

Skeletal Isomerisation of Methyl Oleate Using Zeolite- β : Catalyst Activity and Conversion Assessment

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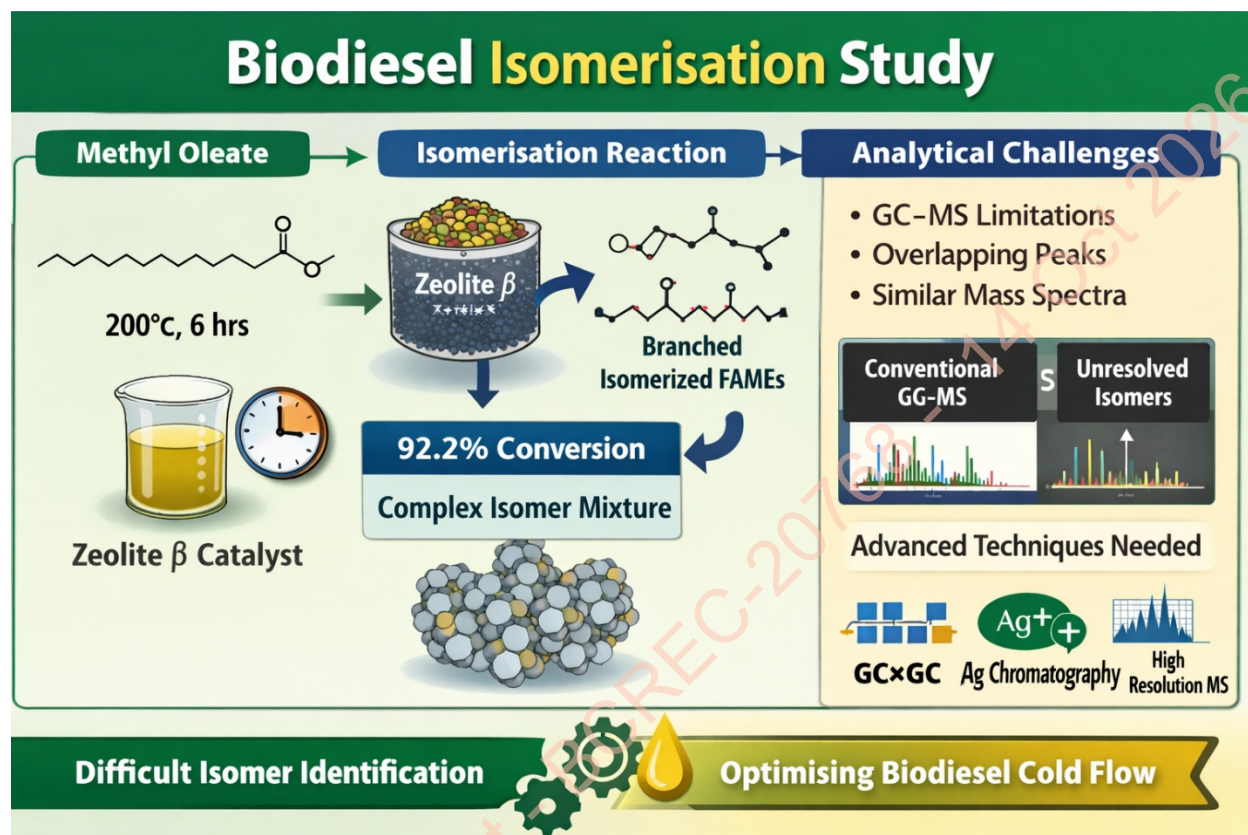
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Graphical Abstract



Abstract

Biodiesel's cold flow properties, limited by the crystallisation of saturated and *trans*-unsaturated fatty acid methyl esters (FAMEs), can be improved through skeletal isomerisation of unsaturated FAMEs to branched-chain isomers. Zeolite- β has shown promise for this reaction, but detailed characterisation of the complex isomer mixture remains challenging. This study investigates the isomerisation of methyl oleate over Zeolite- β (H- β) in a batch reactor at 200 °C and atmospheric pressure for 6 h, using 10 wt% catalyst. A conversion of 92.2% was achieved, confirming high catalyst activity. However, analysis by gas chromatography–mass spectrometry (GC–MS) with a polar BPX70 column revealed significant limitations: the product chromatogram showed extensive peak overlap, and the mass spectra of positional, geometric, and branched-chain isomers were nearly identical, preventing reliable determination of selectivity and yield. The work highlights that conventional GC–MS is inadequate for full isomer speciation in such complex mixtures. Advanced techniques such as comprehensive two-dimensional GC (GC $\times$ GC), silver-ion chromatography, or high-resolution mass spectrometry are required for accurate isomer identification. These findings establish a performance baseline for Zeolite- β and delineate the analytical challenges that must be addressed to enable rational catalyst and process optimisation for improved biodiesel cold flow properties.

Keywords: Biodiesel, Cold flow properties, Skeletal Isomerisation, Zeolite- β , fatty acid methyl esters

1. Introduction

Biodiesel, composed of fatty acid methyl esters (FAMES), offers a renewable alternative to petroleum diesel but its widespread adoption is limited by poor cold flow properties, primarily high cloud and pour points resulting from the crystallisation of saturated and *trans*-unsaturated FAMES at low temperatures [1]. Skeletal isomerisation of unsaturated FAMES to branched-chain isomers has emerged as a promising strategy to improve cold flow behaviour, as the introduction of methyl branches disrupts molecular packing without compromising oxidative stability [2,3]. However, recent studies have shown that isomerisation reactions can also generate undesirable by-products—namely dimers, saturates, free fatty acids (FFAs), and *trans* isomers—that may actually increase the melting point of the mixture [4,5]. Among these, *trans* isomers are particularly problematic; for C18:1 FAME, the *trans* isomer melts approximately 30 °C higher than its *cis* counterpart (Figure 1). While dimerisation, hydrogenation, and hydrolysis can be mitigated by using zeolites with narrower pores or by starving the system of hydrogen and water, the formation of *trans* isomers remains a major challenge in efforts to enhance cold flow properties.

Zeolites, owing to their shape-selective pore architectures and tuneable Brønsted acidity, have been extensively investigated as catalysts for FAME isomerization [6-8]. Zeolite- β , a large-pore zeolite with three-dimensional 12-membered ring channels, has shown particular promise. Reaume and Ellis [5] optimised the isomerisation of methyl oleate over Zeolite- β , achieving substantial conversion and cloud point reduction under varied conditions.

