OPTIMIZATION OF LIPIDS EXTRACTION, SEPARATION, AND TRANSESTERIFICATION FROM DISCARDED BEEF TALLOW USING SUPERCRITICAL CARBON DIOXIDE FOR BIODIESEL PRODUCTION

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

%	Percentage
∑	Sigma, the summation symbol
°C	Celsius, a unit of temperature.
3D	Three-dimensional
A	Frequency factor
bar	bar, a unit of pressure
cm^3	Cubic centimeter, a unit of volume
cm^{-1}	Inverse centimeter
Cs	Equilibrium concentration
E_a	Activation energy
g	Gram, a unit of weight
h	Hour, a unit of time
h	Plank constant (6.626×10^{-34} J/s)
h_s	Initial extraction rate
J	Joule, a unit of energy
k	Rate constant
K	Kelvin, a unit of absolute temperature
k_B	Boltzmann constant (1.38×10^{-23} J/K)
kg	Kilogram, a unit of weight
L	Litter, a unit of volume
m^3	Cubic meter, a unit of volume
meq/kg	Milliequivalents per kilogram, a unit of peroxide value
mg	Milligram, a unit of weight
min	Minute, a unit of time

MJ/kg	Megajoule per kilogram, a unit of heating value
ml	Milliliter, a unit of volume
mm	Millimeter, a unit of length
mm^2/s	Millimeter squared per second, a unit of kinematic viscosity
mol	Mole, a unit of substance concentration
MPa	Megapascal, a unit of pressure
MW	Molecular weight
N	Normality
nm	Nanometer
R	Universal gas constant (8.314 J/mol.K,)
R^2	Correlation coefficient
R^2_{adj}	Adjusted coefficient of determination
R^2_{pred}	Predicted coefficient of determination
s	Second, a unit of time
T	Absolute temperature
t	Time
$t_{1/2}$	Half-life of the reactant
V	Volume
W	Watt, a unit of power
wt	Weight
wt%	Weight percent
ΔE	Energy change
ΔG	Gibbs free energy change
ΔH	Enthalpy change
ΔS	Entropy change
μm	Micrometer

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Collaboration
ASTM	American Society for Testing and Materials
B100	100% biodiesel
B50	50% biodiesel and 50% petroleum diesel
B10	10% biodiesel and 90% petroleum diesel
B5	5% biodiesel and 95% petroleum diesel
BIS	Bureau of Indian Standards
BTU	British thermal unit
C.V.	Coefficient of variation
C:D	Number of carbon atoms: number of double bonds
CCD	Central composite design
CEN	European Committee for Standardization
CFIs	Cold flow improvers
CO	Carbon oxide
CO_2	Carbon dioxide
DBT	Discarded beef tallow
DoE	Design of Experiments
df	Degree of freedom
DSM	Department of Standards Malaysia
EIA	Energy Information Administration
FAMEs	Fatty acid methyl esters
FFAs	Free fatty acids
FTIR	Fourier Transform Infrared

GC-fid	Gas Chromatography with flame ionization detector
GHGs	Greenhouse gases
HC	Hydrocarbons
HCl	Hydrochloric acid
ICl	Wijs solution
IEA	International Energy Agency
IR	Infrared radiation
JISC	Japanese Industrial Standards Committee
KI	Potassium iodide
KOH	Potassium hydroxide
LCA	Life cycle assessment
MAE	Microwave-assisted extraction
MUFAs	Monounsaturated fatty acids
NaOH	Sodium hydroxide
NO_x	Nitrogen oxides
PUFAs	Polyunsaturated fatty acids
Eq	Equation
RCCD	Rotatable central composite design
rpm	Round per minute
RSM	Response surface methodology
SAC	Standardization Administration of China
SC-CO_2	Supercritical carbon dioxide
SCF	Supercritical fluid technique
SD	Standard deviation
SDGs	Sustainable development goals
SFAs	Saturated fatty acids
SHV	Superheated vapor technique

SO_2	Sulfur dioxide
Temp.	Temperature
UAE	Ultrasonic-assisted extraction
UFAs	Unsaturated fatty acids
UN	United Nations
US	United States
WTE	Waste-to-energy principle

LIST OF APPENDICES

PENGOPTIMUMAN PENGEKSTRAKAN, PEMISAHAN, DAN TRANSESTERIFIKASI LIPID LEMAK DAGING LEMBU TERBUANG DARIPADA MENGGUNAKAN KARBON DIOKSIDA SUPERKRITIKAL UNTUK PENGHASILAN BIODIESEL

ABSTRAK

Kajian ini menyelidik penggunaan teknologi karbon dioksida superkritikal (SC-CO_2) untuk mengekstrak lipid daripada lemak lembu terbuang (DBT) bagi penghasilan biodiesel. DBT merupakan bahan suapan yang tidak boleh dimakan dan kos efektif, serta kaya dengan asid lemak dan mempunyai kandungan haba yang tinggi serta kestabilan oksidatif yang baik. Untuk pengekstrakan lipid DBT, kaedah pengekstrakan haba dan pelarut secara konvensional telah digunakan. Namun, kaedah ini terhad oleh ketoksikan pelarut dan suhu tinggi yang boleh merendahkan kualiti asid lemak. Walaupun SC-CO_2 merupakan teknologi pengekstrakan yang cekap dan mesra alam, aplikasinya terhadap DBT masih terhad dalam literatur sedia ada. Bagi menangani jurang penyelidikan ini, kajian ini telah menggunakan dan mengoptimumkan parameter operasi SC-CO_2 untuk mendapatkan pemulihan lipid yang berkesan daripada DBT. Dalam proses SC-CO_2, sampel DBT yang telah disediakan dimasukkan ke dalam bekas pengekstrakan. CO_2 dipanaskan dan dimampatkan melebihi titik kritikalnya, kemudian dipam melalui sampel tersebut. SC-CO_2 menembusi matriks DBT dan melarutkan lipidnya. Setelah proses selesai, CO_2 dinyahtekan dan kembali kepada keadaan gas, meninggalkan lipid yang telah diekstrak. Parameter pengekstrakan termasuk suhu (32–80 °C), tekanan (10–50 MPa), dan masa (15–150 min) telah dioptimumkan menggunakan metodologi permukaan tindak balas (RSM). Pemulihan lipid maksimum sebanyak 86.10% dicapai pada 60 °C,

30 MPa, dan 120 min. Lipid yang diekstrak kemudiannya melalui proses transesterifikasi di bawah pelbagai keadaan nisbah molar lipid kepada metanol (1:3–1:9), nisbah pemangkin (0.25–1.5 wt%), suhu (50–70 °C), dan masa (10–90 min). Hasil biodiesel optimum sebanyak 95.15% dicapai pada 1:7.5 mol/mol, 1.16 wt%, 58 °C, dan 72 min. Analisis kinetik dan termodinamik mengesahkan bahawa kedua-dua proses SC-CO_2 dan transesterifikasi bergantung kepada suhu, bersifat endotermik, dan tidak spontan. Biodiesel yang dihasilkan mematuhi piawaian ASTM D6751 dan EN 14214, sekali gus mengesahkan potensi DBT sebagai bahan suapan yang lestari. Kajian ini membuktikan bahawa SC-CO_2 merupakan kaedah mesra alam dan cekap untuk pengekstrakan lipid dalam penghasilan biodiesel.

