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Integrated Biodiesel Production and DES-Based Purification from Animal Fats: Comparative Effects of Feedstock, Alcohol, and Catalyst

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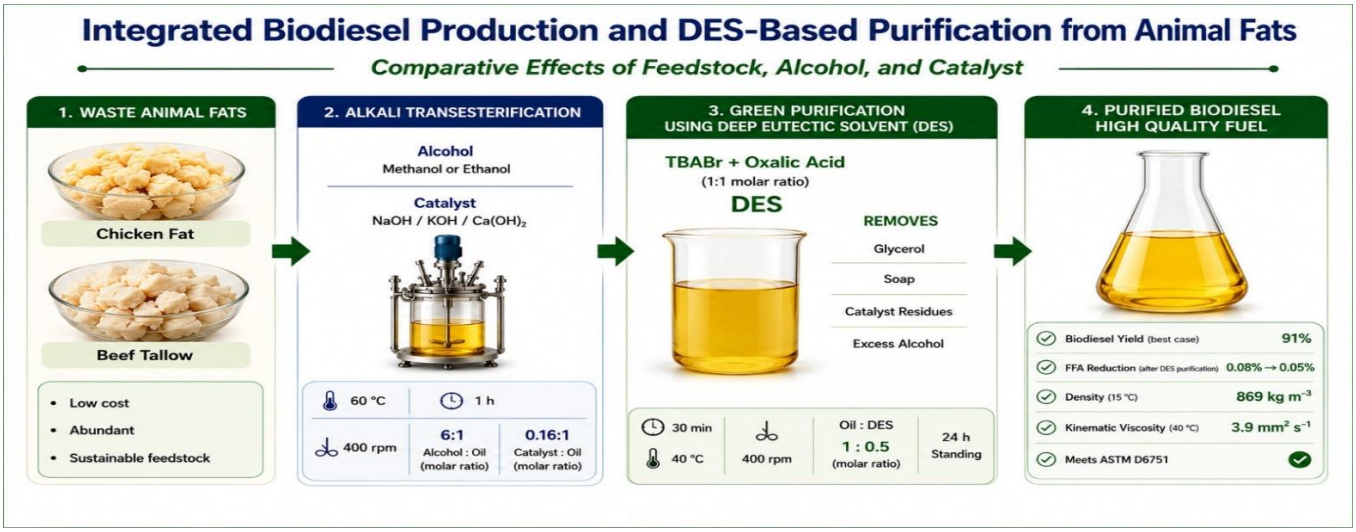
Abstract

Biodiesel is a sustainable alternative to fossil fuels; however, the high cost of feedstock and downstream purification processes remains a significant challenge. This work presents an integrated approach for biodiesel production from chicken and beef fats using an alkali-catalyzed transesterification process followed by purification with a deep eutectic solvent (DES) composed of tetrabutylammonium bromide (TBABr) and oxalic acid. The effects of feedstock type, alcohols (methanol and ethanol), and catalysts (KOH, NaOH, and $Ca(OH)_2$) were comparatively evaluated. The highest biodiesel yield, 91 ± 1%, was achieved using chicken fat, methanol, and NaOH under optimized conditions (60 ^oC, 1 hour, 400 rpm, a 6:1 alcohol-to-oil molar ratio). DES purification reduced acid value by ~37%, demonstrating effective impurity removal. Product quality was further validated through FT-IR, GC-FID, and physicochemical property analysis based on ASTM standards. The study demonstrates that the selection of feedstock, alcohol, and catalyst significantly influences reaction kinetics, phase separation, and mass transfer during process performance. The proposed integrated approach provides an efficient and environmentally friendly strategy for biodiesel production and purification.

Keywords: Biodiesel, Animal Fat, Deep Eutectic Solvent, Transesterification, Purification



Graphical Abstract

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1. Introduction:

Renewable energy is receiving increasing attention as a strategy to address natural and financial challenges. The overuse of fossil fuels and their rising costs have impacted international markets. Furthermore, excessive reliance on fossil fuels intensifies greenhouse gas concentrations, ultimately driving global climate change [1]. Over the next quarter century, a 60% increase in fuel demand is projected. Consequently, many communities worldwide are shifting toward recovering biofuels from renewable energy sources—such as wind, hydro, and biomass—to meet energy requirements [2].

Biodiesel is a mono-alkyl ester that is safer, more sustainable, and easily derived from renewable sources. Its combustion properties and efficiency surpass those of conventional fossil fuels [3]. Vegetable oils cannot be used directly due to their elevated viscosity and limited oxidation stability. Therefore, vegetable oils are converted into biodiesel via dilution, micro-emulsification, pyrolysis, and transesterification [4], [5]. Researchers prioritized the transesterification method for biodiesel generation because it is economical, highly efficient, and versatile regarding raw materials. It entails reacting triglycerides with methanol or ethanol in a catalyzed environment and significantly reduces oil viscosity, ensures the fuel blends well with petroleum diesel, and produces glycerol as a valuable byproduct [6]. The biodiesel production process comprises five key stages: raw material treatment, alcohol-catalyst mixing, transesterification reaction, product separation, and purification.

Biodiesel can be synthesized from feedstock of first, second, third, and fourth generations, as shown in Figure 1. The source of first-generation feedstock is edible oil crops and animal fats, and directly competes with the agrifood value chain [7]. The feedstock in second-generation biofuel covers non-nutritional crops, spent cooking oil, and residual bovine fats that are inappropriate for dietary use due to the presence of harmful materials [8]. Although algae and microorganisms, which are the source of third-generation biofuel, offer various advantages over other feedstocks, the oil extraction process requires significant modification [9]. Fourth-generation feedstock utilizes genetically engineered biomass that is cultivated using high solar energy input and integrated carbon dioxide (CO_2) capture technology. Despite the numerous benefits of fourth-generation biofuel, this technology is the least mature due to its significant capital investment and extended processing time [10]. Among second-generation feedstock, waste animal fats are attractive as a result of being inexpensive and readily available; however can be harder to handle by virtue of being sensitive to saponification depending on FFA (free fatty acids) content, phase separation problems, high cloud point, and reaction conditions [11]. The catalyst is incorporated to enhance the transesterification reaction by increasing the contact area between two liquid reactants (oil and alcohol). These catalysts are broadly classified as chemical or biological (enzymatic) [12]. Chemical catalysts are categorized into two main types: Homogeneous catalysts contain base or acid catalysts, and Heterogeneous catalysts contain solid acid, base, acid–base bifunctional, biomass waste-based, and nano catalysts [13]. Enzymatic catalysts occur in free and immobilized lipase, with immobilized lipase on nanomaterial support being a current prominent technology [14]. The catalyst selection is determined by the oil quality, percentage of FFA in oil, operating parameters, catalyst efficiency needed, price, and availability [15]. Alkali catalysts are still favored in biodiesel production because of the highest yield with the fastest synthesis time under the mildest operational demands [16].

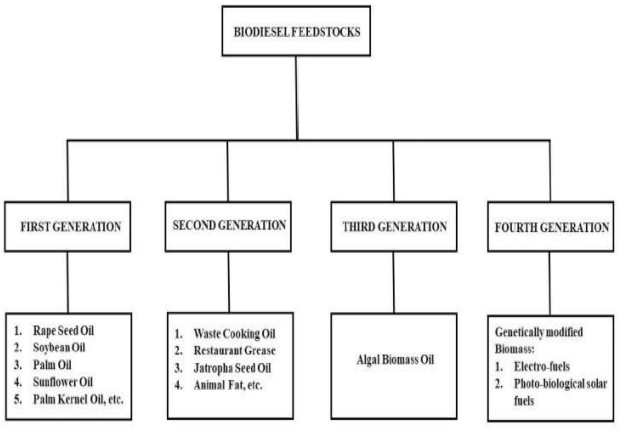

Figure 1: Biodiesel Feedstocks [17]

Biodiesel purification is a mandatory stage in the overall processing sequence, as it directly determines the final quality of the biodiesel produced. This is because the crude biodiesel resulting from the transesterification contains glycerol, unreacted alcohol, and lipids, residual

catalyst, soaps, and water as impurities [18]. The search for the best and most efficient ways to purify biodiesel has intensified, resulting in the development of numerous purification methods. The summary of each method has been reported in Table 1 [19]. A fundamental challenge in modern biodiesel processing is the cost-intensive and ecological concerns of established purification techniques (wet and dry washing). In contrast, Deep Eutectic Solvents (DESs) offer greener, low-cost, biodegradable, and highly selective substitutes of traditional solvents [16].