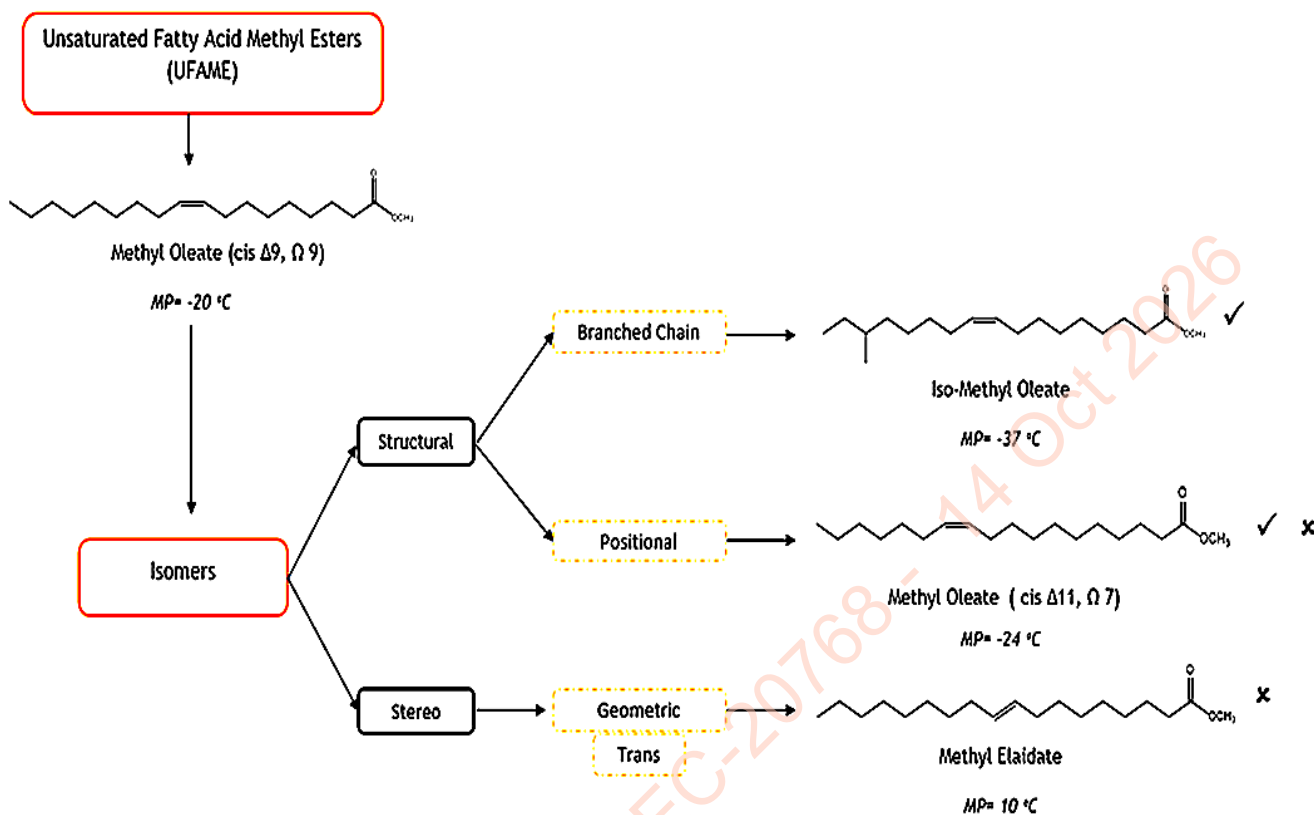


Figure 1. Isomerisation possibilities of cis C18:1 (methyl oleate)

Ha et al. further elucidated the role of calcination temperature and water additives on Beta zeolite activity, establishing that Brønsted acid sites generated at moderate calcination temperatures (ca. 450 °C) are critical [4]. More recently, hierarchical zeolites have been reported to achieve conversions approaching 90% with enhanced stability [9,10].

The present study investigates the isomerisation of methyl oleate over Zeolite- β in a batch reactor system at 200 °C and atmospheric pressure. Although several studies have reported methyl oleate, and other model compounds, isomerisation using Zeolite- β , relatively little attention has been given to the analytical limitations associated with identifying the resulting isomer mixture. Previous studies commonly report catalyst performance without critically evaluating whether

conventional GC-MS can reliably distinguish positional, geometric and skeletal isomers. This study provides one of the first systematic assessments of these analytical limitations while simultaneously establishing a conversion benchmark for Zeolite- β under batch reaction conditions.

The objectives are twofold: first, to quantify the conversion achievable under these conditions, thereby establishing a performance benchmark for comparison with previous work; second, to critically evaluate the capabilities of conventional GC-MS analysis—using a polar capillary column (BPX70)—to resolve and identify the complex mixture of positional, geometric, and branched-chain isomers formed. In this work, only conversion was calculated, as the analytical limitations described below prevented reliable determination of selectivity and yield. By explicitly documenting both the conversion attained and the analytical obstacles encountered (co-elution and spectral similarity), this work delineates the challenges that must be addressed before reliable selectivity and yield can be determined. Such a foundation is a necessary prerequisite for future studies, particularly those aiming to develop continuous flow reactor systems.

2. Experimental

2.1 Materials

Methyl oleate (C18:1 cis, 70% purity) was purchased from Sigma-Aldrich. Zeolite- β (CP814E, Si/Al = 12.5, ammonium form) was obtained from Zeolyst International. Dichloromethane (DCM, $\geq 99.8\%$) and all other reagents were used as received without further purification. Gas (air) used for catalyst activation and reaction had purities $\geq 99.99\%$. Prior to GC-MS analysis, all experimental samples were diluted to 0.2% (v/v) in DCM.

2.2 Catalyst Preparation

The as-supplied Zeolite- β powder (NH_4 - β) was pressed into pellets, crushed, and sieved to obtain particles of 425–600 μm . The sieved catalyst was calcined in a glass tube under a constant air flow of 30 $\text{mL}\cdot\text{min}^{-1}$. The temperature was increased from ambient to 450 $^{\circ}\text{C}$ at a ramp rate of 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$ and held isothermally for 6 h, followed by natural cooling to room temperature. This calcination step converted the ammonium form to the protonic form (H- β) and removed any chemisorbed water or organic residues. After calcination, the catalyst was transferred immediately to the reactor, with the tube ends sealed to minimise moisture adsorption. Activation in air is also often referred to as calcination.

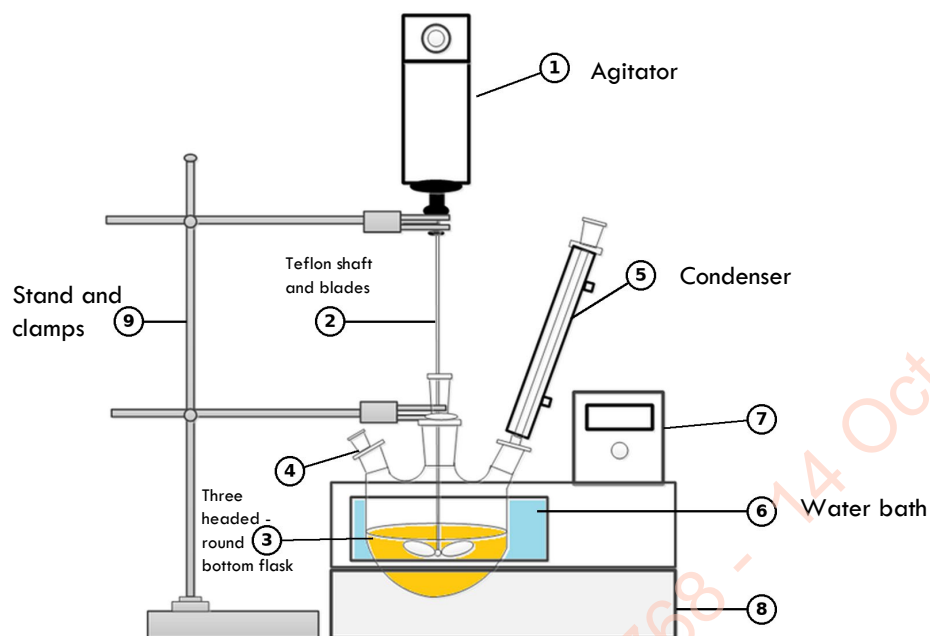


Figure 2. Schematic of the batch reactor set-up used for methyl oleate isomerisation: (1) overhead stirrer motor; (2) stirrer shaft with PTFE (Teflon) agitator blades (500 rpm), passing through a stirrer guide in the central neck; (3) three-necked round-bottom flask containing methyl oleate and H- β catalyst (10 wt%); (4) side neck used as the nitrogen inlet for purging and inert blanketing; (5) water-cooled reflux condenser on the third neck; (6) heating bath; (7) digital temperature controller; (8) heating unit; (9) retort stand and clamps supporting the stirrer motor and flask.