OPTIMIZATION OF LIPIDS EXTRACTION, SEPARATION, AND TRANSESTERIFICATION FROM DISCARDED BEEF TALLOW USING SUPERCRITICAL CARBON DIOXIDE FOR BIODIESEL PRODUCTION

ABSTRACT

This study investigates the use of supercritical carbon dioxide (SC-CO_2) technology to extract lipids from discarded beef tallow (DBT) for biodiesel production. DBT, a non-edible and cost-effective feedstock, is rich in fatty acids and offers high thermal content and oxidative stability. For extracting DBT lipids, conventional thermal and solvent based extraction methods were employed. However, these methods are constrained by solvent toxicity and high temperatures that can degrade fatty acid quality. Although SC-CO_2 is an efficient and eco-friendly extraction technology, its application to DBT remains limited in existing literature. To address this research gap, this study employed and optimized SC-CO_2 operating parameters for effective lipid recovery from DBT. In the SC-CO_2 process, a prepared DBT sample was placed in an extraction vessel. CO_2 was heated and pressurized above its critical point, and pumped through the sample. The SC-CO_2 penetrated the DBT matrix, dissolving its lipids. Upon depressurization, CO_2 returned to its gaseous state, leaving the extracted lipids behind. Extraction parameters including temperatures (32–80 °C), pressures (10–50 MPa), and times (15–150 min) were optimized using response surface methodology (RSM). The maximum lipid recovery of 86.10% was achieved at 60 °C, 30 MPa, and 120 min. The extracted lipids were transesterified under varying conditions of lipid to methanol molar ratios (1:3–1:9), catalyst ratios (0.25–1.5 wt%), temperatures (50–70 °C), and times (10–90 min). The optimized biodiesel yield of 95.15% was achieved at 1:7.5 mol/mol, 1.16 wt%, 58 °C, and 72 min. Kinetic and

thermodynamic analyses confirmed that both SC-CO_2 extraction and transesterification were temperature-dependent, endothermic, and non-spontaneous. The resulting biodiesel met ASTM D6751 and EN 14214 standards, affirming potential of DBT as a sustainable feedstock. This study demonstrates SC-CO_2 as a green and efficient method for lipid extraction in biodiesel production.

CHAPTER 1

INTRODUCTION

1.1 Background

Energy demand is rising globally due to the expanding economy driven by the growth of population worldwide. Despite the various energy sources, fossil fuel remains dominant where it covers 88.69% of needs (EIA, 2025), including 18 million barrels of diesel per day in 2023 (Gadore et al., 2025). Reliance on fossil fuel as the main source of energy raises economic and environmental concerns. Energy prices increase dramatically due to high demand and limited supply, while reserves risk depletion from over-extraction. Besides, combustion of fossil fuel contributes to severe environmental impacts. The emissions of carbon dioxide (CO_2), which was estimated at 37.4 billion tons in 2024, and greenhouse gases (GHGs) escalated the global warming and climate change. Additionally, environmental pollution poses serious risks to human health. Hence, transition to renewable energy offers promising solutions to enhance energy security, boost economic growth, and reduce environmental pollution (Friedlingstein et al., 2024).

Biodiesel is a promising alternative fuel that can substitute fossil diesel. It is defined as the alkyl esters of long-chain fatty acids derived from plant and animal-based feedstocks. Biodiesel can be sourced from four generations of feedstocks which are edible, inedible, algae, and genetically modified crops (Abeysinghe et al., 2025). Typically, it is synthesized through a transesterification reaction in which triglycerides react with alcohol in the presence of a strong base catalyst to produce alkyl esters, known as biodiesel, and glycerol as a byproduct, as illustrated in Figure 1. The physicochemical properties of biodiesel are very similar to those of conventional diesel, allowing it to be used either in pure form or blended with petroleum diesel in

diesel engines without significant modifications. Since biodiesel is composed of biodegradable and renewable materials, it generates lower and less harmful emissions than conventional diesel (Elgharbawy et al., 2025). Due to these eco-friendly and sustainable characteristics, global demand for biodiesel fuel is increasing rapidly where it is expected to reach 60.6 billion liters in 2025 with a 34.37% increase from 2020 (IEA, 2023).

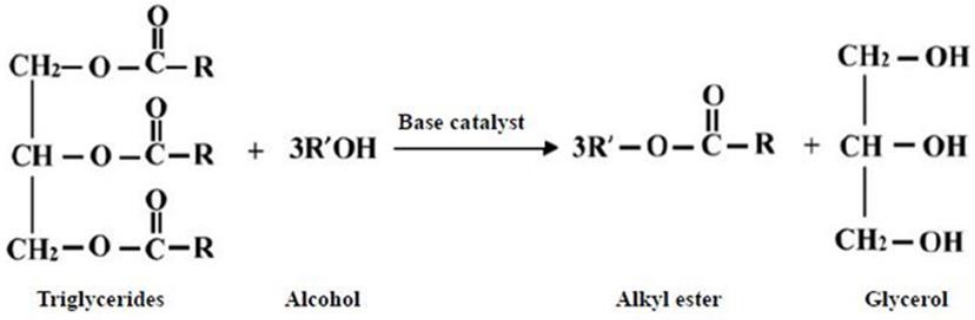

Figure 1.1 Transesterification reaction for biodiesel production

Discarded beef tallow (DBT) is a low-cost feedstock for biodiesel production. It is sourced from waste byproducts of beef slaughterhouses, tanneries, meat processing units, and wet markets. With global beef production estimated at 74 million tonnes in 2023, DBT is increasingly available and affordable (Greenwood, 2021). DBT contains triglycerides with high portions of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), which are ideal components for biodiesel fuel (Zeng et al., 2024; Zhou, Zhang, et al., 2024). Utilizing these lipid-rich wastes not only helps manage accumulated byproducts but also supports environmental health by reducing risks. Additionally, it promotes the ethical and sustainable waste-to-energy (WTE) principle by avoiding competition with food resources. Despite its low cost, DBT offers favorable characteristics for biodiesel like higher productivity, higher thermal content, oxidative stability, and improved safety, Hence, advancing lipid

extraction and biodiesel synthesis techniques is crucial for better utilization of DBT in biodiesel production. (Nagappan et al., 2021; Olubunmi et al., 2022)

Supercritical carbon dioxide (SC-CO_2) is an advanced and efficient technology for extracting lipids from plant and animal-based feedstocks. It eliminates the need for organic solvents, making it cost-effective and non-toxic. Instead, it uses CO_2 in a supercritical state which is a condition occurs at a temperature of 31.3 °C and a pressure of 7.38 MPa. At supercritical state, CO_2 exhibits both liquid and gas characteristics and acts as a solvent for lipids. Hence, the extraction efficiency depends primarily on CO_2 rate, temperature, pressure, and time. Due to the wide range of applicable temperatures and pressures, SC-CO_2 is capable of selective extraction depending on the feedstock (Guo et al., 2022). The current research project utilized SC-CO_2 capabilities to extract lipids from DBT feedstock and investigate the effects of the operating parameters on the lipid yield toward biodiesel production.