Table 1: Summary of the market and ecological effects of common purification processes [19]

Type of purification method		Efficiency (%)	Cost	Energy consumption	Environmental impact
Wet washing		> 90	Moderate	Low	Significant (if untreated)
Dry washing	Distillation	100	High	High	Moderate to High
	Adsorption	> 95	Variable	Low to Moderate	Low
	Membrane separation	90 - 95	Moderate to High	Moderate	Low
	SAC	98 - 99	High	High	Moderate to High

SAC = Solvent Aided Crystallization

From a chemical engineering perspective, the transesterification process involves both reaction kinetics and mass transfer limitations [20]. The immiscibility of oil and alcohol phases initially restricts reaction rate; however, adequate mixing improves interfacial contact and enhances conversion. The reaction follows pseudo-first-order kinetics under excess alcohol conditions [21]. However, side reactions such as saponification reduce catalyst efficiency, particularly in high-FFA feedstocks. Phase equilibrium plays a critical role during separation [22]. The DES system improves separation by extracting polar impurities, thereby enhancing phase disengagement and reducing processing time. A recent study has highlighted the utilization of deep eutectic solvents as a catalyst in oil esterification [23] and in other research publications, DESs are used as purification media for the removal of soap in biodiesel [16]. In previous studies, DESs have been applied as a catalyst or co-catalyst for the investigation of the transesterification reaction [24], [25]. Most of the work has focused on biodiesel derived from vegetable oils or has examined DES systems in a generalized manner, without considering the strong influence of feedstock type on separation behavior. In particular, biodiesel produced from waste animal fats presents distinct challenges, such as high free fatty acid (FFA) content and difficulties in downstream purification [11]. In addition, although alcohol and catalyst selection are known to significantly affect transesterification efficiency [15], [16], their combined impact on downstream DES-assisted purification has received little detailed attention [16], [19].

In practical biodiesel processing, the stages of production and purification are closely linked rather than completely separate, since the reaction conditions often influence phase separation behavior, impurity distribution, and the overall efficiency of the process [19]. However, this relationship has not been explored in detail, particularly for animal fat-based feedstock, which tends to behave differently from vegetable oils due to their more complex composition [11]. Most of the available studies focus either on the transesterification step or on purification techniques independently [16], [24], and therefore do not fully reflect the interconnected nature of real biodiesel processing systems. DES-assisted extraction has been reported as an effective route for removing polar contaminants (e.g., glycerol, soaps, and residual catalyst) from biodiesel [26], which aims to replace the traditional water-washing technique that results in emulsion formation, increased wastewater generation, and higher processing costs [19], [27]

Despite significant progress in biodiesel production, limited studies have focused on integrating production and purification into a single optimized framework. Furthermore, comparative evaluation of feedstock type, alcohol, and catalyst in conjunction with DES-based purification remains insufficiently explored. This study aims to address these gaps by developing an integrated and comparative approach for efficient biodiesel processing.

2. Experimental Procedure:

All experiments were conducted in duplicate, and the reported values represent the average of the measurements.

2.1. Materials:

Analytical grade methanol (CH_3OH), ethanol (C_2H_5OH), sodium hydroxide (NaOH), potassium hydroxide (KOH) and calcium hydroxide ($Ca(OH)_2$), Tetrabutyl ammonium bromide (TBABr) ($[(CH_3CH_2CH_2CH_2)_4N]^+ Br^-$) and Oxalic acid ($(COOH)_2$) were supplied from E.Merck and specimens of chicken and beef fats were purchased from domestic markets in Karachi. All chemicals were used as received.

2.2. Oil Recovery from Animal Fat:

In the current study, oil was separated from animal fat using a thermal melting method. Initially, raw animal fats were acquired from local slaughterhouses, washed, and air-dried to exclude dust and other impurities. Decontaminated fats were then placed in a vessel and heated over a direct flame. In alignment with established literature, the fat was melted by careful monitoring of temperature at 100 °C to facilitate evaporation of residual moisture. This heating phase continued until the moisture was removed, though prolonged heating was avoided to prevent the hydrolytic breakdown of lipids into free fatty acids (FFAs) [28]. Following extraction, the liquid oil was decanted, its acid value (free fatty acids) was determined, and it was placed in sealed vessels for further use. To assure optimal reaction yield, it is widely suggested that the FFA content of the feedstock remains less than 2%, as a high content of free fatty acids (FFAs) interferes with the main reaction through saponification and suppresses ester yield [29]. Beef and Chicken fats used in this research have FFA values of 1.32 ± 0.005% and 0.96 ± 0.005%, respectively.

2.3. Biodiesel Production:

Biodiesel was produced via the transesterification process using chicken and beef fat as feedstocks.

First, the required amount of alkali catalyst (NaOH, KOH, or $Ca(OH)_2$) was dissolved in methanol to prepare the alcohol–catalyst solution. The extracted fat was then added to this solution. The same process was repeated using ethanol.

The reaction mixture was stirred continuously and maintained at a temperature of 60 °C for 1 hour [30], [31], [32]. An alcohol-to-oil molar ratio of 6:1 [32] and an oil-to-catalyst molar ratio of 1:0.16 were used to ensure effective conversion.

After completion of the reaction, the mixture was allowed to settle for 24 hours to enable the separation of biodiesel and glycerol. The upper biodiesel layer was separated from the lower glycerol layer by decantation [33].

Finally, the obtained biodiesel was subjected to purification.

Table 2: Summary of Experimental Parameters and Specifications

Parameter	Specification
Feedstock	Chicken fat, Beef fat
Alcohol Type	Methanol, Ethanol
Catalysts	NaOH, KOH, $Ca(OH)_2$
Molar Ratio (Alcohol : Oil)	6:1
Catalyst Loading (Oil : Alkali)	1:0.16 (molar ratio)
Reaction Temperature	60 ^oC
Reaction Time	1 hour
Reaction stirring speed	400 rpm
Product separation time	24 hours

2.4. Biodiesel Purification:

The purification of the synthesized biodiesel was done by Deep Eutectic Solvent (DES). Tetrabutylammonium bromide (TBABr) as the hydrogen bond acceptor (HBA) and oxalic acid as the hydrogen bond donor (HBD) were combined in a 1:1 molar ratio to form a DES. This mixture was swirled at a constant temperature of 80 ^oC until a transparent, pale-yellow liquid was generated, signaling the accomplishment of the DES synthesis.

For the purification stage, the biodiesel was treated with the prepared DES at a specified oil-to-DES molar proportion of 1:0.5. The treatment was carried out for 30 minutes at 40 ^oC. Following the treatment, the resulting mixture was held to stand for 24 hours to ensure the complete clarification of biodiesel from the DES phase.

Table 3: Summary of DES Preparation and Treatment

Process	Variable	Specification
DES Composition	HBA : HBD	TBABr : Oxalic

Molar Ratio	HBA : HBD	1:1
Synthesis Conditions	Temp / Appearance	80 ^{o}C / Transparent pale-yellow
Purification Ratio	Oil : DES	1:0.5 (molar ratio)
Treatment Conditions	Time / Temp	30 minutes / 40 ^{o}C
Phase Separation	Standing Time	24 hours
Mixing	Stirring speed	400 rpm