2.3 Isomerisation Reaction

Isomerisation of methyl oleate was carried out in a batch reactor system (Figure 2). In a typical experiment, 50 mL of methyl oleate (70% purity) and 10 wt% (based on methyl oleate mass) of calcined Zeolite- β (H- β) were charged into a three-necked round-bottom flask. The reactor was purged with nitrogen for 15 min to remove oxygen, then heated to 200 °C under a nitrogen atmosphere at atmospheric pressure. The mixture was stirred continuously at 500 rpm using a Teflon agitator shaft and blades. The reaction was allowed to proceed for 6 h, after which the reactor was cooled to room temperature. The physical appearance of the reaction mixture before and after the reaction is shown in Figure 3.



Figure 3. Appearance of the reaction mixture (a) before and (b) after isomerization

2.4 GC–MS Analysis

Product samples were analysed using an Agilent Technologies 6890N Network Gas Chromatograph coupled with a 5973 Inert Mass Spectrometer. Separation was achieved on a BPX70 capillary column (60 m $\times$ 250 μ m I.D. $\times$ 0.25 μ m film thickness), a highly polar stationary phase suitable for FAME isomer analysis. The GC operating conditions are summarised in Table 1.

Helium ($\geq 99.999\%$) was used as the carrier gas in constant flow mode at 1.2 mL $\cdot$ min $^{-1}$. The injector was operated in split mode at 250 $^{\circ}$ C with a split ratio of 50:1. The injection volume was 1 μ L. The oven temperature programme was as follows: initial temperature 60 $^{\circ}$ C held for 4 min, increased to 170 $^{\circ}$ C at 30 $^{\circ}$ C $\cdot$ min $^{-1}$, held for 10 min, then increased to 210 $^{\circ}$ C at 3 $^{\circ}$ C $\cdot$ min $^{-1}$ and held for 6 min.

The mass spectrometer was operated in electron ionisation (EI) mode at 70 eV. The transfer line temperature was maintained at 280 $^{\circ}$ C, the ion source at 230 $^{\circ}$ C, and the quadrupole at 150 $^{\circ}$ C. A solvent delay of 4.5 min was applied to protect the filament. Data were acquired in full-scan mode over the range m/z 50–550. Compound identification was performed by comparison of mass spectra with the NIST 08 mass spectral library and by retention time comparison with available standards where applicable.

The GC–MS method was developed based on prior studies of FAME isomer separation [11,12], following a systematic approach that employed a polar capillary column to optimise resolution of positional and geometric isomers.

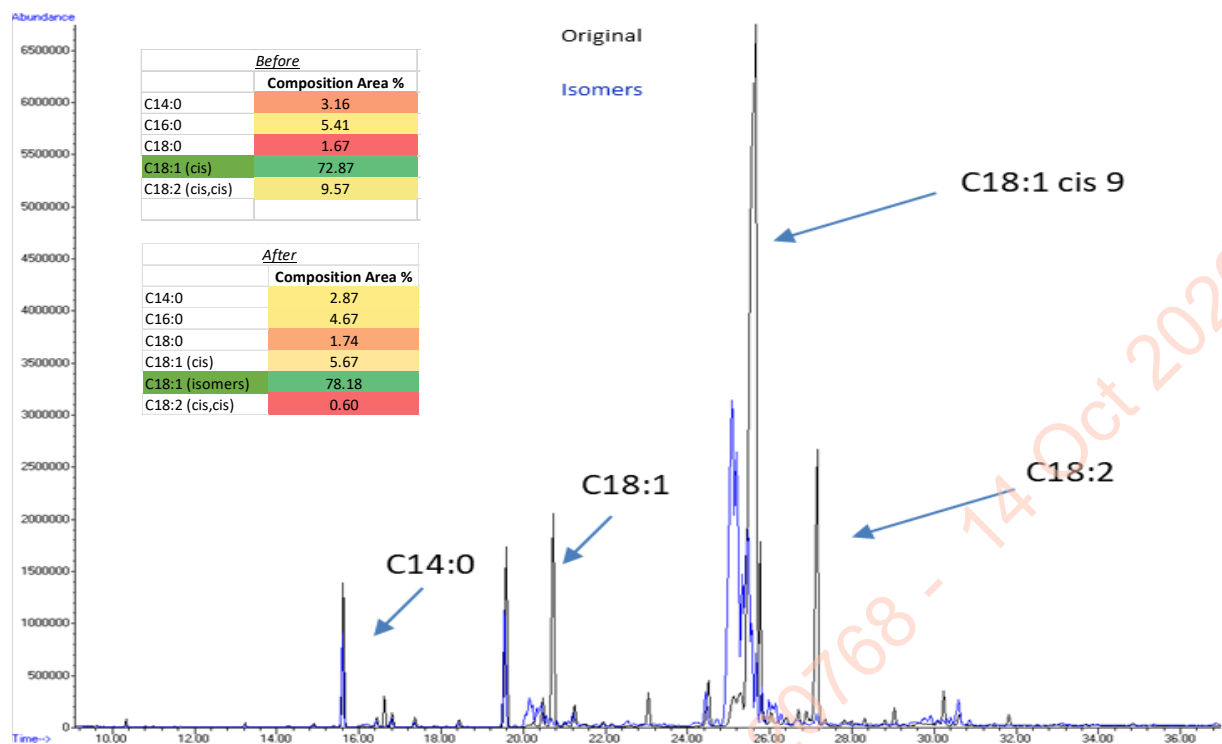


Figure 4. GC–MS chromatograms of the original methyl oleate and of the product mixture after isomerisation over H- β (200 °C, 6 h, 10 wt% catalyst)

Table 1. Liquid sample GC operating conditions and column specification.

Section	Parameter	Specification
Column details	Stationary phase	BPX70 (70% cyanopropyl polysilphenylene-siloxane)
	Dimensions	60 m 250 μ m I.D. $\times$ 0.25 μ m film thickness
Method name		FameIsomAnalysis
Injector	Temperature	250 °C
	Injection volume	1 μ L
	Injector wash	2 $\times$ solvent (dichloromethane)
Inlets	Solvent delay	4.5 min
	Split ratio	50:1
	Split flow	60 mL/min

Column (Helium flow)	Mode	Constant flow
	Flow rate	1.2 mL/min
	Pressure	13 psi (programmed)
	Average velocity	39 cm/sec
Oven	Initial	60 °C, hold 4 min
	Ramp 1	30 °C/min to 170 °C, hold 10 min
	Ramp 2	3 °C/min to 210 °C, hold 6 min
Mass Spectrometer	Ionisation mode	Electron ionisation (EI), 70 eV
	Transfer line temp.	280 °C
	Ion source temp.	230 °C
	Quadrupole temp.	150 °C
	Scan range	<i>m/z</i> 50–550

2.5 Calculations

Conversion, selectivity, and yield were defined according to Equations (1)–(3). However, because individual branched, positional and trans isomers could not be assigned unambiguously, any selectivity or yield values would be scientifically misleading. Therefore, only conversion is reported. Analytical limitations described in Section 3 (co-elution and near-identical mass spectra preventing definitive isomer identification) expand on this.

$$\text{Conversion}(\%) = \frac{\text{moles of methyl oleate reacted}}{\text{moles of methyl oleate initially}} \times 100 \quad (1)$$

$$\text{Selectivity}(\%) = \frac{\text{moles of desired isomers formed}}{\text{moles of methyl oleate reacted}} \times 100 \quad (2)$$

$$\text{Yield}(\%) = \frac{\text{moles of desired isomers formed}}{\text{moles of methyl oleate initially}} \times 100 \quad (3)$$

3. Results and Discussion

3.1 Conversion of Methyl Oleate

The isomerisation of methyl oleate over Zeolite- β (H- β) was carried out at 200 °C for 6 h under atmospheric pressure in a batch reactor. After the reaction, a clear change in the physical appearance of the mixture was observed (Figure 3), indicating that a transformation had occurred. GC–MS analysis of the starting material and the product mixture is shown in Figure 4.