1.2 Problem statement

Biodiesel production from DBT represents a sustainable and cost-effective alternative to fossil fuels. DBT is rich in fatty acids, the essential building blocks of biodiesel fuel, and offers high thermal content and oxidative stability. Additionally, it is an affordable feedstock that does not compete with food resources. Lipids of DBT were extracted using various thermal (Fard et al., 2019; Kleinberg et al., 2019) and solvent-based methods (Aminullah et al., 2018; Gokul and Jambulingam, 2019). However, they are constrained with the toxicity of organic solvents and high temperature that may degrade fatty acid composition. SC-CO_2 is an economical and ecofriendly lipid extraction technology since it eliminates the need for toxic organic solvents, high temperatures, and additional solvent-lipid separation steps (Guo et al.,

2022). Although SC-CO_2 proved efficiency in extracting lipids from animal-based feedstocks, its application to DBT represents a clear gap in the existing literature. This research project aims to fill this research gap by utilizing SC-CO_2 to extract DBT lipids for biodiesel synthesis. To the best of the researcher knowledge, this is the first study to do so. Accordingly, this investigation addresses the following research gaps:

SC-CO_2 extraction of lipids from DBT is influenced by key operating parameters which are temperature, pressure, and treatment time (Alsaedi, Sohrab, et al., 2022; Ilias et al., 2023). While extraction temperature enhances the diffusivity of CO_2, pressure improves interactions between CO_2 and lipid molecules, thereby increasing solubility (Asl et al., 2020; Yang et al., 2019). Sufficient treatment time is also essential for effective extraction. The lipid yield is significantly affected by adjustments to these parameters. Optimization is a mathematical process used to determine the most desirable outcome by systematically adjusting controllable variables (Asaad et al., 2024). However, optimizing SC-CO_2 operating parameters for lipid extraction from DBT remains a gap in the existing body of knowledge. Therefore, this study aims to optimize the temperature, pressure, and time conditions of SC-CO_2 for maximum lipid yield from DBT feedstock.

Biodiesel is synthesized via a transesterification reaction, in which triglycerides react with alcohol in the presence of a catalyst to produce biodiesel and glycerol as a byproduct. Transesterification is the most reliable, cost-effective, and widely used method for biodiesel synthesis, as it yields biodiesel with more favorable properties compared to other methods such as blending, pyrolysis, and microemulsion. This chemical process is influenced by various factors, namely lipid to methanol molar ratio, catalyst ratio, temperature, and reaction time (Jambulingam et al., 2020;

Olubunmi et al., 2022). Each factor enhances yield up to an optimal point, beyond which negative effects occur. For instance, excess molar ratio can dissolve oleic acid and glycerol, reducing conversion, while too much catalyst may form gel-like materials that hinder the reaction (Gohain et al., 2017). Higher temperatures improve solubility and reaction rate but may lead to methanol loss through evaporation (Omkaresh et al., 2023). Adequate reaction time is essential. However, prolonged reaction time can cause hydrolysis and reversibility, lowering biodiesel yield (Yahya et al., 2020). Determining the optimal values of transesterification factors for maximizing biodiesel yield from DBT lipids extracted by SC-CO_2 was not yet investigated, which this study aims to address.

A notable gap in the existing literature is the lack of kinetic and thermodynamic analyses for both SC-CO_2 extraction and the subsequent transesterification reaction in biodiesel production from DBT feedstock. These analyses are essential for understanding reaction rates, energy requirements, and equilibrium concentrations, as well as for determining key parameters such as activation energy, enthalpy, entropy, and Gibbs free energy. These parameters indicate reaction characteristics including degree of disorder, spontaneity, and whether the reaction is endothermic or exothermic (Devaraj and Udayakumar, 2023; Omkaresh et al., 2023; Yang et al., 2019). To address this gap, the present study models SC-CO_2 extraction using second-order kinetics and transesterification using first-order kinetics, complemented by thermodynamic modeling.

Lastly, biodiesel fuel must comply with established standards such as ASTM D6751 and EN 14214. Characterization of both lipids and biodiesel typically involves analysis of their chemical composition and physicochemical properties. The chemical composition reveals the fatty acid profile, which forms the basis of biodiesel fuel,

while physicochemical properties help assess the fuel quality (Bôas and Mendes, 2022; Singh et al., 2019). Given the limited data on the characteristics of DBT lipids extracted using SC-CO_2 and their corresponding biodiesel, this study analyzes both the chemical composition and physicochemical properties to evaluate their conformity with international biodiesel standards. Therefore, the significance of this study lies in utilizing SC-CO_2 to extract lipids from DBT for the production of biodiesel that complies with established quality standards.

1.3 Research objectives

The general aim of this research project is to utilize SC-CO_2 technology to extract lipids from DBT as an affordable feedstock for biodiesel production. The specific objectives are as follows:

1. To optimize the conditions of temperature, pressure, and time that affect lipid extraction from DBT using the SC-CO_2 method.
2. To determine the optimal values of the lipid to methanol molar ratio, catalyst ratio, temperature, and time for the transesterification reaction influencing biodiesel synthesis from the extracted lipids.
3. To analyze the kinetic and thermodynamic behavior of lipid extraction and biodiesel synthesis.
4. To characterize the chemical composition and physicochemical properties of the extracted lipid and the synthesized biodiesel.

1.4 Scope of research

This study is limited to lab-scale experiments, focusing on the use of SC-CO_2 technology to extract lipids from DBT as a sustainable and affordable feedstock generated from slaughterhouse and meat industry byproducts. The study explores how

key operating parameters in SC-CO_2 extraction, namely temperature, pressure, and treatment time affect the yield of lipids from the feedstock. To identify the most effective combination of these variables, Response Surface Methodology (RSM) is utilized as a statistical optimization technique, aiming to maximize the extraction rate for the highest yield of lipids.

The research project also utilizes DBT lipids extracted via SC-CO_2 technology for the production of biodiesel fuel through base-catalyzed transesterification. Potassium hydroxide (KOH) serves as the base catalyst to promote the chemical reaction. The research investigates the impact of the transesterification variables including lipid to methanol molar ratio, catalyst ratio, reaction temperature, and reaction time on the produced biodiesel yield. To enhance process efficiency, RSM is employed to statistically optimize these parameters, aiming to achieve the highest possible biodiesel output under the most favorable reaction conditions.

