International Year of CHEMISTRY 2011

Biomass conversion into biofuels by non-classical methods

Yolandi Nortjé
(B.Sc. Industrial Science)

Dissertation submitted in partial fulfilment of the degree
Magister Scientiae (Chemistry)

School of Physical and Chemical Sciences
North-West University (Potchefstroom Campus)

Supervisor: Professor E.L.J. Breet

May 2011

Innovation through diversity ™

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Contents

Chapter 2

Biomass Sources for Biofuel Production

Chapter 3

Non-Classical Extraction Methods

Chapter 4

The Toolbox

Chapter 5

Results and Discussion

Chapter 6

Evaluation and Future Perspective

Abstract

This investigation was launched in view of two imminent needs in industry today, viz. development of an alternative fuel to replace rapidly dwindling fossil fuel resources, preferably by biomass conversion, and production of a biofuel as a new energy source by implementing clean technology complying with the requirements of green chemistry.

Seed from the diesel tree (*Jatropha curcas* L.*)* and sawdust from pine (*Pinus taeda* L.) were selected as biomass sources since their properties, like rich oil content or diversity of constituents, met the suitability criteria for eventual conversion to biofuels.

Extracts from the two selected biomass sources were derived by three different non-classical methods, viz. supercritical carbon dioxide (sc-CO_2) extraction performed with a laboratory-scale supercritical extractor (LECO TFE2000), microwave-assisted extraction using a closed-vessel industrial microwave system (MARS 5) to produce superheated water, and ultrasound-supported extraction performed in n-hexane or water sonicated by a FINNSONIC soundwave emitter. The extracted material was compared to that obtained by traditional soxhlet extraction using n-hexane as solvent.

One-dimensional and two-dimensional gas chromatography with time-of-flight mass spectrometric detection using a LECO Pegasus 4D GCxGC-TOFMS and different column configurations were employed to cope with the analysis of derivatised samples of the complex, component-rich botanical extracts derived by the non-classical methods adopted.

The oil content of *Jatropha* seed (at least 55% m/m) and the solubility of *Jatropha* oil in sc-CO_2 (nominally 3 x 10^{-3} g per g CO_2 at 313 K and 30 MPa) were determined by utilising the dynamic and static modes of the supercritical extractor, respectively, and extrapolating the resulting yield-time graphs to infinity. These figures proved that *Jatropha* seed is a favourable feedstock for biofuel production, and that sc-CO_2 is an efficient solvent to extract oil from seed while avoiding harsh solvents and unwanted solvent residues in agreement with green chemistry principles.

C16-C18 triglycerides were detected as major constituents of *Jatropha* oil obtained by soxhlet and sc-CO_2 extraction, whereas free fatty acids dominate in extracts by microwave and ultrasound extractions due to thermal degradation and partial hydrolysis of triglycerides at the extraction conditions concerned.

A standard solution of triolein, the most abundant C18 triglyceride in *Jatropha* oil, was used as a reference for the identification of mixed C16-C18 triglycerides present in the oil. By comparing the mass spectrum of each oil sample to the mass spectrum of triolein, some of the triglycerides in the oil samples could be identified with a satisfactory match factor (70 % or higher). Among these were triolein (C18:1), tripalmitin (C16:0), trilinolein (C18:2) and tristearin (C18:0).

The triglycerides could be converted by means of base-catalysed transesterification to a crude biodiesel containing primarily C16-C18 but also some C13-C15 fatty acid methyl esters (FAMEs), the principal building blocks of biodiesel. The crude product could be benchmarked against an SABS approved biodiesel according to the SANS1935 standard in terms of its content of these long-chain esters.

sc-CO_2 and superheated water were found to be equally efficient solvents next to acetone used in soxhlet extraction to retrieve material from pine sawdust samples. Extracts were shown to comprise, among others, hydrocarbons, fatty acids, terpenoids, flavonoids and phenolics. These substances were either dissolved or desorbed by the solvent, and a "bulk solubility" of pine extractables in sc-CO_2 could be determined as 7×10^{-3} g per g CO_2 at 358 K and 60 MPa in a similar way as for *Jatropha* oil. Superheated water was the only solvent capable of cleaving the polymeric cellulose and hemicellulose chains held together by lignin in wood into a series of differently structured sugar entities, resulting in a highly complex two-dimensional chromatogram.

Quantitative analysis of triglycerides had to be aborted since the low volatility of these high molar mass, high boiling point compounds necessitated modification of the instrument's inlet, despite using pseudo on-column injection and special high-temperature columns. To the contrary, qualitative analysis of extracts and converted products demonstrated the powerful identification capability of the chromatographic system used and the diversity of substances available for conversion to biofuel. The chromatographic results published in this dissertation on the two selected biomass sources have been acquired by novel combinations of separation mode (one-dimensional or two-dimensional) and column type/configuration not specifically found in the literature.