The chromatogram of the original methyl oleate (70% purity) shows a dominant peak corresponding to cis-C18:1 Δ^9 , along with minor peaks attributable to other FAMES present as impurities (e.g., C14:0, C18:2). After 6 h of reaction, the intensity of the cis-C18:1 peak decreased substantially, and a broad envelope of new peaks appeared in the region between approximately 19 and 23 min. These new peaks are attributed to isomerisation products.

The conversion of methyl oleate was calculated based on the reduction in peak area of the cis-C18:1 methyl ester relative to the starting material. The GC–MS peak areas of the feed and of the product mixture, together with the step-by-step conversion calculation, are provided in the Supporting Information (Tables S1–S3). A conversion of 92.2% was achieved, demonstrating that Zeolite- β is highly active under the conditions employed. This value is comparable to or higher than those reported in previous studies using similar zeolite catalysts [3,5], confirming the suitability of H- β for skeletal isomerisation of unsaturated FAMES.

Table 2. Comparison of the present methyl oleate isomerisation study with previously reported fatty-acid and FAME isomerisation studies

Study	Feedstock	Catalyst and loading	Principal reaction conditions	Reported conversion, yield or selectivity	Main findings and comparison with the present study
Ngo et al. (2007) [13]	Oleic acid, approximately 91.5% purity	Proton-exchanged H-Ferrierite, typically 2.5–5 wt%, with water	250 °C; 6 h; batch reaction	Up to 99% conversion and approximately 82–85% branched-product selectivity under selected conditions	Modified H-Ferrierite gave high conversion and identifiable branched products after hydrogenation, methylation and GC–MS analysis of picolinyl derivatives. However, the reaction required a higher temperature and extensive post-reaction derivatisation.
Zhang and Zhang (2007) [3]	Oleic acid containing approximately 80 wt% unsaturated C18 fatty acid	Commercial H-Beta zeolites; 3 wt% catalyst	250 °C; 5 h; approximately 100 psig N ₂	Isomerisation selectivity generally 55–80%; activity varied considerably between commercial H-Beta samples	Mesopore volume, rather than Si/Al ratio or crystallinity alone, was identified as the main property controlling H-Beta activity. The study supports the use of Beta zeolite but also demonstrates that commercial Beta samples cannot be compared solely on nominal composition.
Ha et al. (2009) [4]	Oleic acid containing approximately 80 wt% monounsaturated C18 fatty acid	H-Beta, 3 wt%; calcined at different temperatures	250 °C; 6 h; 0.3 MPa N ₂	67.1% conversion for H-Beta calcined at 400 °C, compared with 45.7% after calcination at 550 °C	Calcination at approximately 400–450 °C retained strong Brønsted acidity and produced higher activity than conventional 550 °C calcination. Small water additions promoted conversion, whereas excess water inhibited activity. The present catalyst was also calcined at 450 °C, consistent with their optimum range.
Ngo et al. (2011) [14]	Oleic acid	H-Ferrierite; skeletal isomerisation followed by hydrogenation and esterification	Typically around 250 °C; batch reaction	Greater than 95% conversion and approximately 85% selectivity to saturated branched-chain fatty acids	The resulting isostearic acids and esters exhibited improved low-temperature properties and oxidative stability. Unlike the present work, the desired products were quantified after hydrogenation and derivatisation, simplifying their analytical identification.
Ngo et al. (2012) [15]	Technical-grade oleic acid	Modified H-Ferrierite with triphenylphosphine; generally 5 wt% catalyst	Typically 260 °C; 4 h; pressurised N ₂	Up to approximately 80 wt% branched-product yield; catalyst reused up to ten cycles without significant loss of activity	The additive suppressed dimer formation and improved branched-product yield. Detailed product distributions were resolved using GC×GC–TOF-MS, illustrating the advantage of multidimensional chromatography over the conventional one-dimensional GC–MS used in the present study.
Macias et al. (2011) [16]	Methyl oleate and commercial biodiesel	Mesoporous Ga–Nb oxides; H-Beta, H-Y, H-ZSM-5 and SAPO-11 for comparison; 5 wt% catalyst for methyl oleate tests	220 °C; 6 h for methyl oleate experiments	Branched FAMES were produced, but positional, cis/trans, dehydrogenated, hydrolysed and dimeric products were also detected	The study showed that methyl oleate undergoes several parallel reactions and that high activity does not necessarily imply high skeletal-isomerisation selectivity. This agrees with the complex product envelope and uncertainty in product assignment observed in the present work.
Yori et al. (2006) [17]	Soybean-oil biodiesel	Sulfated zirconia and H-Mordenite	125–275 °C; liquid-phase batch treatment	Cloud-point reductions of approximately 4–6.5 °C at 150–200 °C	Isomerisation improved cold-flow behaviour but also increased viscosity, decreased cetane index and promoted coking above 200 °C. These results demonstrate that conversion alone is insufficient to establish fuel improvement; product identity and fuel-property measurements are also required.
Reaume and Ellis (2012) [18]	Oleic acid/palmitic acid mixtures converted to methyl esters	Beta zeolite for isomerisation; Pt/Beta for hydroisomerisation	250 °C and 1 MPa H ₂ for isomerisation; 300 °C and 4 MPa H ₂ for hydroisomerisation	Maximum cloud-point reduction of approximately 7.5 °C for a 55:45 oleic acid/palmitic acid mixture	Branching lowered cloud point, whereas cis-to-trans isomerisation increased it. The study confirms that the benefits of skeletal branching can be offset by geometric isomerisation, supporting the present manuscript's concern regarding probable trans-isomer formation.
Reaume and Ellis (2013a) [19]	Methyl palmitate and palmitic acid	0.5 wt% Pt-loaded Beta zeolite	200–285 °C; 1–4 MPa H ₂ ; up to 16 h	Methyl palmitate conversion reached approximately 16%; branched-product yield approximately 42% under the reported optimum; cloud point reduced from 30 to 20 °C	Hydroisomerisation successfully improved the cold-flow behaviour of saturated FAMES but required high hydrogen pressure, higher temperature and long reaction times. The present atmospheric-pressure process is operationally simpler but acts on an unsaturated FAME and does not quantify cold-flow improvement.
Reaume and Ellis (2013b) [2]	Eight vegetable oils and fats	Beta zeolite and 0.5 wt% Pt/Beta	Isomerisation: 260 °C, 1.5 MPa H ₂ ; hydroisomerisation: 300 °C, 4 MPa H ₂	Maximum reported cloud-point reduction of approximately 12.9 °C for coconut-oil-derived biodiesel; high-unsaturation biodiesels sometimes showed increased cloud points	The outcome depended strongly on feed composition. Hydrogenation and trans-isomer formation could negate the benefits of branching in unsaturated feeds.
Present study	Methyl oleate, 70% purity	H-Beta CP814E, Si/Al = 12.5; 10 wt% catalyst	200 °C; 6 h; atmospheric pressure; N ₂ atmosphere	92.2% apparent conversion of cis-C18:1 Δ ⁹ ; selectivity and desired-product yield not reported because individual isomers could not be assigned reliably	The conversion is higher than that reported for several H-Beta/oleic-acid systems and approaches values achieved with modified H-Ferrierite. It was obtained at lower temperature and without elevated pressure. However, unlike studies using hydrogenation, derivatisation, GC×GC–TOF-MS or picolinyl esters, conventional BPX70 GC–MS could not distinguish branched, positional and geometric C18:1 isomers.