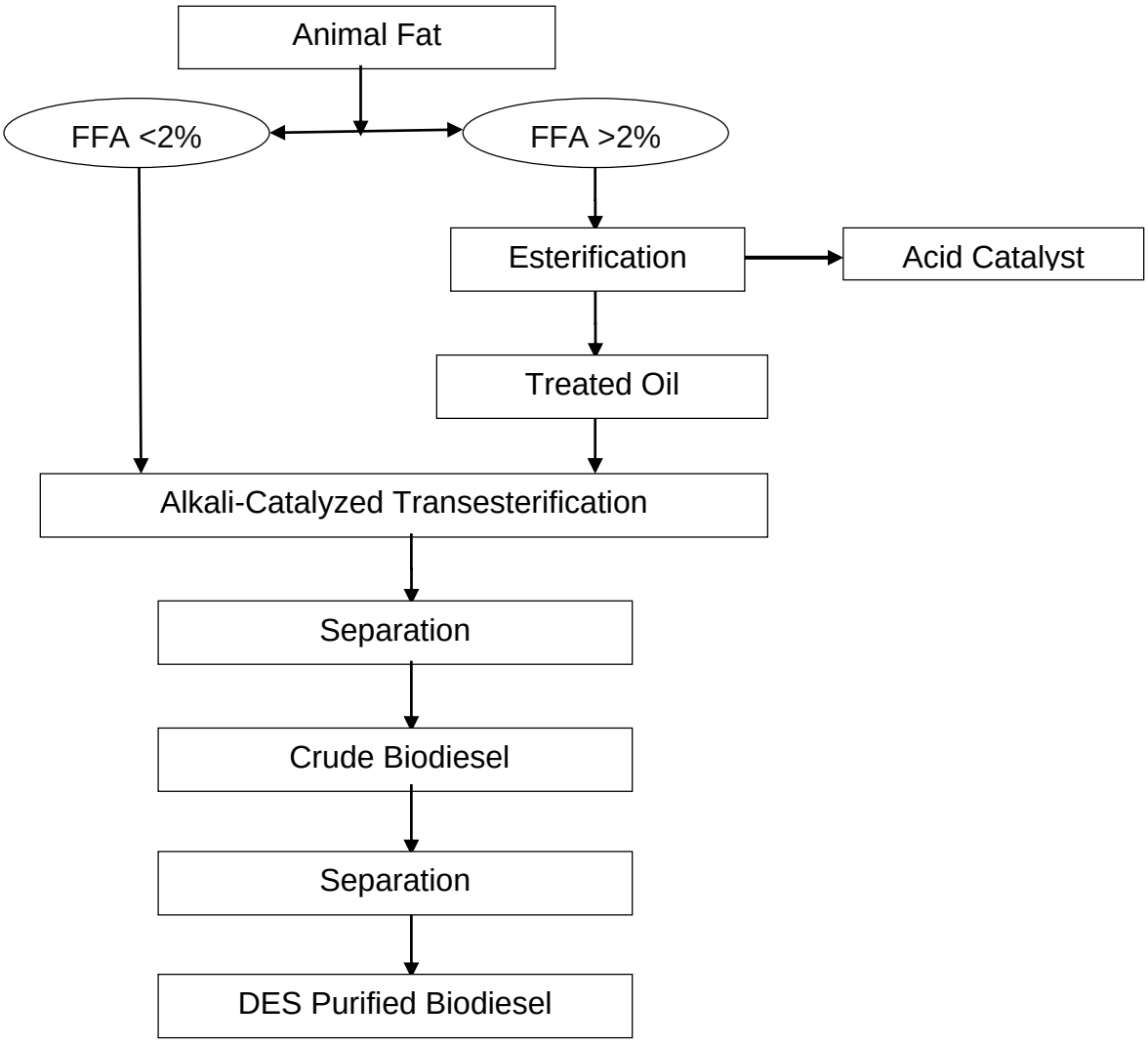

Figure 2: Schematic flow diagram for the integrated process of biodiesel production and purification [34]

2.5. Analytical Methods for Raw Materials, Crude Biodiesel, and Purified Biodiesel:

Acid value (AV) was determined using an acid number method consistent with standard potentiometric titration practice for biodiesel (ASTM D664). Fourier Transform Infrared (FT-IR) spectra were recorded in the range of 4000–500 cm^{-1} to track ester formation and impurity-related functional groups. An FT-IR spectrometer (Thermo Model Nicolet iS20) was used to record spectra of raw animal fat, crude biodiesel, and purified biodiesel. Samples were analyzed on a neat basis using SMART iTX ATR. These were recorded by co-adding 16 scans with 4 cm^{-1} resolution from 4000 cm^{-1} to 400 cm^{-1}. The spectrometer was controlled by OMNIC software. Gas Chromatographic (GC) analysis was employed to verify the formation of fatty acid alkyl esters (FAAE) and to compare crude versus purified biodiesel compositions. The experiments were carried out on a GC 2010 gas chromatograph (Schimadzu), which was installed with an FID detector, a column length of 100 m, and a temperature range of 20 to 250 ^{o}C.

2.6. Fuel Properties of Selected Biodiesel:

The selected biodiesel sample was characterized physicochemically to follow regulations with international standards. Density, kinematic viscosity, acid value, cloud point, flash point, and calorific value were included as key parameters and assessed by applying ASTM standard methods. To ensure operational suitability of fuel, the mentioned properties were then evaluated against ASTM D6751 specifications [35].

3. Results and Discussions:

3.1 Conversion Yield of Unrefined Biodiesel Generated by Chicken Fat Feedstock:

Chicken fat, methanol with potassium hydroxide, showed a balanced performance between biodiesel yield and ease of phase separation, making it suitable for practical operation. Although calcium hydroxide achieved the highest theoretical yield (~95%), its practical applicability remained limited due to poor phase separation (SES ≈ 2) and difficulty in recovering a clear biodiesel layer. This behavior can be attributed to the heterogeneous nature of $Ca(OH)_2$, which slows reaction kinetics [36] and promotes emulsion or soap formation under the given conditions. On the other hand, when ethanol was used, sodium hydroxide performed more effectively than potassium hydroxide, as it facilitated the formation of moderately stiff soaps that improved phase boundary definition and biodiesel recovery [37]. This indicates that alcohol–catalyst interactions significantly influence not only conversion but also product separation behavior. A cross-comparison interprets that chicken fat, methanol with sodium hydroxide, attained a superior conversion yield of 91% than methanol and potassium hydroxide, a system with a separation efficiency score (SES) of 5. (Figures 3a & 3b).

The quantitative analysis of the present study reveals a strategic trade-off between peak chemical efficiency and operational throughput. While established methanol–KOH systems achieve high-tier yields of approximately 96% [31], and extended reaction protocols reach 95.2% [38], the current work operates at a calculated yield of roughly 91% to 92%. This represents a marginal decrease of 4% to 5% in total product output. However, this numerical deficit is offset by significant gains in process economy. By substituting Potassium Hydroxide (M_w ≈ 56.11 g/mol) with Sodium Hydroxide (M_w ≈ 40.00 g/mol), the process utilizes a reagent with a lower molecular weight, effectively reducing the required catalyst mass by roughly 28% on a molar basis. Furthermore, the reduction in reaction time suggests a higher volumetric productivity, where the slight loss in yield is compensated by the ability to process more batches per unit of time. The accompanying data visualization confirms these findings, showing consistent performance above the 90% threshold with narrow error bars, indicating that the shift to a more cost-effective, shorter process remains statistically robust and commercially viable for large-scale integration with downstream DES purification.

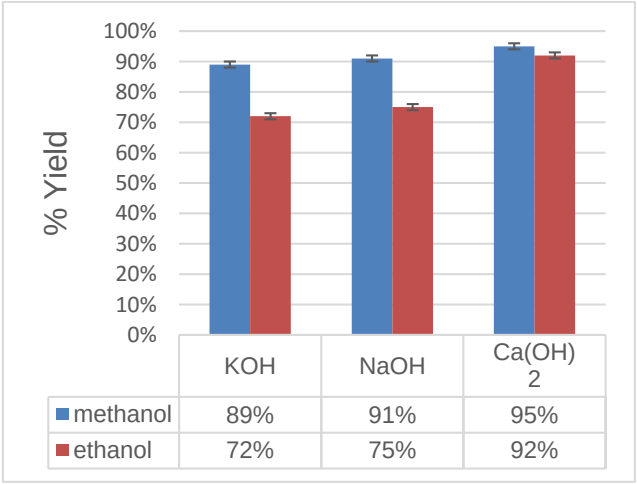

Figure 3 (a): Effect of catalyst type and alcohol on biodiesel yield (Chicken fat)