The second order kinetic and thermodynamic model is used to determine the rate constant, initial extraction rate, equilibrium concentration, activation energy, and changes in enthalpy, entropy, and Gibbs free energy for the SC-CO_2 extraction of DBT lipids. Additionally, the first order kinetic and thermodynamic model is applied to determine the rate constant, pre-exponential factor, activation energy, and changes in enthalpy, entropy, and Gibbs free energy for the transesterification reaction. Based on the obtained parameters, both processes were described in terms of molecular disorder, reaction spontaneity, and endothermic or exothermic characteristics.

The SC-CO_2 extracted lipids and synthesized biodiesel derived from DBT are characterized in terms of both chemical composition and physicochemical properties. The analysis of chemical composition focuses on identifying the fatty acid profile,

detailing the types of fatty acids and their respective percentages. The physicochemical properties are thoroughly examined to evaluate key characteristics essential for compliance with biodiesel standards and performance metrics. These properties include density at 15 °C, kinematic viscosity at 40 °C, cold flow properties namely, cloud point and pour point, flash point, heating value, iodine value, cetane number, acid number, free fatty acids (FFAs), saponification value, peroxide value, and moisture content. This comprehensive characterization allows for a detailed assessment of the quality and compliance of the DBT biodiesel with ASTM D6751 and EN 14214 standards.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

This chapter provides a comprehensive review of the relevant literature that aligns with the scope of the current research project. It begins with an exploration of global energy demand and consumption, emphasizing the critical role of various energy sources. The challenges associated with extensive consumption of fossil fuel as a main source of energy that include environmental concerns, resource depletion, and economic implications are thoroughly discussed. This section also highlights the need for alternative and sustainable energy sources to mitigate these challenges. Next, biodiesel fuel is introduced and examined in detail. This includes its definition, key characteristics, and its role as a renewable energy source. The section also covers global biodiesel production trends and the diverse feedstock generations used for its synthesis. Given that this study focuses on beef tallow as a biodiesel feedstock, a dedicated subsection discusses beef production, the composition of beef tallow, and its characteristics that influence its suitability for biodiesel synthesis. Following this, lipid extraction methods are reviewed extensively. The various techniques are categorized into four main groups which are thermal rendering, solvent-based extraction, instrument-assisted extraction, and supercritical carbon dioxide which is given particular emphasis since the current research project employs this technique to extract lipids from DBT feedstock efficiently. Detailed examples of lipid extraction from animal-based feedstock using each method are presented, along with a thorough comparison of their advantages and limitations. Similarly, biodiesel conversion techniques are examined, with a focus on methods applicable to animal-based feedstocks. The transesterification process is discussed in depth, covering both catalytic

and non-catalytic approaches. Additionally, alternative biodiesel conversion techniques, such as pyrolysis, microemulsion, and blending, are comprehensively explained. Findings from previous studies that document the effectiveness of each method are integrated into this discussion. A comparative analysis of the advantages and disadvantages of each conversion technique is also provided. Beyond that, optimization is discussed as a research tool for achieving desirable outcomes in lipid extraction and biodiesel synthesis. Furthermore, the classifications, techniques, and applications of optimization are elaborated upon in accordance with the objectives of the current study. The chapter also includes a review of kinetic and thermodynamic analyses related to the processes of lipid extraction and transesterification. Factors influencing reaction rates and energy changes in lipid extraction and biodiesel conversion are detailed. Key parameters including activation energy (E_a), rate constant (k), equilibrium concentration (Cs), correlation coefficient (R^2), enthalpy change (ΔH), entropy change (ΔS), and Gibbs free energy change (ΔG) are defined and contextualized within the study. The final section explores the characteristics of lipids and biodiesel, particularly focusing on analytical techniques used to determine their composition and quality. Instrumental methods such as Gas Chromatography with a flame ionization detector (GC-fid) and Fourier Transform Infrared Spectroscopy (FTIR) are elucidated, showcasing their role in identifying and quantifying key compounds in biodiesel. Additionally, the physicochemical properties of biodiesel fuel, along with the industry standards that govern its quality (such as ASTM D6751 and EN 14214), are outlined. By synthesizing these aspects, this chapter establishes a strong foundation for understanding the theoretical and experimental framework of the study, ensuring better evaluation of biodiesel production from animal-based feedstocks.

2.2 Outlook of energy demand and consumption

The current trends of life rely substantially on energy consumption since almost every aspect of human activity requires a continuous supply of energy. With the rapid expansion of global populations and increasing urbanization, the demand for energy increased at an unprecedented rate. This rising demand is primarily driven by diversified economic activities such as industry, transportation, and electricity generation. As cities expand and populations grow, the need for energy to sustain infrastructure, public services, and technological advancements continues to rise (Raihan et al., 2025). Figure 2.1 provides an outlook on global energy consumption from 2022 to 2050. The energy consumption for the current year, 2025, is estimated to reach 661.37 quadrillion British thermal units (BTU), reflecting a 3.69% increase compared to 2022. The estimated growth in energy consumption is expected to reach 9.46% by 2030 and 21.07% by 2040, ultimately reaching 854.74 quadrillion BTU by 2050, representing a 34% rise in global energy consumption (EIA, 2023).

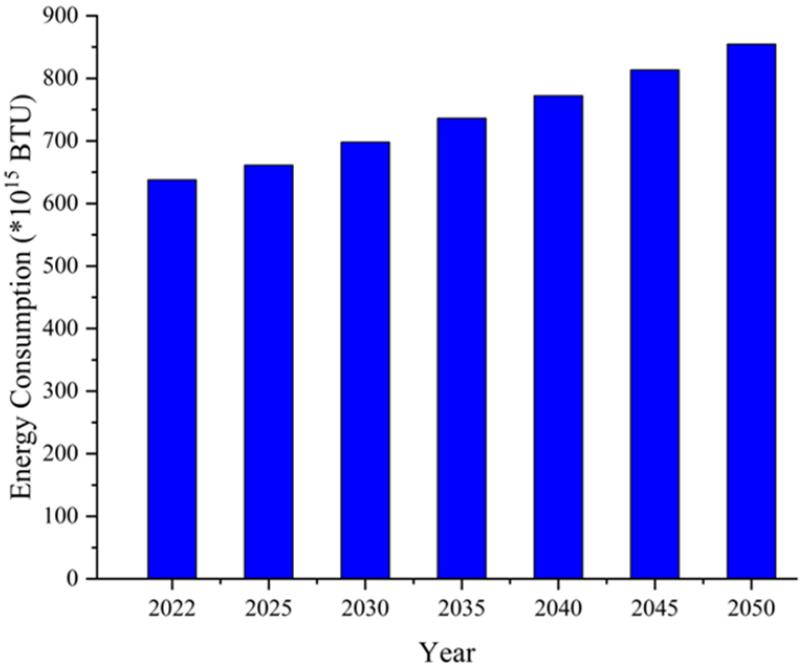

Figure 2.1 Outlook of global energy consumption from 2022 to 2050 (EIA, 2023)

