemistry**, [*s. l.*], v. 26, n. 16, p. 1781–1802, 2005.

POPPE, Jakeline Kathiele *et al.* Transesterification of Waste Frying Oil and Soybean Oil by Combi-lipases Under Ultrasound-Assisted Reactions. **Applied Biochemistry and Biotechnology**, [*s. l.*], v. 186, n. 3, p. 576–589, 2018.

POURZOLFAGHAR, Hamed *et al.* A review of the enzymatic hydroesterification process for biodiesel production. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 61, p. 245–257, 2016.

PRETO, Fernando; ZHANG, Frank; WANG, Jinsheng. A study on using fish oil as an alternative fuel for conventional combustors. **Fuel**, [*s. l.*], v. 87, n. 10–11, p. 2258–2268, 2008.

PUDTIKAJORN, Khamtorn; BENJAKUL, Soottawat. Simple Wet Rendering Method for Extraction of Prime Quality Oil from Skipjack Tuna Eyeballs. **European Journal of Lipid Science and Technology**, [*s. l.*], v. 122, n. 8, 2020.

PULLEN, James; SAEED, Khizer. An overview of biodiesel oxidation stability. **Renewable**

and Sustainable Energy Reviews, [*s. l.*], v. 16, n. 8, p. 5924–5950, 2012.

QIAN, Jingya *et al.* Ultrasound-Assisted Enzymatic Protein Hydrolysis in Food Processing: Mechanism and Parameters. **Foods**, [*s. l.*], v. 12, n. 21, p. 4027, 2023.

QIN, Xiaoli; ZHONG, Jinfeng; WANG, Yonghua. A mutant T1 lipase homology modeling, and its molecular docking and molecular dynamics simulation with fatty acids. **Journal of Biotechnology**, [*s. l.*], v. 337, n. February, p. 24–34, 2021.

QU, Pingping *et al.* Improved thermostability of Thermomyces lanuginosus lipase by molecular dynamics simulation and in silico mutation prediction and its application in biodiesel production. **Fuel**, [*s. l.*], v. 327, 2022.

QUISPE, César A.G.; CORONADO, Christian J.R.; CARVALHO JR., João A. Glycerol: Production, consumption, prices, characterization and new trends in combustion. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 27, p. 475–493, 2013.

RAGUNATHAN, Adhithya; MALATHI, Kullappan; ANBARASU, Anand. MurB as a target in an alternative approach to tackle the Vibrio cholerae resistance using molecular docking and simulation study. **Journal of Cellular Biochemistry**, [*s. l.*], v. 119, n. 2, p. 1726–1732, 2018.

RAI, Amit Kumar *et al.* Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. **Enzyme and Microbial Technology**, [*s. l.*], v. 46, n. 1, p. 9–13, 2010.

RAMBABU, K. *et al.* Enhancing biodiesel production from waste date seed oil through ultrasonic-assisted optimization: A sustainable approach using non-edible feedstocks. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 194, p. 109601, 2023.

RAMÍREZ-VERDUZCO, Luis Felipe; HERNÁNDEZ-SÁNCHEZ, Mirna Jimena. Group contribution method for predicting viscosity of alkyl esters and biodiesel. **Fuel**, [*s. l.*], v. 357, p. 129666, 2024.

RAMOS, Marta *et al.* Biodiesel Production Processes and Sustainable Raw Materials. **Energies**, [*s. l.*], v. 12, n. 23, p. 4408, 2019.

RATHORE, Dheeraj *et al.* Bioengineering to Accelerate Biodiesel Production for a Sustainable Biorefinery. **Bioengineering**, [*s. l.*], v. 9, n. 11, p. 618, 2022.

RIAZ, Iqra *et al.* A review on catalysts of biodiesel (methyl esters) production. **Catalysis Reviews**, [*s. l.*], v. 66, n. 4, p. 1084–1136, 2024.

RIGOGLIUSO, Salvatrice *et al.* Recovery of Bioactive Compounds from Marine Organisms: Focus on the Future Perspectives for Pharmacological, Biomedical and Regenerative Medicine Applications of Marine Collagen. **Molecules**, [*s. l.*], v. 28, n. 3, p. 1152, 2023.

RIZWANUL FATTAH, I. M. *et al.* State of the Art of Catalysts for Biodiesel Production. **Frontiers in Energy Research**, [*s. l.*], v. 8, 2020.

ROBALO, Silvino Sasso *et al.* Review: Enzymatic hydrolysates of fish by-products: technological advantages and bioactive properties. **Ciência Rural**, [*s. l.*], v. 54, n. 8, 2024.