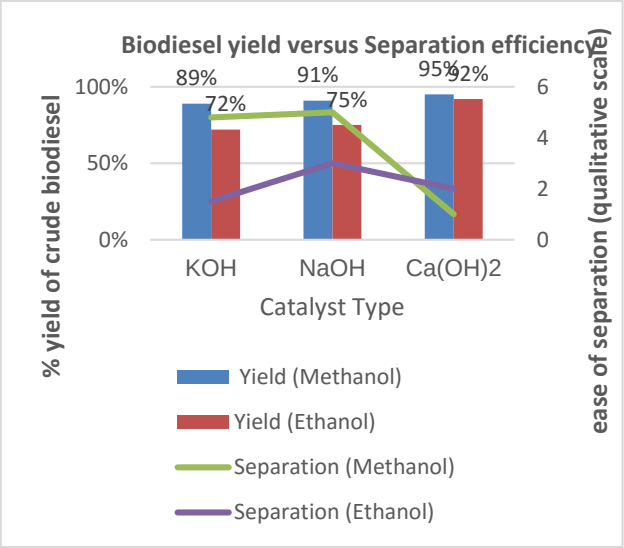

Figure 3 (b): Comparative analysis of percentage yield and separation efficiency of crude biodiesel produced from chicken fat using different catalysts (KOH, NaOH, $Ca(OH)_2$) and alcohols (Methanol vs. Ethanol)

3.2. Conversion Yield of Unrefined Biodiesel Generated by Beef Fat Feedstock:

The results indicate that the simple and quickest way to produce biodiesel from beef fat is the methanol-KOH system, while methanol with calcium hydroxide provides the 96% apparent biodiesel yield, suggesting superior chemical conversion (Figures 4a & 4b). Nevertheless, the separation of the reaction product was complicated (SES ≈ 2), limiting its practical usefulness. Methanol with potassium hydroxide proposed a more harmonized behavior, coupling a reasonable yield of 90% with steady and restorable phase separation (SES ≈ 4), which is crucial for the downstream refining process. For quick recovery of biodiesel, the use of methanol with sodium hydroxide, as well as ethanol with any of the alkaline catalysts examined, is not recommended for this feedstock, as these conditions consistently resulted in the formation of a solid mass rather than a separable biodiesel phase (SES ≈ 1). This behavior is likely associated with the higher free fatty acid content of beef tallow, which accelerates saponification reaction under unsuitable alcohol–catalyst combinations [39]. In previous studies, researchers follow two step transesterification method or varying process parameters when dealing with beef fat because of its sensitive nature [39], [40]. However, in this work, a yield of about 90% has been obtained despite the crucial handling issues of beef fat, which is higher than that of other scientists, as mentioned in Table 5 [30], [39].

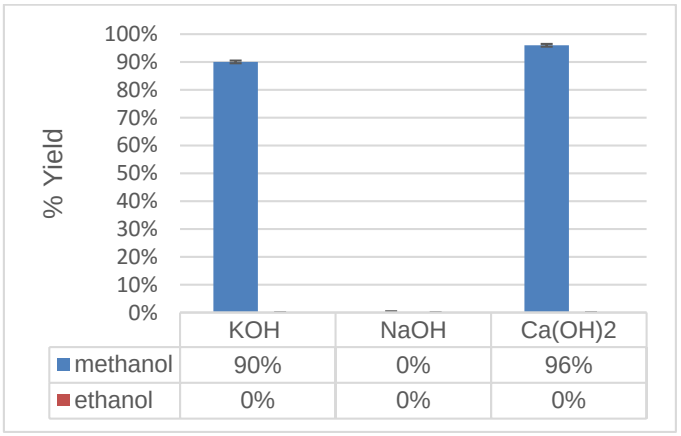

Figure 4 (a): Effect of catalyst type and alcohol on biodiesel yield (Beef fat)

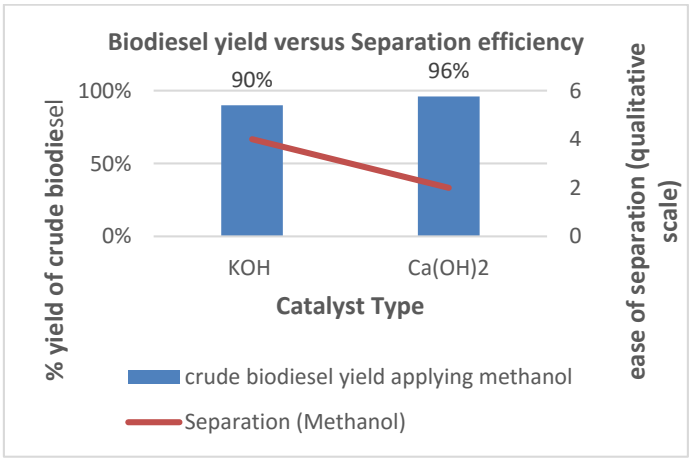

Figure 4(b): Comparative analysis of percentage yield and separation efficiency of crude biodiesel produced from beef fat using KOH, $Ca(OH)_2$, and methanol

3.3. Comparative Analysis of Unrefined Biodiesel Generated by Both Chicken and Beef Feed Stocks:

The investigation reveals that chicken fat exhibits greater process stability and versatility, as biodiesel formation was achieved using both methanol and ethanol, while beef fat declared sensitivity towards ethanol. For practical production, methanol with potassium hydroxide remains the most efficient and reliable choice for both feedstocks due to its ease of product separation and having a minimum tendency toward saponification [30]. Furthermore, chicken fat can be regarded as the more stable feedstock, as it retains biodiesel-forming capability even when paired with ethanol and sodium hydroxide, whereas, regardless of

alcohol type, beef fat malfunctioned with sodium hydroxide; this point is also validated by a researcher [40]. This contrast suggests that chicken fat possesses a more favorable fatty acid profile for alkali-catalyzed transesterification under a wider range of operating conditions, and it worked best with the methanol-NaOH system. The key comparative outcomes are summarized in Table 4.

Table 4: Comparative Outcomes of Successful Biodiesel samples

S. No.	Feedstock	Alcohol	Catalyst	Yield	Separation Ease	Separation Efficiency Score (SES)
1	Beef Fat	Methanol	KOH	90 ± 1%	Successful	4
2	Chicken Fat	Methanol	KOH	89 ± 1%	High	4.8
3	Chicken Fat	Methanol	NaOH	91 ± 1%	High/Clear	5
4	Chicken Fat	Ethanol	NaOH	75 ± 1%	Moderate	3

The following table compiles the data for biodiesel production yield and parameters reported by some researchers.

Table 5: Homogeneous base catalysts used in Transesterification

Homogeneous base catalysts	Feedstock	Alcohol type	Alcohol to oil molar ratio	Catalyst weight %	Reaction temperature (^{0}C)	Reaction time (h)	Biodiesel Yield	References
KOH	Chicken and Beef fats	Methanol	2.1:1	0.5%	60	1	88%	[30]
NaOH	Chicken Fat	Methanol	Not found	Not found	65	120 minutes	95.2%	[38]
KOH	Chicken Fat	Methanol	4:1	0.5%	60	1	96%	[31]
NaOH	Chicken by-product waste	Methanol	6:1	1%	60	1	79%	[32]
NaOH	Beef Fat	Methanol	6:1	1.5%	65	1	43.64%	[39]

3.4. Refining of Biodiesel:

The TBABr–oxalic acid deep eutectic solvent (DES) operates through a combination of acid–base neutralization and selective extraction mechanisms. The hydrogen bond donor (oxalic acid) and hydrogen bond acceptor (TBABr) form a structured hydrogen-bonding network, resulting in a highly polar medium. This polarity enables selective interaction with polar impurities such as glycerol, residual catalyst, soaps, and free fatty acids.

The mechanism primarily involves:

- Hydrogen bonding interactions between DES components and hydroxyl-rich glycerol molecules.
- Electrostatic interactions between bromide ions and charged soap species.
- Acid-base neutralization of residual alkaline catalyst.

The selection of TBABr–oxalic acid was based on its stability, low cost, ease of preparation, and strong affinity for polar contaminants, as reported in recent literature [23]. Furthermore, the use of TBAB/Oxalic acid as a DES aided a swifter and more reliable phase separation without the emergence of emulsion and improved optical clarity, proving to be a green and superior replacement to the ordinary water washing method. Literature showed that a minimum of three cycles of water is needed to purify contaminated biodiesel, so a large amount of wastewater would be generated, and a small amount of water in the biodiesel layer can deplete fuel quality [41], [42]. The results are compiled in Table 6.