2.2.1 Sources of energy

The global energy demand is met through three main categories of energy sources which are non-renewable, renewable, and nuclear energy. The contribution of each source is illustrated in Figure 2.2 according to the US Energy Information Administration (EIA) in 2023. Fossil fuels as non-renewable energy sources continue to dominate the global energy supply, providing an overwhelming 525.30 quadrillion BTU, which accounts for 88.69% of the total world energy production. These fossil fuel sources are primarily divided into petroleum liquids, which make up 32.58% of the global production, followed closely by solid coal at 30.25% and natural gas at 25.87%. In contrast, nuclear energy gained attention as a cleaner and economically viable alternative. It contributes 27.68 quadrillion BTU, covering 4.67% of the total energy production. Meanwhile, renewable energy sources such as biofuels, hydropower, geothermal, wind, and solar offer a sustainable approach to meeting global energy needs. Despite their environmental benefits, renewables currently account for only 6.64% of the total energy production, generating 39.32 quadrillion BTU (EIA, 2025).

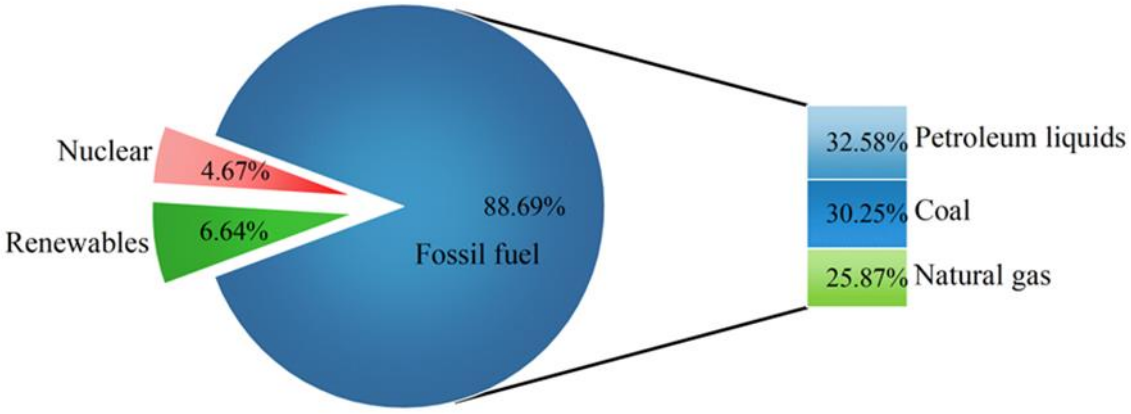

Figure 2.2 Distribution of world energy production by sources (EIA, 2025)

2.2.2 Addressing the challenges of the current growing energy demand

Regardless of the availability of energy for human activities, the extensive consumption of carbon-based materials as the primary source of energy led to significant economic and environmental challenges. Fossil fuels were naturally formed over millions of years. However, they are consumed at a much faster rate than their formation due to the sharp rise in energy demand. This rapid depletion of reserves threatens energy security and disrupts supply chains. As fossil fuel reserves decline, production and transportation costs increase, leading to higher energy prices. In addition to production limitations, fluctuations in supply and demand further contribute to energy price volatility, affecting industries and economies. Moreover, rising energy costs prevent millions of people, particularly in developing countries, from accessing a reliable energy source (Avazkhodjaev et al., 2024).

Beyond economic challenges, the extensive consumption of fossil fuels poses serious environmental concerns. Burning carbon-based fuels releases vast amounts of CO_2 and GHGs that significantly contribute to global warming and climate change. In 2024, global CO_2 emissions from fossil fuel combustion were estimated at 37.4 billion tons (Friedlingstein et al., 2024). Additionally, byproducts such as sulfur dioxide (SO_2) and nitrogen oxides (NO_x) play a key role in air pollution, leading to environmental issues such as smog and acid rain. Beyond combustion, fossil fuel extraction and transportation also pose severe environmental risks. Chemical waste and oil spills contaminate water and soil, while drilling and mining operations destroy natural habitats. The pollution associated with fossil fuel consumption not only degrades ecosystems but also poses serious health risks, increasing the prevalence of chronic diseases such as cancer, cardiovascular conditions, and respiratory disorders (Woodruff, 2024).

Given these economic and environmental challenges, transitioning to renewable energy becomes a necessity to ensure a sustainable and secure energy future. Governments worldwide are implementing policies and regulations to accelerate the shift toward green and renewable energy sources. These efforts include financial incentives, subsidies, and stricter environmental regulations to reduce dependence on fossil fuels. Furthermore, significant investments are directed toward developing modern infrastructure, smart grids, and advanced clean energy technologies to enhance efficiency and reliability. Encouraging research and innovation in energy storage, hydrogen fuel, and carbon capture technologies is also crucial to overcoming current limitations and ensuring long-term energy sustainability (Kyei et al., 2025).

2.3 Biodiesel as a renewable energy

Biodiesel is the alkyl esters of long-chain fatty acids derived from vegetative and animal-based feedstocks. Lipids are extracted from these feedstocks and chemically processed to convert triglycerides in the extracted oil into alkyl esters. This conversion occurs through a chemical reaction known as transesterification, which involves the use of alcohol and a base catalyst along with lipids to produce alkyl esters and glycerol. The resulting biodiesel can be used for heating, electricity generation, and fueling diesel engines, which is the most common application since it does not require significant modifications in diesel engines due to the similarity in physicochemical properties. Biodiesel can be used either as a pure fuel biodiesel or blended with petroleum-based diesel to enhance physicochemical properties and reduce the environmental impact of conventional diesel fuel. Hence, biodiesel is a promising alternative to conventional diesel, which drives its global demand as a sustainable substitute for petroleum-based diesel (Elgharbawy et al., 2025).

2.3.1 Global demand of biodiesel

The global biodiesel industry is experiencing significant growth as nations and industries seek sustainable alternatives to fossil fuels. This expansion is driven by the increasing global demand for biodiesel, which plays a crucial role in the transition toward renewable sources. Figure 2.3 presents the global biodiesel demand from 2016 to 2028, as estimated by the International Energy Agency (IEA) under the accelerated case scenario. In 2016, the global demand for biodiesel was recorded at 33.30 billion liters. By 2020, this demand increased by 35.44%, reaching 45.10 billion liters, reflecting a steady growth trend. Estimations indicate that demand will continue to rise, reaching 60.60 billion liters by 2025. By 2028, the demand is expected to increase by 60% compared to 2020 levels, reaching approximately 68.10 billion liters. These figures highlight biodiesel growing importance as a viable fuel alternative (IEA, 2023). Despite its potential, biodiesel commercialization as an alternative to petroleum diesel faces challenges. The availability and cost of feedstocks significantly impact production, accounting for 70–95% of total expenses. Addressing these challenges is critical to ensuring the long-term sustainability of biodiesel (Bhuiya et al., 2020).