Note. The numerical values are not directly equivalent in every case because the studies used different starting materials (oleic acid, methyl oleate, saturated FAMES or whole biodiesel), analytical definitions of conversion, yield and selectivity, post-reaction treatments, catalyst loadings, additives, temperatures, pressures and analytical methods. The table should therefore not be interpreted as a strict catalyst ranking.

The 92.2% disappearance of cis-C18:1 Δ^9 observed in the present study confirms the substantial activity of H-Beta for the transformation of methyl oleate. As summarised in Table 2, the value is higher than the oleic acid conversions of 67.1% and 45.7% reported by Ha et al. for H-Beta calcined at 400 and 550 °C, respectively, under otherwise more severe reaction conditions of 250 °C and 0.3 MPa N₂. Their results showed that calcination at approximately 400–450 °C generated a catalyst rich in strong Brønsted acid sites, whereas calcination at 550 °C caused a considerable reduction in activity [4]. The present H-Beta catalyst was calcined at 450 °C, which lies within the activity-maximising range identified by Ha et al. This may partly explain the high conversion obtained despite operating at only 200 °C.

The high apparent conversion may also be related to the use of methyl oleate rather than oleic acid. Ha et al. found that the methyl ester of oleic acid exhibited higher conversion than the corresponding free fatty acid over the same Beta catalyst, which they attributed to the poisoning effect of the carboxylic acid group [4]. Consequently, the present conversion should not be compared uncritically with results obtained using oleic acid, because methyl oleate may access and react at the catalyst acid sites more readily.

Modified H-Ferrierite catalysts have produced even higher or comparable conversions. Ngo et al. reported oleic acid conversions of up to approximately 99%, with branched-product selectivities approaching 82–85%, at 250 °C for 6 h [13]. However, their product mixture was hydrogenated and methylated before analysis, and branching positions were determined using picolinyl derivatives. These additional steps converted the unsaturated products into more readily distinguishable saturated derivatives. Therefore, the reported selectivity cannot be compared directly with the present untreated C18:1 product mixture.

Later work by Ngo et al. achieved branched-chain fatty acid yields of up to approximately 80 wt% using modified H-Ferrierite with triphenylphosphine. That study also employed comprehensive two-dimensional GC coupled with time-of-flight mass spectrometry to characterise the complex isomer distribution [15]. The contrast with the current findings is analytically important: although conventional GC–MS detected extensive conversion and the appearance of numerous new peaks, it lacked the chromatographic resolution and spectral discrimination required to identify individual products. The literature therefore indicates that reliable selectivity determination requires either chemical derivatisation, multidimensional chromatography, or both [20,21].

The complex chromatographic behaviour observed here is also consistent with Macias et al., who reported that methyl oleate and biodiesel underwent several simultaneous reactions over acidic and mesoporous catalysts. Their products included skeletal isomers, positional and cis/trans isomers, diolefins, fatty acids formed by hydrolysis and dimers [16]. Thus, disappearance of the methyl oleate peak cannot automatically be equated with formation of beneficial branched FAMES. It represents an overall transformation that may include desired skeletal isomerisation and undesirable competing reactions.

The likely formation of trans products is particularly relevant to the intended improvement in cold-flow properties. Reaume and Ellis showed that branching lowered the cloud point, whereas cis-to-trans isomerisation produced the opposite effect. A cloud-point reduction of 7.5 °C was achieved for methyl esters derived from an oleic acid/palmitic acid mixture, but the benefit depended on the balance between branching and trans-isomer formation [18]. Similarly, in whole-biodiesel studies, high-unsaturation feedstocks sometimes exhibited increased rather than decreased cloud points because competing hydrogenation and isomerisation reactions generated less favourable products [2].

The present conversion of 92.2% is therefore significant because it was achieved at 200 °C, atmospheric pressure and without hydrogen, which are milder conditions than many of the reported isomerisation and hydroisomerisation processes. Nevertheless, high conversion alone does not prove improved cold-flow performance. Reliable evaluation requires separate quantification of branched, positional and trans isomers, followed by direct measurement of cloud point, pour point or cold-filter-plugging point. The present study consequently establishes a useful activity benchmark for H-Beta while demonstrating that conventional one-dimensional GC–MS is inadequate for determining the selectivity needed to assess the true cold-flow benefit.

3.2 Formation of trans Isomers

A key concern in the isomerisation of unsaturated FAMES is the potential formation of trans isomers, which have significantly higher melting points than their cis counterparts (e.g., approximately 30 °C higher for C18:1) and therefore worsen cold flow properties. As shown in Figure 5, heating of vegetable oils at temperatures below 240 °C for short periods typically generates only small amounts of trans isomers (<5%) in the absence of catalysts [22]. However, the presence of an acidic zeolite can accelerate trans formation even at lower temperatures. In the present study, the reaction was conducted at 200 °C with H- β , a Brønsted acid catalyst, making the formation of trans isomers highly probable. Indeed, previous work on methyl oleate isomerisation over Zeolite- β has reported substantial trans formation under similar conditions [3,5].