Table 6: Comparative study on quality of purified Biodiesel

S. No.	Feedstock	Alcohol / Catalyst	Purification Method	Final Product Quality
1	Beef Fat	Methanol+ KOH	TBAB/Oxalic DES	High (significant Acid Value reduction)
2	Chicken Fat	Methanol+ KOH	TBAB/Oxalic DES	Excellent
3	Chicken Fat	Methanol+ NaOH	TBAB/Oxalic DES	Superior
4	Chicken Fat	Ethanol+ NaOH	TBAB/Oxalic DES	Improved (lowered soap residue)

3.5. Acid Value or Free Fatty Acid of Unrefined and Refined Biodiesel:

The acid value and free fatty acids (FFAs) are key parameters for identifying the quality of biodiesel; minimal values typically refer to a superior quality end product [43]. In this study, the coupled work of biodiesel production and purification from beef tallow and chicken fat reveals an impactful reduction in Acid Value (AV) when utilizing the selected TBAB/Oxalic acid Deep Eutectic Solvent (DES) method. The Laboratory analysis illustrates that while preliminary production process results in relatively high %FFA levels fluctuating from 0.08 to 0.42%, the terminal purification process predominantly brings these values down to a range of 0.05 to 0.26% (Table 7). It is a pivotal change, as it ensures the reaction end products meet the international fuel compliance ASTM D6751, which requires a maximum acid value of 0.5 mg KOH/g (equivalent to approximately 0.25% FFA). Except for beef fat, it is slightly higher than the reference point. By obtaining values as low as 0.05%, the purification method not only meets but significantly exceeds these safety and quality standards, ensuring the fuel is non-corrosive and chemically stable for engine use, as most of the washing techniques did not satisfy the standard acid value criteria, and it is permissible up to 0.4% in Austria [42].

Table 7: Comparative Analysis of Acid Value Reduction

S. No.	Feedstock and Catalyst	Initial %FFA	Final %FFA	% Reduction	ASTM D6751
1	Beef Tallow (Methanol+ KOH)	0.42 ± 0.005%	0.26 ± 0.005%	~38%	<0.25%
2	Chicken Fat (Methanol+ KOH)	0.38 ± 0.005%	0.24 ± 0.005%	~36.8%	<0.25%
3	Chicken Fat (Methanol+ NaOH)	0.08 ± 0.005%	0.05 ± 0.005%	~37.5%	<0.25%
4	Chicken Fat (Ethanol+ NaOH)	0.2 ± 0.005%	0.14 ± 0.005%	~30%	<0.25%

3.6. FT-IR Analysis of Raw Materials, Unrefined and Refined Biodiesel:

FT-IR analysis was performed on the raw lipid feedstock and the relevant crude and purified biodiesel samples in the spectral range of 4000 cm^{-1} to 500 cm^{-1} to observe changes in functional group composition during the transesterification and refining process. The spectra of the biodiesel samples indicated characteristic absorption or transmittance bands associated with ester functional groups, satisfying the successful conversion of triglycerides into fatty acid alkyl esters. In comparison with the contaminated biodiesel, the refined samples showed reduced intensity of bands associated with hydroxyl-containing impurities, manifesting effective removal of residual glycerol and soap species following DES treatment. These peak shifts further account for the role of TBAB/oxalic acid DES in improving biodiesel purity without altering the basic ester structure of the biodiesel.

Table 8: Individual functional groups found in standard biodiesel

Component	Location	Significance	References
Carbonyl Stretch (C=O)	1742 cm^{-1}	Represents the ester group	[44]
Methyl (O-CH3)	Around 1170 cm^{-1}	Fingerprint region for methyl esters.	[2]
C-O alkoxy esters, ethers, and C-O-C stretching	1030.98 - 1240.08 cm^{-1}	Indication of fatty acid methyl esters	[45]
Methyl group – CH_3 / CH_2 bending	1425-1447 cm^{-1}	Related to the methyl group of the ester	[46]
Alkanes (C-H) asymmetrical or symmetrical stretching	2800-3000cm^{-1}	Long hydrocarbon chains are inherited from the original fats or oils.	[45]

Table 9: Recognition of fundamental impurities in biodiesel

Component	Location	Significance	References
Residual glycerine	1260-900 cm-1	Peaks indicate the presence of a byproduct in this region	[47]
Soap formation	1550 – 1600 cm^{-1}	Peak in this region indicates the formation of soap.	[48]
O-H stretching	3300-3500 cm^{-1}	moisture or residual alcohol indicates in this region	[45]

3.6.1. FT-IR Analysis of Beef Fat-Derived Biodiesel:

The infrared spectra (FT-IR) of Fatty Acid Methyl Esters (FAME) successfully verify the characteristic functional groups, distinctly at 1742 cm^{-1}, 1170 cm^{-1}, 1030.98 - 1240.08 cm^{-1}, and 1425 - 1447 cm^{-1} [44]. The spectra for beef fat and biodiesel reveal significant convergence by reason of their similar hydrocarbon backbones, key transition shifts validate the transesterification (Figure 5), particularly, the peak observed at approximately 1435 cm^{-1} corresponds to the asymmetric stretching of the $–CH_3$ group, signaling the effective attachment of the methoxy group from methanol to the fatty acid chains [47]. Furthermore, the distinct morphological change in the peaks within the 1030.98 - 1240.08 cm^{-1} region serves as primary evidence of notable transesterification from triglycerides to methyl esters [45].

For purified biodiesel (Figure 5), the absence of broad absorption bands in the 3300 - 3500 cm^{-1} region indicates removal of residual methanol or water, in addition, absence of sharp peaks in both 1550 – 1600 cm^{-1} and 1260-900 cm^{-1} regions validates the removal of soap and residual glycerin respectively supports that the biodiesel has been refined successfully [45], [47], [48].

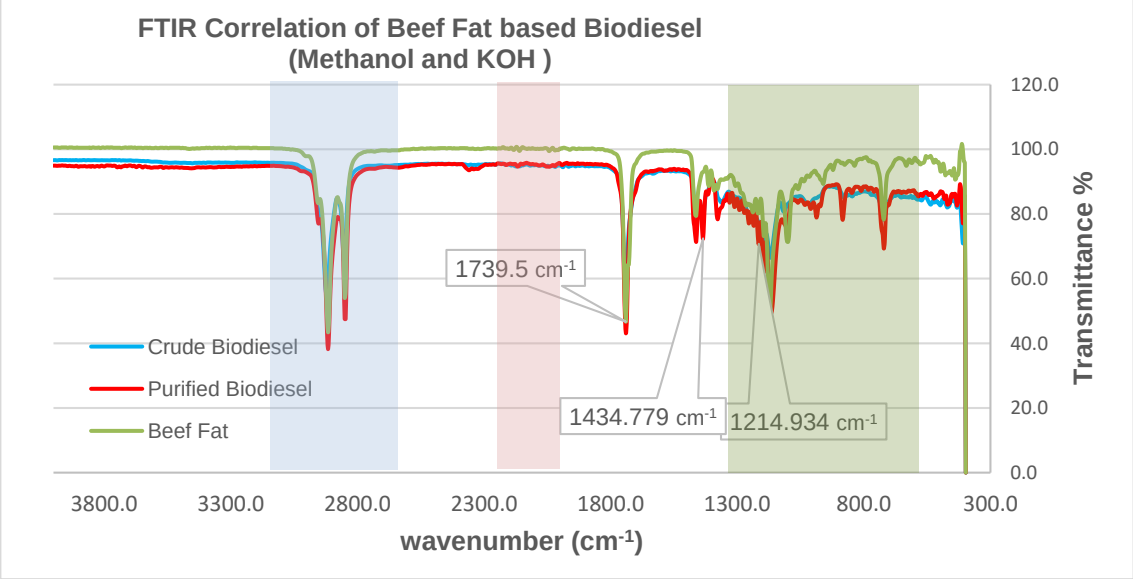

Figure 5: FT-IR spectra comparison of raw beef fat, crude biodiesel (Methanol/KOH), and DES-purified biodiesel

3.6.2. FT-IR Analysis of Chicken Fat-Derived Biodiesel in Methanol-KOH and Methanol-NaOH Systems:

The FTIR spectra of chicken fat-derived biodiesel produced using methanol with KOH and NaOH catalysts confirm successful transesterification in both systems. In each case, a strong ester carbonyl (C=O) stretching band appears at approximately ~1740 cm^{-1}, along with characteristic C–O stretching vibrations, indicating the formation of fatty acid methyl esters (FAMEs) (Figures 6 and 7) [44]. Moreover, the fundamental signal of efficient transesterification is observed in the fingerprint region (1000–1500 cm^{-1}), where the distinct transmittance or absorption patterns of the triglyceride's glycerol backbone in the original chicken fat shift to reflect the methoxy ($-OCH_3$) group characteristic of methyl esters [45], [46].