ROE, Daniel R.; BROOKS, Bernard R. A protocol for preparing explicitly solvated systems for stable molecular dynamics simulations. **Journal of Chemical Physics**, [*s. l.*], v. 153, n. 5, p. 1–9, 2020.

ROY, Dalim Kumer; ABEDIN, Mohammad Zoynal. Potentiality of biodiesel and bioethanol production from feedstock in Bangladesh: A review. **Heliyon**, [*s. l.*], v. 8, n. 11, p. e11213, 2022.

RYU, Young-Jin *et al.* Development of Carbon-Based Solid Acid Catalysts Using a Lipid-Extracted Alga, Dunaliella tertiolecta, for Esterification. **Journal of Microbiology and Biotechnology**, [*s. l.*], v. 28, n. 5, p. 732–738, 2018.

SADANA, Ajit. Enzyme deactivation. **Biotechnology Advances**, [*s. l.*], v. 6, n. 3, p. 349-IN2, 1988.

SAINI, Ramesh Kumar *et al.* Advances in Lipid Extraction Methods—A Review. **International Journal of Molecular Sciences**, [*s. l.*], v. 22, n. 24, p. 13643, 2021.

SALAHELDEEN, Mohammed *et al.* Current State and Perspectives on Transesterification of Triglycerides for Biodiesel Production. **Catalysts**, [*s. l.*], v. 11, n. 9, p. 1121, 2021.

SALES, Misael B. *et al.* Sustainable Feedstocks and Challenges in Biodiesel Production: An Advanced Bibliometric Analysis. **Bioengineering**, [*s. l.*], v. 9, n. 10, p. 539, 2022.

SÁNCHEZ-SOLÍS, Alejandra *et al.* Biodiesel Production Processes with Yeast: A Sustainable Approach. **Energies**, [*s. l.*], v. 17, n. 2, p. 302, 2024.

ŠÁNEK, Lubomír *et al.* Pilot-scale production of biodiesel from waste fats and oils using tetramethylammonium hydroxide. **Waste Management**, [*s. l.*], v. 48, p. 630–637, 2016.

SANTOS, Antonio Lucas R. *et al.* Study of molecular arrangement and density estimation of soybean oil biodiesel-diesel blends employing molecular dynamic simulation. **Fuel**, [*s. l.*], v. 377, p. 132760, 2024.

SARVE, Antaram N.; VARMA, Mahesh N.; SONAWANE, Shriram S. Ultrasound assisted two-stage biodiesel synthesis from non-edible Schleichera triguga oil using heterogeneous catalyst: Kinetics and thermodynamic analysis. **Ultrasonics Sonochemistry**, [*s. l.*], v. 29, p. 288–298, 2016.

SEMWAL, Surbhi *et al.* Biodiesel production using heterogeneous catalysts. **Bioresource Technology**, [*s. l.*], v. 102, n. 3, p. 2151–2161, 2011.

SHAKILANISHI, S.; SHANTHI, C. An overview on preparation of enzymes for industrial use. **Biocatalysis and Biotransformation**, [*s. l.*], v. 42, n. 4, p. 485–496, 2024.

SHAMEER, P. Mohamed; RAMESH, K. Green technology and performance consequences of an eco-friendly substance on a 4-stroke diesel engine at standard injection timing and

compression ratio. **Journal of Mechanical Science and Technology**, [*s. l.*], v. 31, n. 3, p. 1497–1507, 2017.

SHARMA, Binod; SHRESTHA, Ashish. Petroleum dependence in developing countries with an emphasis on Nepal and potential keys. **Energy Strategy Reviews**, [*s. l.*], v. 45, p. 101053, 2023.

SHU, Liwen *et al.* Transesterification of phosphatidylcholine with DHA-rich algal oil using immobilized Candida antarctica lipase B to produce DHA-phosphatidylcholine. **Enzyme and Microbial Technology**, [*s. l.*], v. 169, p. 110266, 2023.

SINGH, Yashvir *et al.* Biodiesel production and exploring properties of Datura stramonium L. oil with its optimization using combined approaches—Taguchi, grey relational analysis, and response surface methodology. **Environmental Science and Pollution Research**, [*s. l.*], v. 31, n. 16, p. 23802–23821, 2024.

SINGH, Narender *et al.* Progress and facts on biodiesel generations, production methods, influencing factors, and reactors: A comprehensive review from 2000 to 2023. **Energy Conversion and Management**, [*s. l.*], v. 302, p. 118157, 2024.