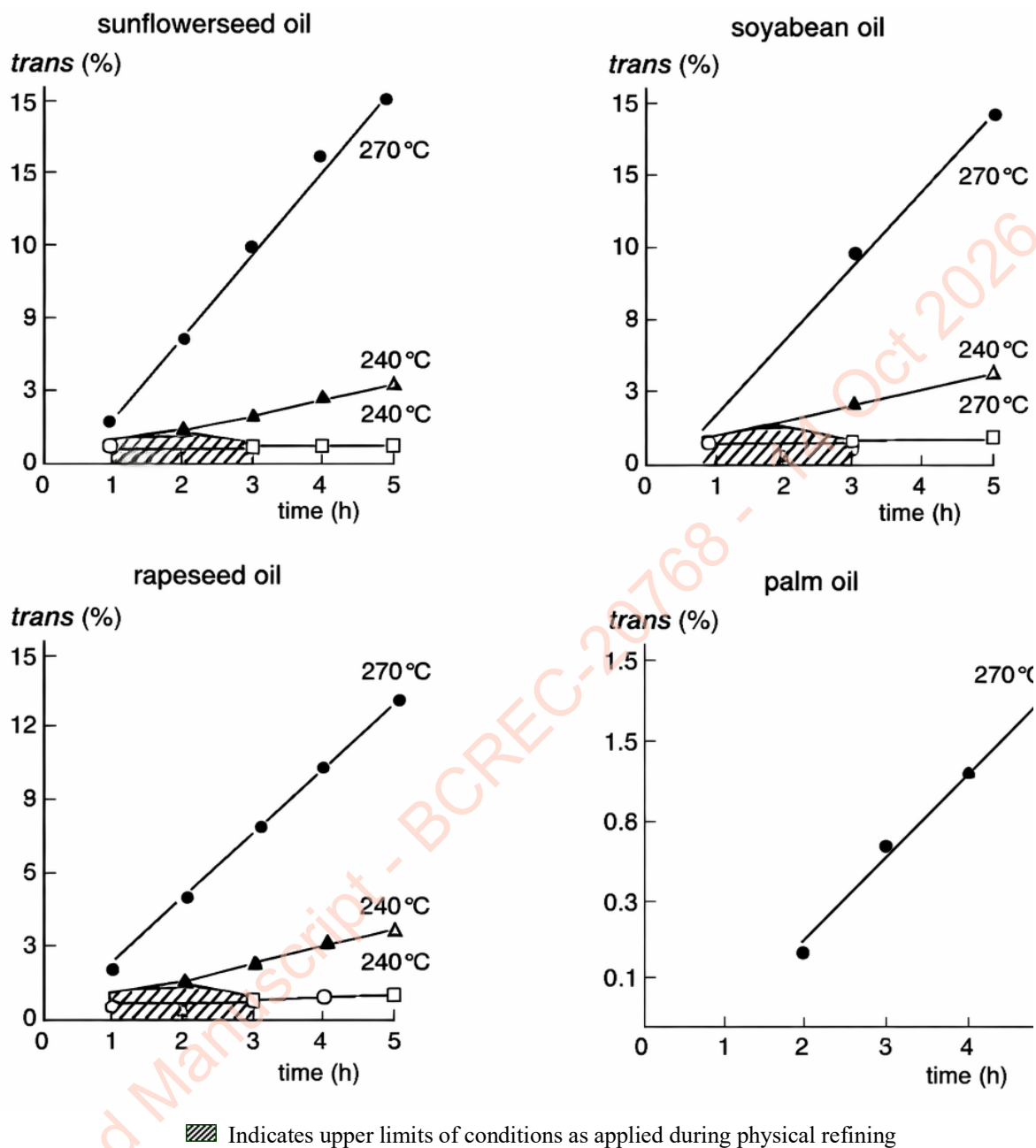


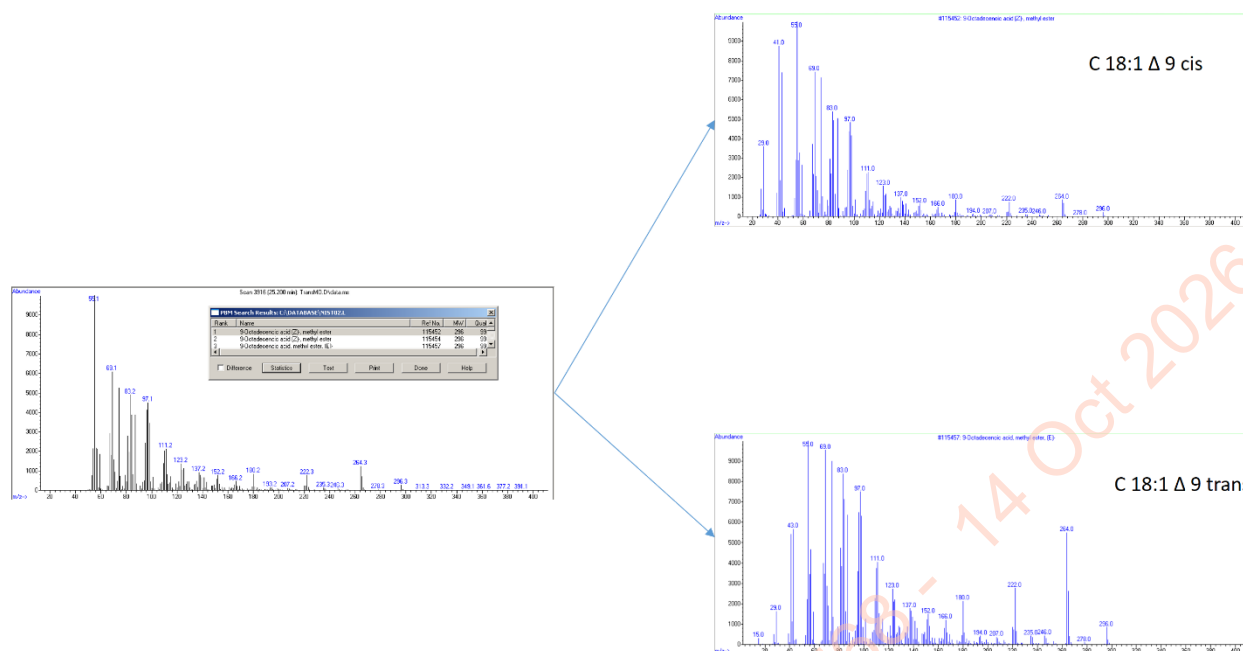
Figure 5. Trans fatty acid formation at various temperatures for different oils upon natural heating (adapted from FAO, 1994) [23].

3.3 Challenges in Isomer Identification

Although conversion was high and trans isomers were likely formed, the detailed characterisation of the isomer mixture proved challenging. The reaction mixture is expected to contain a complex combination of positional isomers (double bond migration), geometric isomers (cis/trans), and branched-chain (skeletal) isomers. In principle, each of these classes influences cold flow properties differently, making accurate speciation essential for evaluating catalyst performance.

Chromatographic resolution. The GC–MS method employed a polar BPX70 capillary column, selected for its reported ability to separate FAME isomers [11,12]. As shown in Figure 4, the product chromatogram exhibits a broad envelope of partially resolved peaks. Baseline separation of all components was not achieved, and many peaks overlapped, preventing the assignment of individual isomer types based solely on retention time.

Mass spectral similarity. Even for peaks that appeared well resolved, the electron ionisation mass spectra of different C18:1 isomers were nearly identical. For example, positional isomers (Δ^8 , Δ^9 , Δ^{11}) and geometric isomers (cis/trans) of C18:1 all show major fragment ions at m/z 180, 222, and 264, with no distinctive features for unambiguous assignment (Figures 6 and 7). Comparison with the NIST mass spectral library did not allow reliable differentiation between geometric and branched isomers.



Theoretical isomer complexity. This analytical difficulty is compounded by the sheer number of possible isomers. As summarised in Table 3 [24], even a saturated C18 fatty acid can theoretically exist as over 2.9 million isomers when branching is considered. With the additional complexity of double bond position and geometry, the number of potential products becomes astronomically large. The current analytical approach—one-dimensional GC with quadrupole MS—lacks the resolving power and spectral discrimination required to identify individual isomers within such a complex mixture [25].

Table 3. Number of theoretical isomer possibilities of saturated fatty acids with 12 to 21 carbon atoms [24].

Number of carbon atoms	Stereoisomers	Non-stereoisomers	Total isomers
12	4,326	110	4,436
13	12,648	184	12,832
14	37,186	310	37,496
15	109,980	520	110,500
16	327,216	876	328,092
17	979,020	1,471	980,491
18	2,944,414	2,475	2,946,889
19	8,897,732	4,159	8,901,891
20	27,004,290	6,996	27,011,286
21	82,287,516	11,759	82,299,275

Consequently, it was not possible to reliably determine selectivity (the fraction of converted methyl oleate that forms desired branched or cis-positional isomers) or yield (the amount of desired isomers relative to starting material). Only the overall conversion could be quantified.

3.4 Shape-Selective Removal of trans Isomers

A complementary strategy to improve the cold flow properties of isomerised biodiesel involves the selective removal of trans isomers using shape-selective zeolites. As proposed by Philippaerts et al. [26] and illustrated in Figure 8, the straight molecular geometry of trans fatty acids allows them to enter the narrow pores of MFI-type zeolites (e.g., ZSM-5), while the bent cis isomers are excluded. When Pt is loaded onto the zeolite, the trans isomers can be selectively hydrogenated to saturated species or fully retained, thereby enriching the product in cis and branched isomers. Figure 9 shows that using Pt-ZSM-5 under mild conditions (100 °C, 10 bar) leads to minimal formation of trans isomers and fully saturated triglycerides during hydrogenation of soybean oil [27].