The study as a whole proved that *Jatropha* oil is a suitable source of biomass for biodiesel production, and that even waste wood shows potential for conversion into liquid fuels. The non-classical extraction methods were found to be capable of retrieving material relevant to biofuel production from these biomass sources.

Opsomming

Hierdie ondersoek is geïnisieer in die lig van twee dringende behoeftes in die nywerheid vandag, nl. ontwikkeling van ’n alternatiewe brandstof om vinnig afnemende bronne van fossielbrandstof te vervang, verkieslik deur biomassa-omsetting, en vervaardiging van ’n biobrandstof as ’n nuwe energiebron deur die implementering van skoon tegnologie wat aan die vereistes van groen chemie voldoen.

Saad van die dieselboom (*Jatropha curcas* L.*)* en saagsels van dennehout (*Pinus taeda* L.) is gekies as biomassabronne omdat hulle eienskappe, soos hoë olie-inhoud of diverse komponente, voldoen aan die geskiktheidsvereistes vir eventuele omsetting na biobrandstowwe.

Ekstrakte van die twee gekose biomassabronne is deur drie verskillende nie-klassieke metodes verkry, nl. ekstraksie met superkritieke koolstofdioksied (sc-CO_2) uitgevoer met ’n laboratorium-grootte superkritieke ekstraktor (LECO TFE2000), mikrogolf gesteunde ekstraksie in geslote monsterhouers in ’n industriële mikrogolfoond (MARS 5) wat oorverhitte water produseer, en ultrasoniese ekstraksie uitgevoer in n-heksaan of water wat deur ’n FINNSONIC klankgolfstraler geaktiveer word. Die geëkstraheerde materiaal is vergelyk met dié verkry deur tradisionele soxhlet-ekstraksie met n-heksaan as oplosmiddel.

Een- en twee-dimensionele gaschromatografie met vlugtydmassaspektrometriese deteksie is ingespan om, deur van ’n LECO Pegasus 4D GCxGC-TOFMS en verskillende kolomkonfigurasies gebruik te maak, gederivatiseerde monsters van die komplekse, komponent-ryke botaniese ekstrakte wat deur die gekose nie-klassieke metodes verkry is, te analiseer.

Die olie-inhoud van *Jatropha*-saad (minstens 55% m/m) en die oplosbaarheid van *Jatropha*-olie in sc-CO_2 (nominaal 3 x 10^{-3} g per g CO_2 by 313 K en 30 MPa) is bepaal deur van onderskeidelik die dinamiese en statiese modusse van die superkritieke ekstraktor gebruik te maak en die opbrengs-tyd-grafieke wat as resultate verkry is na oneindigheid te ekstrapoleer. Hierdie waardes bevestig dat *Jatropha*-saad ’n geskikte hulpbron vir biobrandstofvervaardiging is, en dat sc-CO_2 ’n doeltreffende oplosmiddel is om olie uit saad te ekstraheer terwyl onvriendelike oplosmiddels en ongewenste oplosmiddelreste vermy word in ooreenstemming met die beginsels van groen chemie.

C16-C18-trigliseriede is geïdentifiseer as die belangrikste bestanddele van *Jatropha*-olie wat deur soxhlet- en sc-CO_2-ekstraksie verkry is, terwyl vrye vetsure oorheers in die ekstrakte wat met mikrogolf- en ultraklankekstraksies verkry is as gevolg van termiese ontbinding en gedeeltelike hidrolise van trigliseriede by die betrokke ekstraksiekondisies.

’n Standaardoplossing van triolien, die volopste C18-trigliseried in *Jatropha*-olie, is as ’n verwysing gebruik vir die identifikasie van mengsels van C16-C18-trigliseriede wat in die olie teenwoordig is. Deur die massaspektrum van elke oliemonster met die massaspektrum van triolien te vergelyk, kon sommige van die trigliseriede in die oliemonsters met ’n bevredigende pasfaktor (70 % of hoër) geïdentifiseer word. Hieronder was triolien (C18:1), tripalmitien (C16:0), trilinolien (C18:2) en tristearien (C18:0).

Die trigliseriede kon met behulp van basis-gekataliseerde transverestering omgeskakel word na ’n ru-biodiesel wat hoofsaaklik C16-C18 maar ook sommige C13-C15-vetsuurmetielesters (FAMEs), die hoofbestanddele van biodiesel, bevat. Die ruproduk kon aan die hand van ’n SABS goedgekeurde biodiesel volgens die SANS1935 standaard in terme van die vetsuurmetielester-inhoud daarvan gewaarmerk word.