SINGH, Sakshi; BANI BAKER, Qanita; SINGH, Dev Bukhsh. Molecular docking and molecular dynamics simulation. *In*: SINGH, Dev Bukhsh; PATHAK, Rajesh Kumar (org.). **Bioinformatics: Methods and Applications**. 1. ed. [*S. l.*]: Elsevier, 2022. p. 291–304.

SINGH, Bhim; JANA, Asim Kumar. Agri-residues and agro-industrial waste substrates bioconversion by fungal cultures to biocatalyst lipase for green chemistry: A review. **Journal of Environmental Management**, [*s. l.*], v. 348, p. 119219, 2023.

SMAISIM, Ghassan F. *et al.* Synthesis of biodiesel from fish processing waste by nano magnetic catalyst and its thermodynamic analysis. **Case Studies in Thermal Engineering**, [*s. l.*], v. 35, p. 102115, 2022.

SREEHARSHA, Rachapudi Venkata; DUBEY, Namrata; MOHAN, S Venkata. Orienting biodiesel production towards sustainability and circularity by tailoring the feedstock and processes. **Journal of Cleaner Production**, [*s. l.*], v. 414, p. 137526, 2023.

SREEPRASANTH, P.S. *et al.* Hydrophobic, solid acid catalysts for production of biofuels and lubricants. **Applied Catalysis A: General**, [*s. l.*], v. 314, n. 2, p. 148–159, 2006.

STREET, Arthur G.; MAYO, Stephen L. Pairwise calculation of protein solvent-accessible surface areas. **Folding and Design**, [*s. l.*], v. 3, n. 4, p. 253–258, 1998.

SU, Jung-Jeng; CHOU, Yu-Chun. Biodiesel Production by Acid Methanolysis of Slaughterhouse Sludge Cake. **Animals**, [*s. l.*], v. 9, n. 12, p. 1029, 2019.

SUHARA, Ade *et al.* Biodiesel Sustainability: Review of Progress and Challenges of Biodiesel as Sustainable Biofuel. **Clean Technologies**, [*s. l.*], v. 6, n. 3, p. 886–906, 2024.

SUNDARAM, Thanigaivel *et al.* Advancements in biosurfactant production using agro-industrial waste for industrial and environmental applications. **Frontiers in Microbiology**, [*s.*

l.], v. 15, 2024.

SUTHERLAND, Jeffrey J. *et al.* Lessons in molecular recognition. 2. Assessing and improving cross-docking accuracy. **Journal of Chemical Information and Modeling**, [*s. l.*], v. 47, n. 6, p. 2293–2302, 2007.

SUTTON, Richard S.; MAHMOOD, A. Rupam; WHITE, Martha. An emphatic approach to the problem of off-policy temporal-difference learning. **Journal of Machine Learning Research**, [*s. l.*], v. 17, p. 1–29, 2016.

TAHERI-GARAVAND, Amin *et al.* Application of artificial neural networks for the prediction of performance and exhaust emissions in IC engine using biodiesel-diesel blends containing quantum dot based on carbon doped. **Energy Conversion and Management: X**, [*s. l.*], v. 16, p. 100304, 2022.

TAMRAT, Samuel *et al.* Emission and performance analysis of diesel engine running with CeO2 nanoparticle additive blended into castor oil biodiesel as a substitute fuel. **Scientific Reports**, [*s. l.*], v. 14, n. 1, p. 7634, 2024.

TARIQ, Muhammad; ALI, Saqib; KHALID, Nasir. Activity of homogeneous and heterogeneous catalysts, spectroscopic and chromatographic characterization of biodiesel: A review. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 16, n. 8, p. 6303–6316, 2012.

THAMARAI KANNAN, B. *et al.* Use of waste fish oil biodiesel blended with aluminium oxide nanoparticle in IC engines: an experimental on performance, combustion and emission study. **Scientific Reports**, [*s. l.*], v. 12, n. 1, p. 12930, 2022.

THANIGAIVEL, Sundaram *et al.* Sustainability and carbon neutralization trends in microalgae bioenergy production from wastewater treatment: A review. **Bioresource Technology**, [*s. l.*], v. 364, p. 128057, 2022.

THE STATE OF WORLD FISHERIES AND AQUACULTURE 2024. [*S. l.*]: FAO, 2024.

THIRUMALAI, D.; LORIMER, George H.; HYEON, Changbong. Iterative annealing mechanism explains the functions of the GroEL and RNA chaperones. **Protein Science**, [*s. l.*], v. 29, n. 2, p. 360–377, 2020.