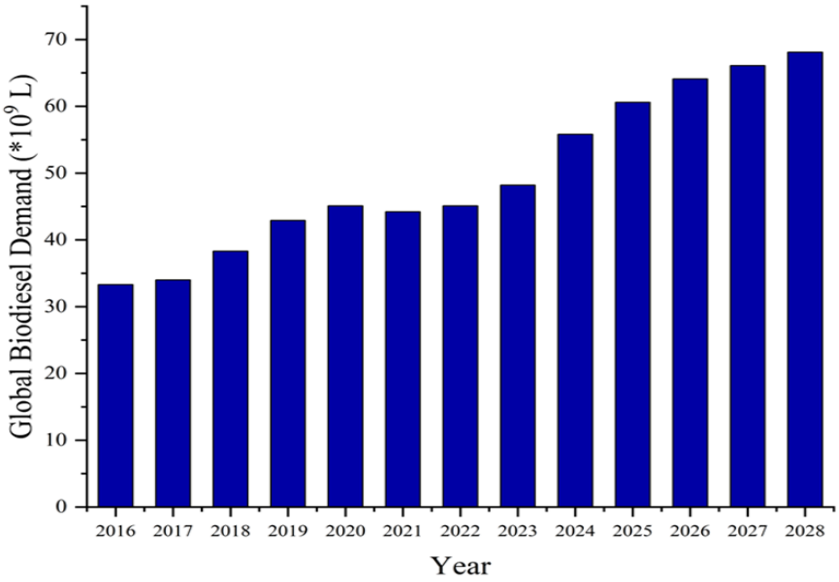

Figure 2.3 The global biodiesel demand from 2016 to 2028 (IEA, 2023).

2.3.2 Biodiesel feedstocks

Biodiesel fuel can be synthesized from four generations of feedstocks which are edible, inedible, algae, and genetically modified crops as presented in Figure 2.4. Elaborations on each generation including definition, examples, advantages, and disadvantages were summarized in Table 2.1. The first generation of edible feedstocks contributes around 75% to the global production of commercial biodiesel. However, this approach raises ethical, economical, and environmental concerns. The use of edible feedstocks for biodiesel production is considered unethical because it threatens food security and contributes to rising food prices. Additionally, the high costs of freshwater, fertilizers, arable lands, and labor work make biodiesel unfeasible economically compared to petroleum-based diesel. Moreover, the consumption of natural resources and emissions released during feedstock cultivation may result in a larger environmental footprint than conventional fossil fuel itself (Abeysinghe et al., 2025).

The second generation of inedible feedstocks includes oils derived from non-food plant sources and waste materials. While inedible plant-based oils such as jatropha and rubber seed oil reduce the competition with edible feedstocks, these resources alone are insufficient to meet the growing demand for biodiesel. Therefore, they cannot fully replace the feedstocks of first generation. Significant biodiesel feedstocks from second generation are oils extracted from waste materials. Given the large volumes of waste generated daily, these sources offer a promising and sustainable alternative for biodiesel production. However, proper pretreatment, oil extraction, and synthesis techniques are critical to efficiently convert waste into biodiesel, adhering to WTE principle which is widely recognized in sustainable waste management as well (Elgharbawy et al., 2021).

Algae oils represent the third generation of biodiesel feedstocks. Although algae offer advantages such as high productivity, fast growth rates, and efficient

photosynthetic activity, they also present several disadvantages which include high consumption of natural resources like water and fertilizers, competition for arable lands, GHGs emissions, and, lower lipid yields compared to first and second generations of feedstocks. For this reason, only algae species with a lipid content of more than 5% are considered viable candidates for biodiesel production (Jeliani et al., 2021).

Lastly, genetic modifications were recently applied to vegetative crops to enhance their benefits and overcome limitations as biodiesel feedstocks. This advanced approach is recognized as the fourth generation of biodiesel feedstocks. Genetic modifications showed significant improvements in characteristics such as CO_2 sequestration, reducing GHGs, boosting productivity, regulating growth, and increasing lipid content. However, serious concerns remain including product toxicity, high capital investment requirements, pathogenicity risks, and threats to indigenous species. Despite these advancements, research outcomes in this area are rarely accessible, as many studies and reports remain classified (Kossalbayev et al., 2025).

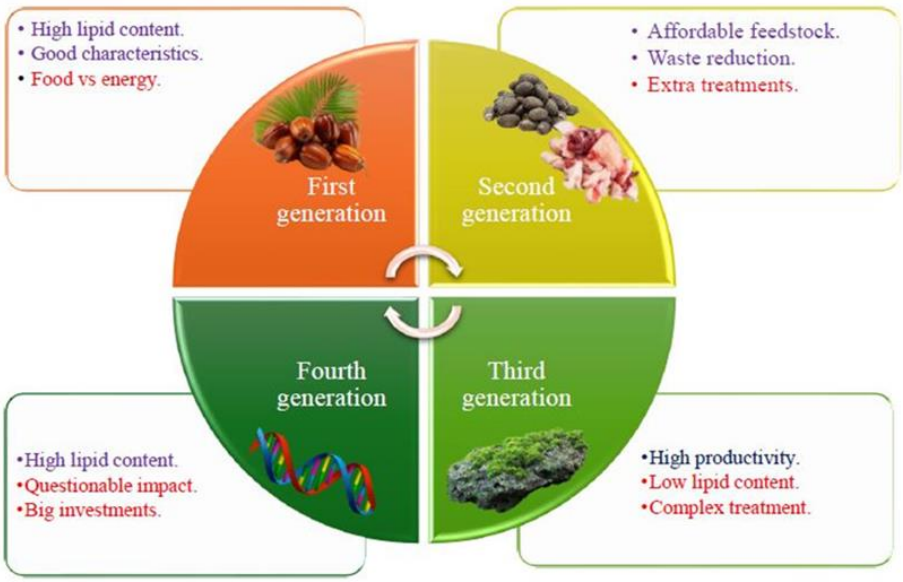

Figure 2.4 The four generations of biodiesel feedstocks

Table 2.1 Comparative overview of the four generations of biodiesel feedstocks (Retrieved from (Aron et al., 2020; Zheng and Cho, 2025))