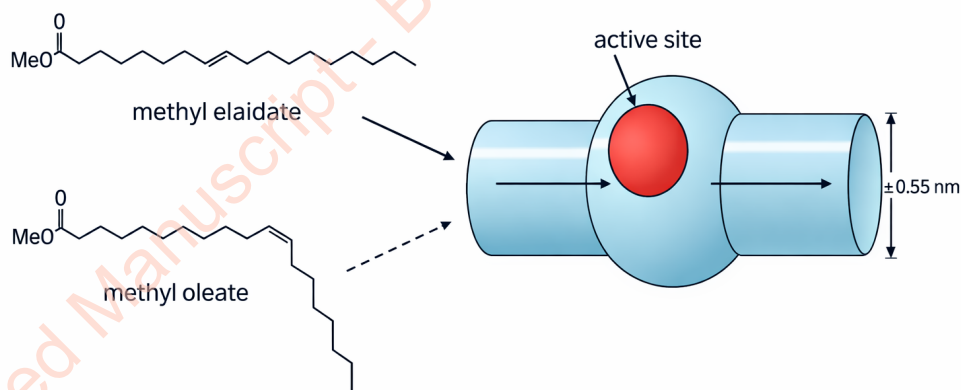


Figure 8. Removal of trans fatty acids in presence of cis isomers by shape selective hydrogenation over Pt-loaded ZSM-5

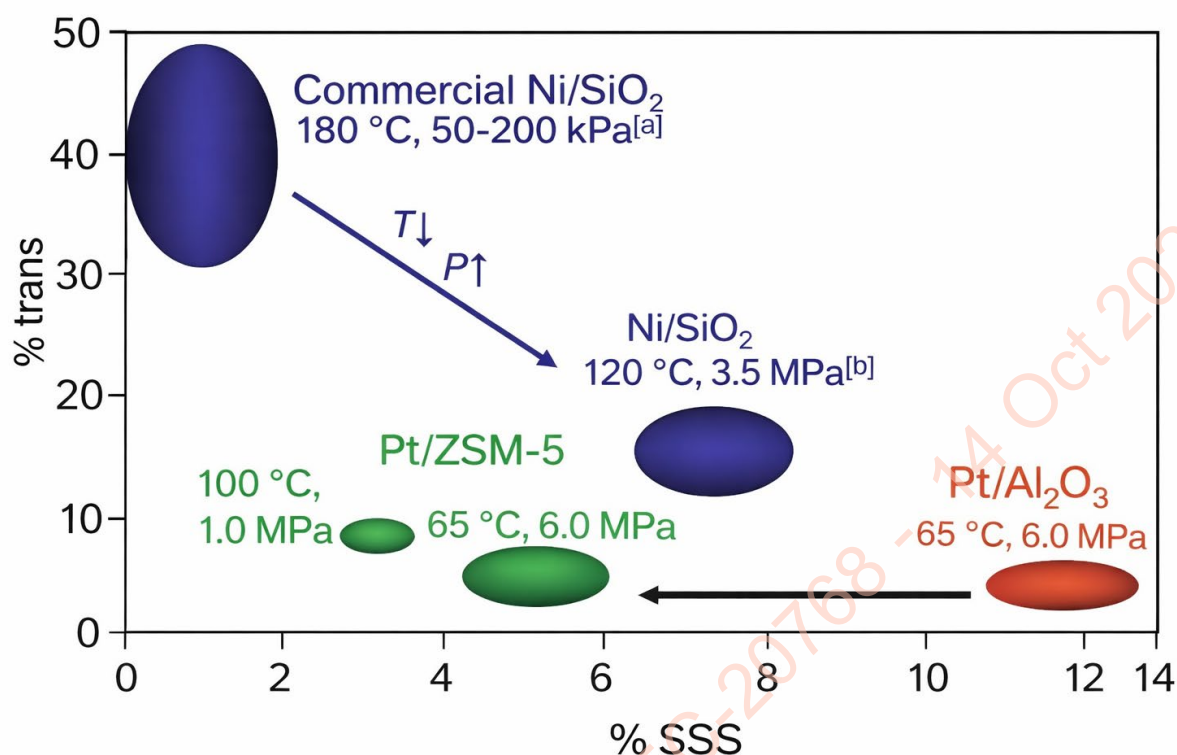


Figure 9. Selectivity of the formation of trans versus fully saturated triglycerides of hydrogenated soybean oil [27].

This concept could be adapted to the present system by incorporating a ZSM-5-based catalyst bed downstream of the isomerisation reactor to remove unwanted trans isomers. Such an approach, combined with improved analytical methods, offers a promising route toward biodiesel with optimised cold flow properties. Moreover, carrying out reactions in a continuous setup could be a significant game changer in enhancing the cold flow properties of biodiesel [28,29].

4. Conclusions

The isomerisation of methyl oleate over Zeolite- β (H- β) was successfully achieved in a batch reactor system at 200 °C and atmospheric pressure. A high conversion of 92.2% was obtained after 6 h, confirming the activity of this catalyst under the conditions employed. This work establishes an analytical benchmark for future catalyst optimisation and demonstrates that improvements in chromatographic methodology are a prerequisite before meaningful catalyst selectivity can be evaluated.

However, the characterisation of the isomeric products using conventional GC–MS with a polar BPX70 column proved insufficient. The product chromatogram showed a broad envelope of partially resolved peaks, and the mass spectra of different C18:1 isomers were nearly identical, preventing reliable differentiation between positional, geometric, and branched-chain isomers. This limitation, combined with the immense number of possible isomers, meant that selectivity and yield could not be quantified.

Future studies aiming to optimise the isomerisation process for improved cold flow properties will require more advanced analytical techniques. Comprehensive two-dimensional gas chromatography (GC $\times$ GC), silver-ion chromatography, or high-resolution mass spectrometry coupled with specialised spectral libraries are recommended to enable definitive isomer identification and accurate determination of selectivity and yield. Additionally, integrating shape-selective zeolites such as Pt-ZSM-5 for the selective removal of *trans* isomers could further enhance the quality of the final biodiesel product.

Availability of data and materials

The authors confirm that the data supporting the findings of this study will be made available upon reasonable request.

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Declaration of Generative AI Use

During preparation of this revision, the authors used ChatGPT (OpenAI) and Claude (Anthropic) to assist with reference formatting, organization of the response to reviewers, and language editing. The authors reviewed and verified all changes and take full responsibility for the accuracy, originality and integrity of the manuscript. No generative AI tool was used to generate, process, analyse or interpret the experimental data.

Credit Statement

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Muhammad Ashraf: Methodology, Investigation, Data analysis Review & Editing.

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All authors have read and agreed to the published version of the manuscript.