TIAN, Li *et al.* Marine microorganisms: natural factories for polyunsaturated fatty acid production. **Blue Biotechnology**, [*s. l.*], v. 1, n. 1, p. 15, 2024.

TIBESIGWA, Timothy; OLUPOT, Peter Wilberforce; KIRABIRA, John Baptist. Assessment of the techno-economic viability of B10 synthesis from second-generation biodiesel feedstocks in Uganda. **International Journal of Sustainable Energy**, [*s. l.*], v. 42, n. 1, p. 351–373, 2023.

TOGUN, Hussein *et al.* A review on recent advances on improving fuel economy and performance of a fuel cell hybrid electric vehicle. **International Journal of Hydrogen Energy**, [*s. l.*], v. 89, p. 22–47, 2024.

TORRES-VALENZUELA, Laura Sofía; BALLESTEROS-GÓMEZ, Ana; RUBIO, Soledad.

Green Solvents for the Extraction of High Added-Value Compounds from Agri-food Waste. **Food Engineering Reviews**, [*s. l.*], v. 12, n. 1, p. 83–100, 2020.

TROTT, Oleg; OLSON, Arthur J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. **Journal of Computational Chemistry**, [*s. l.*], v. 31, n. 2, p. 455–461, 2010.

TUBINO, Matthieu; JUNIOR, José Geraldo Rocha; BAUERFELDT, Glauco Favilla. Biodiesel synthesis with alkaline catalysts: A new refractometric monitoring and kinetic study. **Fuel**, [*s. l.*], v. 125, p. 164–172, 2014.

ULLAH, Zahoor *et al.* Unveiling Biodiesel Production: Exploring Reaction Protocols, Catalysts, and Influential Factors. **ChemBioEng Reviews**, [*s. l.*], v. 11, n. 6, 2024.

UNUGUL, Tuba *et al.* Environmentally friendly processes from coffee wastes to trimethylolpropane esters to be considered biolubricants. **Journal of the Air & Waste Management Association**, [*s. l.*], v. 70, n. 11, p. 1198–1215, 2020.

VALENTI, Wagner C. *et al.* Aquaculture in Brazil: past, present and future. **Aquaculture Reports**, [*s. l.*], v. 19, p. 100611, 2021.

VERMA, Puneet; SHARMA, M.P. Review of process parameters for biodiesel production from different feedstocks. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 62, p. 1063–1071, 2016.

VICTORINO DA SILVA AMATTO, Isabela *et al.* Enzyme engineering and its industrial applications. **Biotechnology and Applied Biochemistry**, [*s. l.*], v. 69, n. 2, p. 389–409, 2022.

VIDAL-LIMON, Abraham; AGUILAR-TOALÁ, José E.; LICEAGA, Andrea M. Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides. **Journal of Agricultural and Food Chemistry**, [*s. l.*], v. 70, n. 4, p. 934–943, 2022.

WAHL, Joel *et al.* Accuracy evaluation and addition of improved dihedral parameters for the MMFF94s. **Journal of Cheminformatics**, [*s. l.*], v. 11, n. 1, p. 1–10, 2019.

WANCURA, João H.C. *et al.* Demystifying the enzymatic biodiesel: How lipases are contributing to its technological advances. **Renewable Energy**, [*s. l.*], v. 216, p. 119085, 2023.

WANG, Hong hai *et al.* Application of Lipase B from *Candida antarctica* in the Pharmaceutical Industry. **Industrial & Engineering Chemistry Research**, [*s. l.*], v. 62, n. 39, p. 15733–15751, 2023.

WANG, Baohua *et al.* Enabling Catalysts for Biodiesel Production via Transesterification. **Catalysts**, [*s. l.*], v. 13, n. 4, p. 740, 2023.

WANG, Lu *et al.* Synthesis, catalysts and enhancement technologies of biodiesel from oil feedstock – A review. **Science of The Total Environment**, [*s. l.*], v. 904, p. 166982, 2023.

WELDESLASE, Mebrhit Gebreyohanes *et al.* Maximizing biodiesel production from waste cooking oil with lime-based zinc-doped CaO using response surface methodology. **Scientific Reports**, [*s. l.*], v. 13, n. 1, p. 4430, 2023.

XIA, Shuaibo *et al.* Lipase-catalyzed production of biodiesel: a critical review on feedstock, enzyme carrier and process factors. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 18, n. 1, p. 291–309, 2024.

XIE, Dongming. Integrating Cellular and Bioprocess Engineering in the Non-Conventional Yeast Yarrowia lipolytica for Biodiesel Production: A Review. **Frontiers in Bioengineering and Biotechnology**, [*s. l.*], v. 5, 2017.