Crude biodiesel shows higher transmittance in several regions due to impurities such as soap, glycerol, unreacted methanol, and residual KOH, whereas purified biodiesel exhibits reduced transmittance, indicating improved purity. In both cases, for isolation of impurities like soap and glycerin, the spectral regions between 1550 – 1600 cm^{-1} [48] and 1260-900 cm^{-1} [47], respectively, could be observed. A comparison of both catalytic systems shows similar spectral profiles; however, the NaOH-catalyzed system exhibits slightly more pronounced ester peak intensity, suggesting a higher degree of conversion under the same reaction conditions (Figures 6 and 7).

The shift between 3300 cm^{-1} and 3600 cm^{-1} [45] interprets the powerful removal of unreacted methanol and residual moisture during the post-reaction washing and drying phases, confirming the finished product with

high-quality biodiesel in methanol-NaOH system (Figures 6 and 7). Overall, FTIR analysis validates effective biodiesel production from chicken fat using both KOH and NaOH catalysts, with NaOH showing marginally improved purification efficiency.

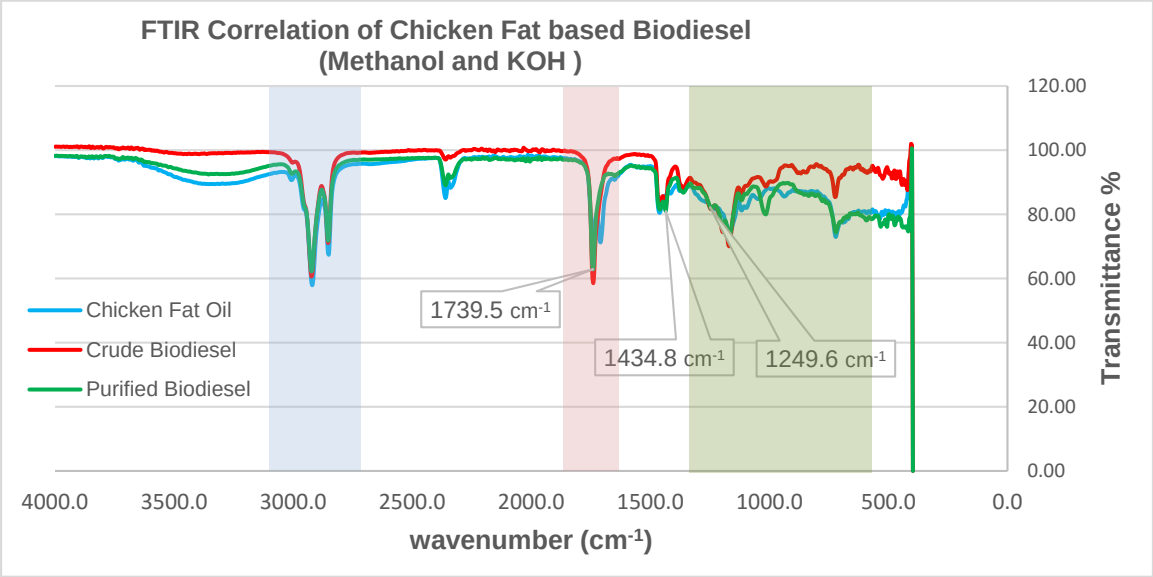

Figure 6: FT-IR spectra comparison of raw chicken fat, crude biodiesel (Methanol/KOH), and DES-purified biodiesel

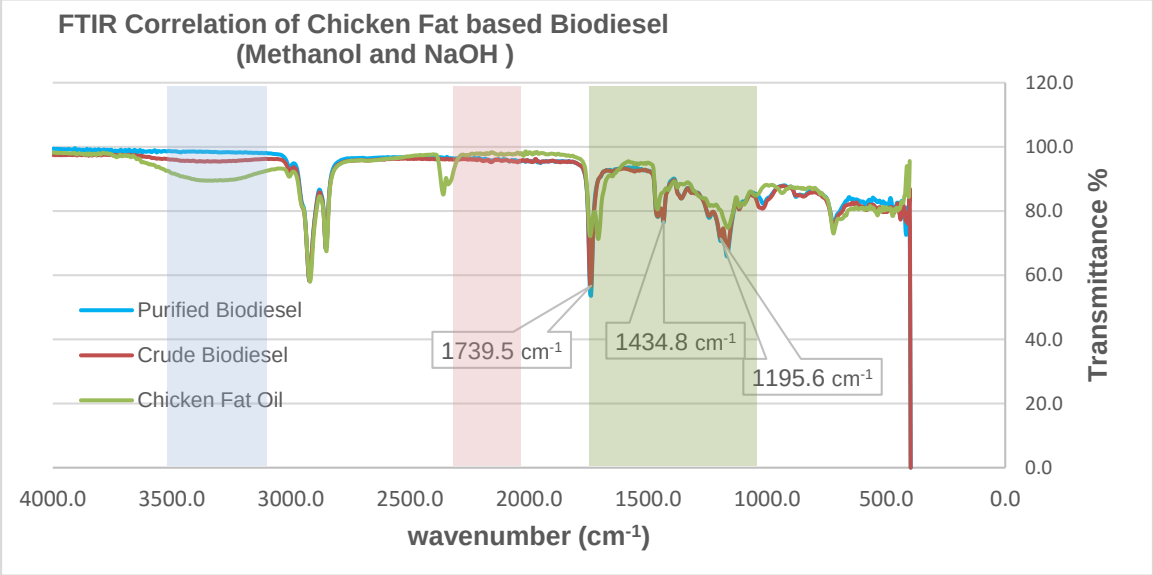

Figure 7: FT-IR spectra of raw chicken fat, crude biodiesel (Methanol/NaOH), and DES purified biodiesel

3.6.3. FT-IR Analysis of Chicken Fat-Derived Biodiesel in Ethanol and NaOH System:

The superimposed spectra of the raw chicken fat, crude and purified biodiesel (Fatty Acid Ethyl Esters, FAEE) were monitored using Fourier Transform Infrared (FT-IR) spectroscopy, and it illustrates the effective transesterification process through the identification of key functional groups (Figure 8). In the refined biodiesel, a sharp and intense peak is observed at 1731.8 cm^{-1}, which is characteristic of the ester functional group in FAEE. The most diagnostic feature of the conversion is the carbonyl (C=O) stretching vibration [44]. Furthermore, the spectral shift from contaminated biodiesel to non-contaminated biodiesel in this region indicates how DES is working effectively to save ester identity. The presence of aliphatic hydrocarbon chains is confirmed by the strong asymmetric and symmetric C-H stretching vibrations at 2927.4 cm^{-1} and 2854.1 cm^{-1}

[45], respectively, and these peaks remain relatively uniform in all spectrums, as the long-carbon chain backbone of the fatty acids remains intact during the reaction. As well as the evidence of the esterification identified specifically in the "fingerprint region" between 1000 cm^{-1} and 1300 cm^{-1} [45] and the peaks at 1195.6 cm^{-1} and 1029.8 cm^{-1} correspond to the C-O stretching vibrations. Additionally, the refined resolution of these peaks in the purified sample, compared to the chicken fat oil, reflects the successful replacement of the glycerol backbone with ethyl groups derived from the ethanol solvent (Figure 8).

The production of biodiesel in this system was a critical challenge as chicken fat has high Free Fatty Acid (FFA) content, which can lead to saponification (soap formation) when using a strong base catalyst like NaOH along with ethanol [49]. Spectroscopically, soap is identified by a strong absorption band in the 1550 - 1600 cm^{-1} range, and in the analyzed spectra [48], the absence of a dominant peak in this region for the refined biodiesel suggests that the washing and purification steps were highly effective. Whereas a minor shoulder is observed at 1627.6 cm^{-1}, this is possibly assigned to C=C stretching in the unsaturated fatty acid chains (such as oleic or linoleic acid) native to chicken fat [50], instead of significant soap residue (Figure 8).