Items	**First generation**	**Second generation**	**Third generation**	**Fourth generation**
Common classification	Edible	Inedible	Algae	Genetically modified crops
Definition	Biodiesel is produced from feedstocks that are also used for human food.	Biodiesel is produced from non-food-based feedstocks.	Biodiesel is derived from high-yield biomass of algae.	Biodiesel is developed using advanced biotechnology for enhanced yield.
Examples	Soybean, sunflower, corn, and palm oils	Jatropha, rubber, waste oil, and waste animal fat	Microalgae, macroalgae, and seaweed	Genetically modified algae, synthetic microorganisms, and carbon-capturing crops
Advantages	• High lipid content • Good Characteristics • Available and accessible	• Affordable • Waste reduction • Sustainable for energy security	• High biomass productivity • Low resources consumption demand • CO_2 capturers.	• High lipid content • carbon-negative biofuels • Highly efficient and sustainable
Disadvantages	• Competitive with food resources • High resource consumption demand • Pricy	• Potential impurities • Extra treatment • Inconsistent supply	• Low content • Complex treatment • High production costs • Contamination risk	• Questionable impact • Big investments • Ethical and regulatory concerns

2.4 Discarded beef tallow as a feedstock for biodiesel production

Beef is one of the primary sources of protein for humans. Global beef production increased to meet high demand, which was estimated at 74 million tonnes in 2023, reflecting a 5.71% increase from 70 million tonnes in 2019. The largest beef producer is The US where it produced 12.30 million tonnes in 2019, accounting for 17% of global beef production. Most of this beef is consumed domestically, while only 11.10% is exported. In Asia, China dominates the beef industry, producing 6.85 million tonnes in 2019, which constituted 9% of global production. India followed, contributing only 4% of the total global beef output. In Southeast Asia, beef production is largely driven by indigenous herd-holders, who supply a significant portion of domestic demand. However, beef production in the region is expected to increase to meet rising demand like the situation in Indonesia that currently imports 55% of its beef needs. As beef production expands, waste byproducts from slaughterhouses, tanneries, meat processing units, and wet markets are also increasing. Managing such waste is essential to mitigate potential environmental and health risks. One effective solution is utilizing these byproducts to create value-added products such as biodiesel (Greenwood, 2021).

Beef tallow refers to the solid fatty tissues of beef cattle. Figure 2.5 shows the deposits of tallow within beef anatomy including subcutaneous, intermuscular, and intramuscular fat. The high melting point, ranging from 45 to 50 °C, is a distinctive characteristic of beef tallow that makes it solid at room temperature. Additionally, it exhibits high oxidative stability, making it resistant to oxidation. It also has a complex crystallization behavior, tending to form large graininess crystals. Beef tallow is used in various applications that include food industry as an ingredient or flavor enhancer,

cooking, soap-making, candle crafting, lubricants, and biodiesel production (Zeng et al., 2024; Zhou, Zhang, et al., 2024).

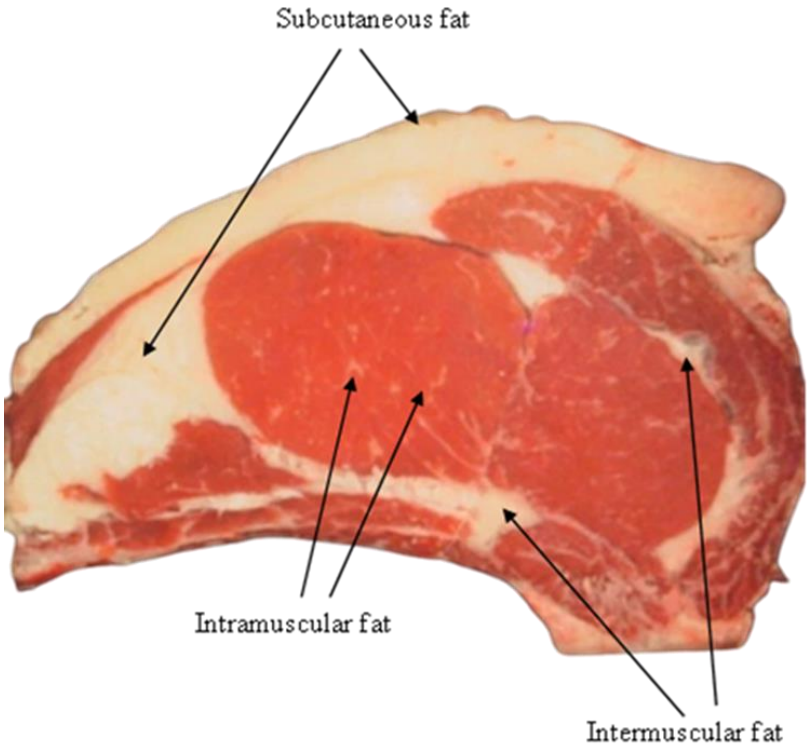

Figure 2.5 Tallow deposits within beef anatomy (Nunes et al., 2015)

Beef tallow is rich in lipid content where it ranges from around 40% (Gokul et al., 2020) to over 80% (Aminullah et al., 2018; Ranjitha et al., 2020), depending on the tallow parts and the cattle diet. The lipids of beef tallow are primarily composed of triglycerides, with high proportions of SFAs and MUFAs, while containing lower amounts of polyunsaturated fatty acids (PUFAs). The lipids of beef tallow have preferable physicochemical properties such as high oxidative stability, low iodine value, and elevated melting points, due to their saturated nature. Additionally, their high thermal content makes them suitable for bioenergy applications, particularly biodiesel production. Furthermore, the low degree of unsaturation improves resistance to rancidity which enhances transportation and storage handling (Zeng et al., 2024).

DBT is a predominant byproduct waste from slaughterhouses, tanneries, wet markets, and meat processing facilities. This lipid-rich waste serves as a cost-effective feedstock for biodiesel production due to its wide availability and affordability, making it an economically viable option for sustainable fuel production. Energy recovery from DBT provides an effective waste management solution, reducing waste accumulation and minimizing harmful emissions. Additionally, using DBT for biodiesel implements WTE principle and supports ethical and sustainable energy practices by avoiding competition with food sources. Despite its low cost, DBT offers several advantages for the biodiesel industry. Its high contents of SFAs and MUFAs enhance biodiesel stability by reducing oxidation reactions with atmospheric oxygen. Its high energy content improves fuel performance, while its elevated flash point enhances the safety of handling, transport, and storage. Recent studies highlight advancements in lipid extraction technologies and biodiesel synthesis techniques for better utilization of DBT toward biodiesel production (Ilias et al., 2023; Nagappan et al., 2021; Olubunmi et al., 2022).

2.5 Methods of lipid extraction

As part of biodiesel synthesis, lipids are separated from feedstock using various extraction methods as illustrated in Figure 2.6. To increase the efficiency of lipid extraction, proper sample preparation is crucial before starting the process. This preparation typically begins with the removal of undesirable components and thorough cleaning of the samples. Additionally, dehydration of the samples is extremely important, as water content hinders access to the lipids and most solvents are immiscible with water. Dehydration can be achieved through thermal drying, typically using a low-temperature vacuum oven, or through freeze-drying using the lyophilization technique,

which is commonly employed for animal-based feedstocks (Hewavitharana et al., 2020). Apart from dehydration, reducing the sample size is another essential step in the extraction process. Smaller particle sizes provide a larger surface area, enabling better contact between the sample and the solvent which improves penetration and increases lipid yield.