Daar is vasgestel dat sc-CO_2 en oorverhitte water benewens asetoon, wat vir soxhlet-ekstraksie aangewend is, ewe doeltreffende oplosmiddels is om materiaal uit dennesaagsels te onttrek. Dit het geblyk dat ekstrakte onder andere koolwaterstowwe, vetsure, terpenoïede, flavonoïede en fenole bevat. Hierdie stowwe is deur die oplosmiddel opgelos of gedesorbeer, en ’n “globale oplosbaarheid” in sc-CO_2 van ekstraheerbare stowwe in dennehout is as 7×10^{-3} g per g CO_2 by 358 K en 60 MPa op ’n soortgelyke wyse as vir *Jatropha*-olie bepaal. Oorverhitte water was die enigste oplosmiddel wat instaat was om die polimeriese sellulose- en hemisellulosekettings wat in hout deur lignien aanmekaar gehou word tot ’n reeks suikerentiteite met verskillende strukture te klief en sodoende tot ’n hoogs ingewikkelde twee-dimensionele chromatogram aanleiding te gee.

Kwantitatiewe analise van trigliseriede is laat vaar omdat die lae vlugtigheid van hierdie verbindinge met hul hoë molmassas en hoë kookpunte wysigings aan die instrument se inlaat genoodsaak het ten spyte daarvan dat gekoppelde kolominspuiting nageboots en spesiale hoë-temperatuur-kolomme gebruik is. Daarteenoor het die kwalitatiewe analise van ekstrakte en omgesette produkte die kragtige identifikasievermoëns van die benutte chromatografiese stelsel en die diversiteit van stowwe beskikbaar vir omsetting na biobrandstof gedemonstreer. Die chromatografiese resultate wat vir die twee gekose biomassabronne in hierdie verhandeling gepubliseer is, is verkry deur ongewone kombinasies van skeidingmodus (een- en twee-dimensioneel) en kolomtipe/kolomkonfigurasie wat nie spesifiek in die literatuur gevind word nie.

Die studie het in geheel getoon dat *Jatropha*-olie ’n geskikte bron vir biobrandstofvervaardiging is, en dat selfs afvalhout belofte inhou vir omskakeling na vloeibare brandstowwe. Die nie-klassieke ekstraksiemetodes het geblyk instaat te wees om uit hierdie biomassabronne materiale te onttrek wat vir die vervaardiging van biobrandstof van belang is.

ACKNOWLEDGEMENTS

"Dear God, thank you for the talents you have entrusted to me. Help me to invest and use them wisely in the service of your Kingdom. Thank you for hearing and answering my prayer and bestowing upon me the abilities and determination to complete my studies."

I would also like to thank:

Professor E.L.J. Breet for his patience, friendliness and advice, and for sharing his knowledge and experience to guide me through this study. I also wish to thank him for the opportunity to present a poster at the 9th International Conference on Supercritical Fluids and their Applications in Italy during 2010. It was a wonderful experience!

My family and friends for all their support, love and enthusiasm and C.J. for continuously motivating me with lots of late-night coffee!

Dr. Pieter van Zyl for training and advice in utilising supercritical equipment.

Dr. Johan Jordaan for support and guidance with the GCxGC-TOFMS.

Dr. Peter Gorst-Allman, Jack Cochran and Jayne de Vos for all the expert advice, training and support with analytical and technical aspects throughout the study.

Dr. John Morris for donating *Jatropha curcas* seed and assisting in the extraction and conversion processes.

Sasol for funding this project and Dr. Reinier Nel for acting as line manager and tutor.

Chapter 0: A Bird's Eye View

Yolandi Nortjé undertook this research study for the purpose of acquiring a Magister Scientiae in Chemistry at North-West University, Potchefstroom. It was sponsored within the Sasol Technology University Support Program by virtue of a bursary and postgraduate project funding. The experimental work was done in the laboratories of the Chemical Resource Beneficiation research focus area of the University. It was the last postgraduate project to be supervised by Prof. Ernst Breet during his 40-year employment at NWU.

0.1 Project orientation

The availability of fossil fuels is declining rapidly, while the demand is increasing, making it imperative to develop sustainable alternative processes for fuel production.

One possible approach is biomass conversion. Botanical material is regarded as a sustainable source of organic carbon from which biofuels can be produced to substitute petroleum resources [1]. Soybean oil with added methanol is used as the most common form of biodiesel produced in the United States [2, 3]. Seed of the so-called diesel tree (*Jatropha curcas)* is a biomass source with a rich oil content and represents another source from which biodiesel may be derived [4, 5]. An introductory investigation has previously been conducted into deriving a biofuel from this source [6], but it is pursued in much more depth in this study with regard to analysis of the oil, its subsequent conversion into a biofuel, and benchmarking of the final product by comparison to an SABS approved biodiesel standard.