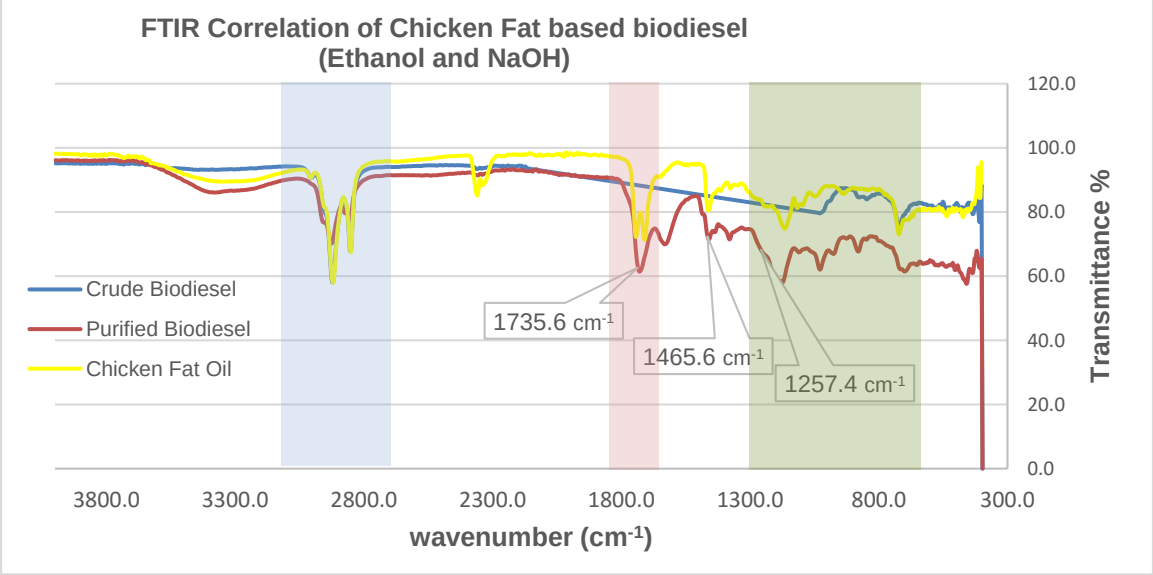

Figure 8: FT-IR spectra comparison of raw chicken fat, crude biodiesel (Ethanol/NaOH), and DES-purified biodiesel

3.6.4. Comparative Study of Purified Biodiesel FT-IR Spectrum:

The meaningful FTIR spectra interpret that the transesterification of animal fats into fatty acid alkyl esters or biodiesel has been achieved in these successful samples, as evidenced by the prominent ester carbonyl peak around 1740 cm^{-1} (Figure 9). The application of methanol, being a smaller molecule than ethanol, offers better kinetic reactivity and clear phase separation from glycerol, whereas NaOH delivers a higher molar potency and more manageable soap formation compared to KOH [49]. The high-quality biodiesel is vital for meeting fuel compliance and inhibiting engine deposits or corrosion therefore best sample is isolated among successful refined biodiesel samples to test the some physicochemical properties and it is reflected in the spectrum of chicken fat, Methanol and Sodium Hydroxide (NaOH) system by exceptionally flat baseline in the 3300-3500 cm^{-1} region, interpreting a drier, superior quality fuel [45].

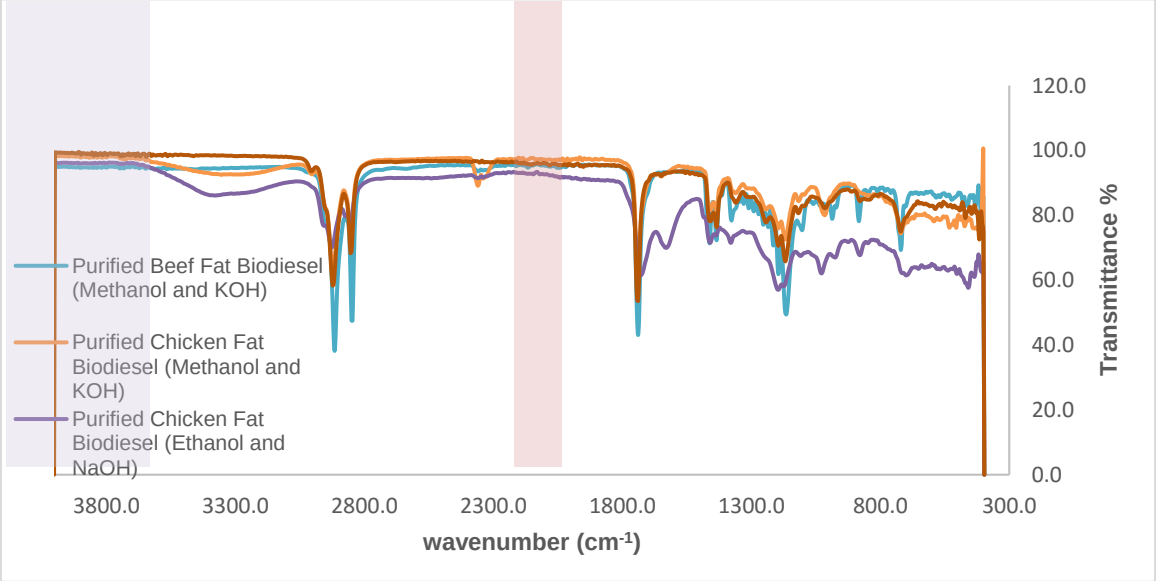

Figure 9: Comparative FT-IR Spectra of Methyl and Ethyl Esters Synthesized from Beef and Chicken Feedstocks

3.6.5 FT-IR Spectrum of Deep Eutectic Solvent:

Deep Eutectic Solvent (DES) synthesis is analyzed spectroscopically, and the major changes are observed in the vibrational spectra, representing the successful fusion of salt (TBABr) and acid into a single liquid phase. It is mechanized by non-covalent interactions, explicitly hydrogen bonding. An extended signal is observed at 3378.7 cm^{-1} (94.7% transmittance), which is a key indicator of the hydroxyl group (O-H stretching) of Oxalic Acid involved in intermolecular hydrogen bonding within the DES [51]. The characteristic peak at 1743.3 cm^{-1} (47.2% transmittance) illustrates the C=O stretching vibration [44] (Figure 10). The unique shift of this peak in the DES spectrum indicates that the carboxylic acid environment has been altered by the existence of the bromide anion. The sharp peak at 1149.4 cm^{-1} (30.1% transmittance) is assigned to the C-O stretching of the acid backbone, showing a high degree of integration in the eutectic mixture [45]. The peaks at 2958.3 cm^{-1} and 2877.3 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the aliphatic C-H bonds, respectively, specifically the methyl (CH_3) and methylene (CH_2) groups in the butyl chains of the $[TBA]^+$ cation [52]. The preservation of these peaks in the DES (green) indicates that the quaternary ammonium structure remains chemically stable (Figure 10). The DES spectrum shows a unique profile in the 1100--1200 cm^{-1} region, likely representing C-O stretching and C-N vibrations, which are distinct from the starting materials, further confirming the successful homogenization of the two components into a single liquid phase [51]. The peaks observed around 1469.5 cm^{-1} and 1380 cm^{-1} range in both the TBABr and DES are attributed to C-H bending, further verifying the detection of the salt component [23], [51], [53].

The robust hydrogen-bonding network observed—specifically the O-H and C=O shifts—creates a highly polar environment within the DES [23]. Biodiesel is non-polar; it does not participate in this polar network. However, glycerol molecules contain three hydroxyl (-OH) groups, readily form new hydrogen bonds with the bromide anion (Br^-) and the oxalic acid in the DES, resulting in a sharp spontaneous extraction, where the polar DES layer captures the glycerol, leaving the pure biodiesel in a distinct upper layer. Furthermore, the acidic nature of the oxalic acid within the DES helps the isolation of residual free fatty acids (FFAs) from impure biodiesel, and DES acts as an extractive agent to minimize the Total Acid Number (TAN) of the fuel. The same O-H stretching interactions identified at 3378.7 cm^{-1} allow the DES to capture the carboxyl groups of the FFAs, pulling them out of the biodiesel phase (Figure 10). Finally, residual methanol and catalysts often remain in crude biodiesel after production, and the extensive H-bonding network described allows the DES to dissolve these excess polar reagents. By utilizing this DES, impurities of biodiesel could be minimized to such levels that meet international quality regulations, such as EN 14214 or ASTM D6751.