References

- [1] Knothe, G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Processing Technology*, 86(10), 1059-1070. DOI: <https://doi.org/10.1016/j.fuproc.2004.11.002>
- [2] Reaume, S.J., Ellis, N. (2013). Use of isomerization and hydroisomerization reactions to improve the cold flow properties of vegetable oil based biodiesel. *Energies*, 6(2), 619-633. DOI: <https://doi.org/10.3390/en6020619>
- [3] Zhang, S., Zhang, Z.C. (2007). Skeletal isomerization of unsaturated fatty acids: The role of mesopores in HBeta zeolites. *Catalysis Letters*, 115(3-4), 114-121. DOI: <https://doi.org/10.1007/s10562-007-9083-z>
- [4] Ha, L., Mao, J., Zhou, J., Zhang, Z.C., Zhang, S. (2009). Skeletal isomerization of unsaturated fatty acids on Beta zeolites: Effects of calcination temperature and additives. *Applied Catalysis A: General*, 356(1), 52-56. DOI: <https://doi.org/10.1016/j.apcata.2008.12.018>
- [5] Reaume, S.J., Ellis, N. (2011). Optimizing reaction conditions for the isomerization of fatty acids and fatty acid methyl esters to their branch chain products. *Journal of the American Oil Chemists' Society*, 88(5), 661-671. DOI: <https://doi.org/10.1007/s11746-010-1718-3>
- [6] Maghrebi, R., Buffi, M., Bondioli, P., Chiaramonti, D. (2021). Isomerization of long-chain fatty acids and long-chain hydrocarbons: A review. *Renewable and Sustainable Energy Reviews*, 149, 111264. DOI: <https://doi.org/10.1016/j.rser.2021.111264>
- [7] Dhar, A., Vekariya, R.L., Bhadja, P. (2018). n-Alkane isomerization by catalysis—a method of industrial importance: An overview. *Cogent Chemistry*, 4(1), 1514686. DOI: <https://doi.org/10.1080/23312009.2018.1514686>
- [8] Kumar, N. (2021). Microporous zeolites and related nanoporous materials: Synthesis, characterization and application in catalysis. *Catalysts*, 11(3), 382. DOI: <https://doi.org/10.3390/catal11030382>
- [9] Ferreira Young, A., Nothaft Romano, P., Aurélio Suller Garcia, M., Alexandre Gomes Aranda, D., Monnerat Araújo Ribeiro de Almeida, J. (2024). Microwave-enhanced preparation of finned-FER zeolites and their application in the skeletal isomerization of FAME. *Fuel*, 373, 132331. DOI: <https://doi.org/10.1016/j.fuel.2024.132331>
- [10] Sierra-Cantor, J.F., Gimello, O., Guerrero-Fajardo, C.A., Di Renzo, F., Petitjean, H., Riviere, M., Gérardin, C., Tanchoux, N. (2024). Synthesis of alkyl-branched fatty acids by isomerization on micro-mesoporous ferrierite-based zeolitic materials. *Applied Catalysis*

- B: Environmental*, 344, 123602. DOI: <https://doi.org/10.1016/j.apcatb.2023.123602>
- [11] Ecker, J., Scherer, M., Schmitz, G., Liebisch, G. (2012). A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. *Journal of Chromatography B*, 897, 98-104. DOI: <https://doi.org/10.1016/j.jchromb.2012.04.015>
- [12] Härtig, C. (2008). Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography-mass spectrometry database. *Journal of Chromatography A*, 1177(1), 159-169. DOI: <https://doi.org/10.1016/j.chroma.2007.10.089>
- [13] Ngo, H.L., Nuñez, A., Lin, W., Foglia, T.A. (2007). Zeolite-catalyzed isomerization of oleic acid to branched-chain isomers. *European Journal of Lipid Science and Technology*, 109(3), 214-224. DOI: <https://doi.org/10.1002/ejlt.200600246>
- [14] Ngo, H.L., Dunn, R.O., Sharma, B., Foglia, T.A. (2011). Synthesis and physical properties of isostearic acids and their esters. *European Journal of Lipid Science and Technology*, 113(2), 180-188. DOI: <https://doi.org/10.1002/ejlt.201000335>
- [15] Ngo, H.L., Hoh, E., Foglia, T.A. (2012). Improved synthesis and characterization of saturated branched-chain fatty acid isomers. *European Journal of Lipid Science and Technology*, 114(2), 213-221. DOI: <https://doi.org/10.1002/ejlt.201000471>
- [16] Macias, E.E., Deshmane, C.A., Jasinski, J.B., Carreon, M.A., Ratnasamy, P. (2011). Catalytic transformations of methyl oleate and biodiesel over mesoporous gallium–niobium oxides. *Catalysis Communications*, 12(7), 644-650. DOI: <https://doi.org/10.1016/j.catcom.2010.12.005>
- [17] Yori, J.C., D'Amato, M.A., Grau, J.M., Pieck, C.L., Vera, C.R. (2006). Depression of the cloud point of biodiesel by reaction over solid acids. *Energy & Fuels*, 20(6), 2721-2726. DOI: <https://doi.org/10.1021/ef060245i>
- [18] Reaume, S.J., Ellis, N. (2012). Synergistic effects of skeletal isomerization on oleic and palmitic acid mixtures for the reduction in cloud point of their methyl esters. *Energy & Fuels*, 26(7), 4514-4520. DOI: <https://doi.org/10.1021/ef3006564>
- [19] Reaume, S.J., Ellis, N. (2013). Use of hydroisomerization to reduce the cloud point of saturated fatty acids and methyl esters used in biodiesel production. *Biomass and Bioenergy*, 49, 188-196. DOI: <https://doi.org/10.1016/j.biombioe.2012.12.008>
- [20] Ngo, H.L., Dunn, R.O., Hoh, E. (2013). C18-unsaturated branched-chain fatty acid isomers: Characterization and physical properties. *European Journal of Lipid Science and Technology*, 115(6), 676-683. DOI: <https://doi.org/10.1002/ejlt.201200323>
- [21] Ngo, H.L., Fox, P.S., Nuñez, A., Moreau, R.A., Haas, M.J. (2014). Catalytic synthesis and characterization of phenol-branched-chain fatty acid isomers. *European Journal of Lipid Science and Technology*, 116(3), 344-351. DOI: <https://doi.org/10.1002/ejlt.201300227>
- [22] Kelley, D.S. (1996). Dietary fat and human immune response. *INFORM: International News on Fats, Oils and Related Materials*, 7(8), 852-858.

- [23] FAO, WHO (1994). *Fats and Oils in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation, Rome, 19-26 October 1993*. FAO Food and Nutrition Paper No. 57. Rome: Food and Agriculture Organization of the United Nations. <https://www.fao.org/4/v4700e/v4700e00.htm>
- [24] Abrahamsson, S., Stållberg-Stenhagen, S., Stenhagen, E. (1964). The higher saturated branched chain fatty acids. *Progress in the Chemistry of Fats and Other Lipids*, 7, 1-157. DOI: [https://doi.org/10.1016/0079-6832\(64\)90009-6](https://doi.org/10.1016/0079-6832(64)90009-6)
- [25] Fu, X., Hafza, N., Götz, F., Lämmerhofer, M. (2023). Profiling of branched chain and straight chain saturated fatty acids by ultra-high performance liquid chromatography-mass spectrometry. *Journal of Chromatography A*, 1703, 464111. DOI: <https://doi.org/10.1016/j.chroma.2023.464111>
- [26] Philippaerts, A., Paulussen, S., Turner, S., Lebedev, O.I., Van Tendeloo, G., Poelman, H., Bulut, M., De Clippel, F., Smeets, P., Sels, B., Jacobs, P. (2010). Selectivity in sorption and hydrogenation of methyl oleate and elaidate on MFI zeolites. *Journal of Catalysis*, 270(1), 172-184. DOI: <https://doi.org/10.1016/j.jcat.2009.12.022>
- [27] Philippaerts, A., Jacobs, P.A., Sels, B.F. (2013). Is there still a future for hydrogenated vegetable oils? *Angewandte Chemie International Edition*, 52(20), 5220-5226. DOI: <https://doi.org/10.1002/anie.201209731>
- [28] Anwar, A., Garforth, A. (2016). Challenges and opportunities of enhancing cold flow properties of biodiesel via heterogeneous catalysis. *Fuel*, 173, 189-208. DOI: <https://doi.org/10.1016/j.fuel.2016.01.050>
- [29] Pradana, Y.S., Makertihartha, I.G.B.N., Indarto, A., Prakoso, T., Soerawidjaja, T.H. (2024). A review of biodiesel cold flow properties and its improvement methods: Towards sustainable biodiesel application. *Energies*, 17(18), 4543. DOI: <https://doi.org/10.3390/en17184543>

Supporting Information

Supporting Information: GC–MS peak tables (retention time, peak area, peak height and area %) for the methyl oleate feed before reaction and for the product mixture after isomerisation over H- β at 200 °C for 6 h, FAME composition summary, and the conversion calculation based on Equation (1) in Supporting_Information_BCREC-2026-20768-R2 document.