XU, Chao *et al.* Experimental and theoretical evidence of enhanced catalytic performance of lipase B from Candida antarctica acquired by the chemical modification with amino acid ionic liquids. **Molecular Catalysis**, [*s. l.*], v. 501, p. 111355, 2021.

YADAV, Vishwanath; BISWAS, Shreya; GOYAL, Arun. Enzymes of Industrial Significance and Their Applications. *In*: VERMA, Pradeep (org.). **Industrial Microbiology and Biotechnology**. 1. ed. Singapore: Springer Singapore, 2024. p. 277–307.

YAHYAEE, R.; GHOBADIAN, B.; NAJAFI, G. Waste fish oil biodiesel as a source of renewable fuel in Iran. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 17, p. 312–319, 2013.

YAN, Wenxin *et al.* Advanced strategies in high-throughput droplet screening for enzyme engineering. **Biosensors and Bioelectronics**, [*s. l.*], v. 248, p. 115972, 2024.

YESILYURT, Murat Kadir; CESUR, Cüneyt. A statistical optimization attempt by applying the Taguchi technique for the optimum transesterification process parameters in the production of biodiesel from Papaver somniferum L. seed oil. **Fuel**, [*s. l.*], v. 329, p. 125406, 2022.

YOSHIOKA, Taichi *et al.* Accelerated acidolysis for triglyceride modification using commercial lipases activated by a hydration–aggregation pretreatment. **Process Biochemistry**, [*s. l.*], v. 132, p. 130–139, 2023.

YUE, Mengmeng *et al.* Recent advances of biodiesel production enhanced by external field via heterogeneous catalytic transesterification system. **Chemical Engineering and Processing - Process Intensification**, [*s. l.*], v. 205, p. 109997, 2024.

YUSUF, Basiru O.; OLADEPO, Sulayman A.; GANIYU, Saheed A. Efficient and Sustainable Biodiesel Production via Transesterification: Catalysts and Operating Conditions. **Catalysts**, [*s. l.*], v. 14, n. 9, p. 581, 2024.

ZENG, Can; ZHONG, Nanjing. Encapsulation of lipases on coordination polymers and their catalytic performance in glycerolysis and esterification. **Grain & Oil Science and Technology**, [*s. l.*], v. 6, n. 3, p. 113–119, 2023.

ZHANG, Yiqi *et al.* Fish Scale Valorization by Hydrothermal Pretreatment Followed by Enzymatic Hydrolysis for Gelatin Hydrolysate Production. **Molecules**, [*s. l.*], v. 24, n. 16, p.

2998, 2019.

ZHANG, Zhuangzhuang *et al.* Lewis Acid-Base Site-Assisted In Situ Transesterification Catalysis to Produce Biodiesel. **Catalysts**, [*s. l.*], v. 14, n. 10, p. 731, 2024.

ZHANG, Yi; NARLIKAR, Geeta J.; KUTATELADZE, Tatiana G. Enzymatic Reactions inside Biological Condensates. **Journal of Molecular Biology**, [*s. l.*], v. 433, n. 12, p. 166624, 2021.

ZHAO, Zhen *et al.* A review of biophysical strategies to investigate protein-ligand binding: What have we employed?. **International Journal of Biological Macromolecules**, [*s. l.*], v. 276, p. 133973, 2024.

ZHAO, Jie *et al.* Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. **Asian-Australasian Journal of Animal Sciences**, [*s. l.*], v. 32, n. 6, p. 783–791, 2019.

ZHAO, Junxin *et al.* Production, purification, properties and current perspectives for modification and application of microbial lipases. **Preparative Biochemistry & Biotechnology**, [*s. l.*], v. 54, n. 8, p. 1001–1016, 2024.

ZHOU, Jiahui; HUANG, Meilan. Navigating the landscape of enzyme design: from molecular simulations to machine learning. **Chemical Society Reviews**, [*s. l.*], v. 53, n. 16, p. 8202–8239, 2024.

ZHU, Jishen *et al.* Taguchi method for optimizing the cation exchange resin catalyzed esterification of oleic acid with ethanol and adsorption kinetics study. **Process Safety and Environmental Protection**, [*s. l.*], v. 182, p. 989–998, 2024.

ZULQARNAIN *et al.* Overview of Feedstocks for Sustainable Biodiesel Production and Implementation of the Biodiesel Program in Pakistan. **ACS Omega**, [*s. l.*], v. 6, n. 29, p. 19099–19114, 2021.