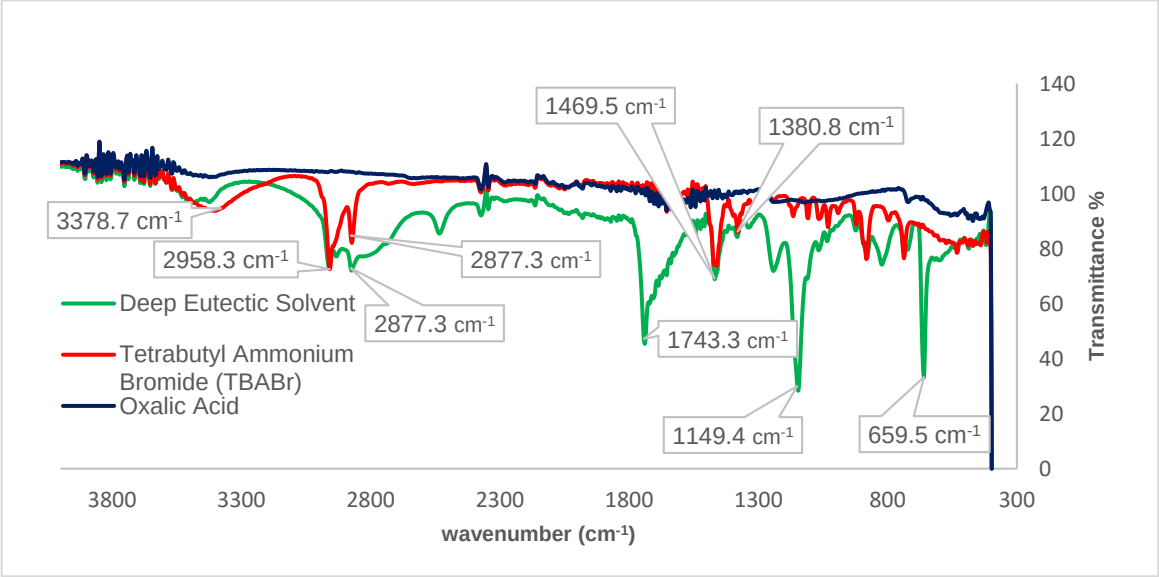

Figure 10: FT-IR spectra of TBABr, Oxalic Acid, and the synthesized Deep Eutectic Solvent (DES) TBABr : Oxalic Acid (1:1)

3.7. Physicochemical Properties of Selected Biodiesel Sample:

The beef-based biodiesel sample obtained a cloud point higher than 12 °C; since that temperature is beyond the ASTM D6751 specification, it was deemed unsuitable for further analysis. Consequently, the purified chicken fat biodiesel sample, produced via methanol and sodium hydroxide (NaOH) catalyzed transesterification process, was selected for property analysis based on FTIR results. The specific physicochemical properties of this sample were experimentally measured and matched with ASTM D6751 international regulations.

Table 10: Physicochemical Properties of Purified Chicken Fat Biodiesel produced via Methanol and NaOH Transesterification

Properties	Biodiesel	ASTM D6751 [34]	Standard Test Methods
Density at 15 °C	869 ± 0.5 Kg/m^{-3}	860-900 Kg/m^{-3}	ASTM D 4052
Kinematic viscosity or viscosity (40 °C)	3.9 ± 0.05 mm^2 s^{-1}	1.9–6.0 mm^2 s^{-1}	ASTM D 445
Acid Value	0.06 ± 0.05 mg KOH g^{-1}	0.50 mg KOH g^{-1} max.	ASTM D 664
Cloud Point (°C)	6 ± 0.5 °C	-3 to 12 °C	ASTM D 2500
Flash Point (°C)	145 ± 0.5 °C	100 to 170 °C	ASTM D 93
Calorific value (MJ/kg)	40 ± 0.5 MJ/kg	-	ASTM D 3338

3.8. GC-FID Analysis of Raw Materials, Unrefined and Refined Biodiesel:

The GC-FID technique was utilized to examine fatty acid composition in raw materials, pure biodiesel, and impure biodiesel. The GC-FID results show that beef fat contains saturated fatty acids more than chicken fat, while beef fat showed resilience towards biodiesel formation, so it could be interpreted that unsaturated fats have a greater tendency for the production of biodiesel. Specifically, crude and purified biodiesel samples of chicken fat with methanol and KOH, and methanol and NaOH systems were taken into consideration for observing reaction progress. As unsaturated fatty acids get easily oxidized [54], it is also noticed that the content of saturated fatty acids is increasing from raw to finished product, which determines that pure and impure biodiesel may have hindrance towards oxidation due to the decline of unsaturated fatty acids more than raw chicken fat. Also, cold flow properties of FAME are influenced by unsaturated fatty acids [55]. The fatty acid profiles of beef and chicken fats align closely with the previous findings [30], [31] and the characteristics of both unrefined and refined biodiesel samples are consistent with the results reported by a scientist [56]. Notably, this process yielded superior outcomes, achieving full compliance with EN 14214 standards, mainly due to a 12% linoleic acid content [57].

Table 11: Fatty Acid Composition of Chicken fat, Beef fat, unrefined chicken fat biodiesel, and refined chicken fat biodiesel

S. No.	Fatty Acid	Carbon Number	Degree of Saturation	Fatty Acid %					
				Beef Fat (Wt.%)	Chicken Fat (Wt.%)	Methanol and KOH		Methanol and NaOH	
						Unrefined Chicken Fat Biodiesel (Wt.%)	Refined Chicken Fat Biodiesel (Wt.%)	Unrefined Chicken Fat Biodiesel (Wt.%)	Refined Chicken Fat Biodiesel (Wt.%)
1	Myristic acid	C14:0	Saturated	1.74	-	-	1.582	-	-
2	Palmitic acid	C16:0	Saturated	21.83	19.36	28.03	27.31	26.66	27.76
3	Stearic acid	C18:0	Saturated	25.51	5.17	6.88	6.808	4.424	7.259
	Total			**49.08**	**24.53**	**34.91**	**35.7**	**31.084**	**35.019**
4	Palmitoleic acid	C16:1	Monounsaturated	2.37	5.37	7.18	5.863	4.424	7.259
5	Oleic acid	C18:1	Monounsaturated	32.56	48.23	45.53	44.522	48.28	44.839
	Total			**34.93**	**53.6**	**52.71**	**50.38**	**52.704**	**52.098**
6	Linoleic acid	C18:2	Polyunsaturated	5.21	15.92	12.39	12.59	14.04	12.4
7	Linolenic acid	C18:3	Polyunsaturated	0.78	1.23	-	1.328	0.98	0.764
	Total			**5.99**	**17.15**	**12.39**	**13.918**	**15.02**	**13.16**
8	others	-		10	4.72	-	-	-	-

4. Conclusion:

This study presents an integrated approach for biodiesel production and purification from waste animal fats, combining alkali-catalyzed transesterification with deep eutectic solvent (DES)-based purification. The results demonstrate that biodiesel yield and quality are strongly influenced by feedstock type, alcohol selection, and catalyst.

Chicken fat exhibited superior adaptability across different reaction conditions, while beef tallow showed sensitivity due to higher FFA content and saponification tendency. Among the tested systems, methanol–NaOH with chicken fat produced the highest quality biodiesel with optimal yield (91 ± 1%) and lowest acid value (0.05 ± 0.005%). Methanol consistently outperformed ethanol, achieving ~10–15% higher conversion efficiency, while NaOH showed the highest catalytic activity among the tested catalysts.

The DES system (TBABr–oxalic acid) proved to be an effective and sustainable purification alternative, reducing acid value by approximately 35–40% and significantly improving phase separation by minimizing emulsion formation. The enhanced purification efficiency is attributed to hydrogen bonding interactions and polarity-driven extraction of impurities such as glycerol, soaps, and residual catalysts.

From a chemical engineering perspective, the study highlights the importance of reaction kinetics, mass transfer limitations, and phase equilibrium behavior in determining overall process efficiency. The integration of production and purification steps provides a more practical and scalable framework compared to conventional multi-step processes.

However, this work is limited to bench-scale experiments. Potential challenges in industrial implementation include mixing efficiency, mass transfer constraints, phase separation dynamics, and recovery/reusability of the DES system. Future work should focus on process optimization, kinetic modeling, and techno-economic analysis to evaluate large-scale feasibility.

Overall, the proposed integrated approach offers a promising, environmentally friendly strategy for efficient biodiesel production and purification from low-cost animal fat feedstocks.

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