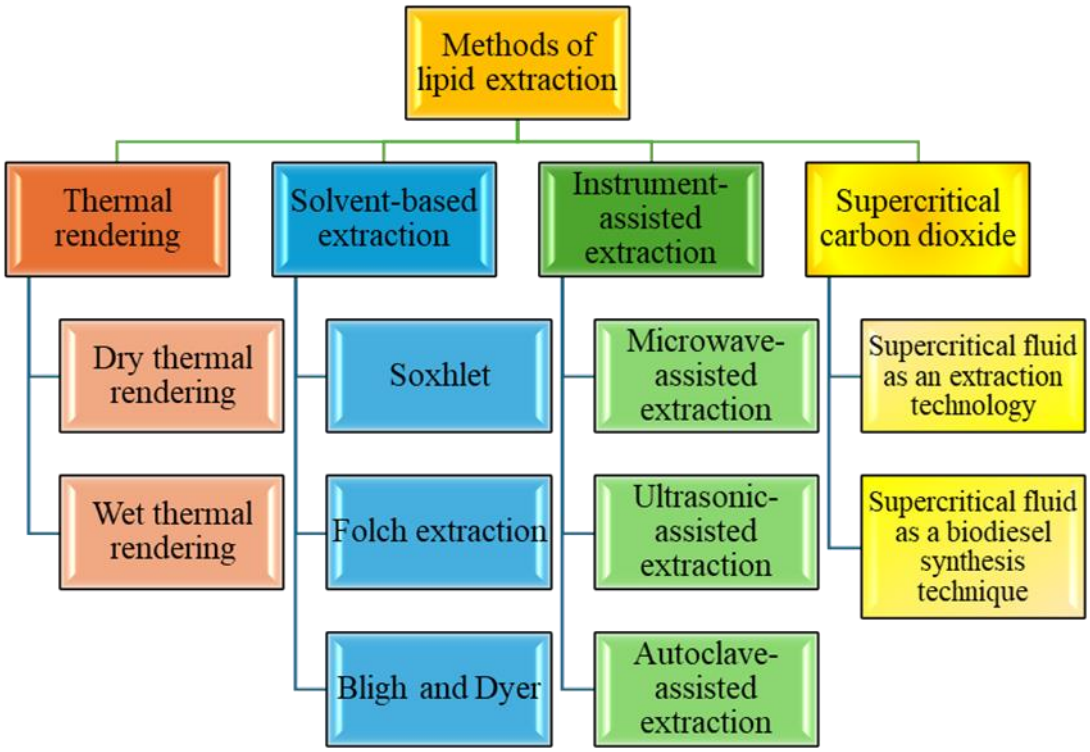

Figure 2.6 Methods of lipid extraction from animal-based feedstock

2.5.1 Thermal rendering

Thermal rendering is an extraction method that relies on heat to separate lipids from feedstock. It is commonly used for animal-based feedstocks. There are two approaches of thermal rendering which are dry and wet rendering.

2.5.1(a) Dry thermal rendering

Dry thermal rendering is one of the simplest lipid separation techniques. It involves exposing feedstock to a direct source of heat. This method is commonly used for fatty samples of animal-based feedstocks that contain abundant adipocytes within

subcutaneous and intramuscular tissues. Such oil-rich samples can be rendered into liquified lipids when exposed to sufficient heat. In some cases, a jacketed steam container is used to provide additional pressure during the rendering process. Temperatures applied for rendering animal-based feedstocks typically range from 70 to 150 °C. However, the quality of the produced lipids can be a concern because high temperatures affect the composition and characteristics of fatty acids contained in the samples. Although dry thermal rendering proved efficient as a lipid separation technique, the total yield mainly depends on factors such as temperature, rendering time, and the lipid content of the sample (Srinivasan et al., 2020). Table 2.2. summarizes previous studies that used the dry thermal rendering for lipid separation from various animal-based feedstocks.

Table 2.2 Lipid separation from animal-based feedstocks using dry thermal rendering

Animal-based feedstock	**Parameters**		**Yield (%)**	**Reference**
	Temp. (°C)	**Time (h)**		
Waste subcutaneous animal fat	120	-	88.47	Srinivasan et al. (2020)
Waste intramuscular animal fat	120	-	69.62	
Subcutaneous beef wastes	120	-	86.95	Ranjitha et al. (2020)
Intramuscular beef wastes	120	-	2.35	
Subcutaneous beef tallow	120	-	86.57	Jambulingam et al. (2019)
Lard fat	-	-	70	Dias et al. (2009)
Sheep tail	150	0.33	19.1	Alajmi et al. (2021)
Chicken fat	95	3	52.06	Hernández-Cruz et al. (2017)

2.5.1(b) Wet thermal rendering

Wet thermal rendering involves using heat and water to separate lipids from animal-based feedstock. In this process, the feedstock is mixed with water in a normal

or jacketed container. Heat is then applied to increase the system temperature, which causes the water to boil and the lipids to melt. Over time, this process produces a mixture of hot water and lipids. The mixture is then allowed to cool before separation. Conventional methods can be used to separate the components of this mixture of lipids, water, and residual solids. Filtration is employed to remove undesirable residual particles. If the lipid and water layers are clearly separated, decanting is an effective approach for separation. Alternatively, evaporation can be used to separate the components by exploiting the difference in boiling points between water and oil. Centrifugation is another effective approach, capable of separating even small amounts of lipids and residual solids. In terms of efficiency, wet thermal rendering generally yields less lipids compared to dry thermal rendering (Ranjitha et al., 2020; Srinivasan et al., 2020). Table 2.3 summarizes previous studies that employed wet thermal rendering for lipid separation from various animal-based feedstocks.

Table 2.3 Lipid separation from animal-based feedstock using wet thermal rendering

Animal-based feedstock	**Water content**	**Parameters**		**Yield (%)**	**Reference**
		Tempe. (°C)	**Time (h)**		
Cowhide fleshing	Water: sample 1:1	75	3	6.2	Abul Hashem and Shahruk (2017)
Mixture of beef waste materials	Sample: water 1:0.8	Boiling temperature	9	15	Chizoo et al. (2017)
Subcutaneous beef wastes	-	70 – 90 °C	-	76.78	Ranjitha et al. (2020)
Intramuscular beef wastes	-	70 – 90 °C	-	1.98	
Subcutaneous beef tallow	-	70 – 90 °C	-	74.32	Jambulingam et al. (2019)
Waste subcutaneous animal fat	-	Slightly below the boiling point	-	75.73	Srinivasan et al. (2020)