Wood was another biomass source considered in this study, since it had been argued that wood could possibly be cleaved by superheated water into liquid products which could be upgraded into a biofuel. Oxygen containing organic molecules undergo reaction in natural superheated water [7, 8] since the less polar solvent (H_2O), non-polar oxidizing agent (O_2) and non-polar organic compound (oxygenate) become increasingly mutually compatible as temperature is increased [9].

There are several new and fairly unexplored methods to obtain biomass extracts for subsequent conversion to biofuels, including supercritical fluid extraction, microwave-assisted superheating and ultrasound-supported extraction. These methods are termed non-

classical [10], since ambient or close-to-ambient conditions are extended, sometimes by orders of magnitude, to result in novel chemistry not accessible under normal conditions. Interest in these methods has resulted from increasing environmental and public health awareness, stricter regulations with regard to chemical disposal and toxic gas emissions, and a need for clean technology complying with the requirements of green chemistry [11]. It was an objective of this investigation to employ the three non-classical methods mentioned above to extract oil from *Jatropha* seed and material from pine sawdust for eventual conversion to a biodiesel, and to compare the results to those obtained by traditional methods such as soxhlet extraction.

In this investigation one-dimensional and two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOFMS) [12] had to be employed to cope with the analysis of the complex, component-rich botanical extracts derived by the non-classical methods used. It turned out to be equally effective for the analysis of the derivatised extracts and of the methylated products, the latter being benchmarked against a commercial biodiesel standard in terms of its content of fatty acid methyl esters (FAMEs), the principal building blocks of biodiesel [13].

0.2 Objectives

The main objectives of this study can in view of the foregoing be more formally stated as follows:

- to explore three non-classical methods (supercritical fluid extraction, microwave-assisted superheating, ultrasound-supported extraction) for the acquisition of extracts from selected biomass matrices (*Jatropha* seed, pine sawdust);
- to perform base-catalysed transesterification to convert botanical extracts into typical biodiesel building blocks (fatty acid methyl esters);
- to develop suitable protocols for chromatographic analysis of derivatised extracts and converted products in terms of suitable composition for biofuel production in comparison to available benchmarking standards;
- to compare results obtained with non-classical methods to those achieved by classical methods of bioconversion (e.g. soxhlet extraction) in order to identify the most suitable method(s) of biomass conversion into biofuels.

In addition to these main objectives, the project also served the purpose to contribute to a lesser extent to the following relevant issues:

- to emphasise the strategic importance of materials derived from plants as alternative energy sources;
- to support the idea of sustainable or green chemistry by implementing "clean" technology [14] based on non-hazardous sc-CO_2 and subcritical H_2O;
- to draw the attention of industry to the viability of non-classical methods and help defeating negative perceptions about extreme conditions often associated with these methods;
- to emphasise the importance of advanced analytical techniques to characterise complex mixtures such as botanical extracts and thereby assist in the development of alternative transportation fuels;
- to promote the research and development of environmentally friendly chemical processes.

0.3 Strategy

In order to achieve the objectives stated above a task list was compiled as outlined below:

- obtain freshly cultivated diesel tree seed and suitable samples of pine sawdust as selected examples of biomass sources for potential subsequent conversion into biofuels;
- develop effective procedures of sample preparation, particle size optimisation and sample handling prior to and after extraction;
- conduct extraction runs with sc-CO_2, superheated H_2O and ultrasound-activated solvents (H_2O, n-hexane) using the selected biomatrices and available infrastructure (supercritical extractor, microwave equipment, ultrasound emitter);
- apply standard procedures to derivatise botanical extracts acquired by different non-classical methods and to convert these by catalysed methylation into fuel related products (FAMEs = fatty acid methyl esters);
- develop suitable protocols to analyse both derivatised extracts and converted products using one-dimensional and two-dimensional gas chromatography with time-

of-flight mass spectrometric detection (GCx GC-TOF/MS) and different specialised column types and configurations;

- benchmark biofuel type products obtained through transesterification against an approved standard (SANS1935) [15];
- compare results of non-classical methods mutually and with those of classical methods to find the most suitable biomass conversion technology.

References

[1] Huber, G.W., Iborra S. and Corma A. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chemical Reviews*, 106 (2006): 4044.

[2] Dunn, R.O. Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel). *Fuel Processing Technology*, 86 No.10 (2005): 1071-1085.

[3] Monyem, M. and Van Gerpen, J.H. The effect of biodiesel oxidation on engine performance and emissions. *Biomass and Bioenergy*, 20 No. 4 (2001): 317-325.

[4] Morris, J. Making biodiesel with high profit from *Jatropha curcas* L, Batho Pele Group Developmental Plan, Syringa Institute, Centurion (South Africa), 2006.

[5] Palgrave, K.C. Trees of Southern Africa. Struik Publishers, Cape Town, 2002, 512.

[6] Van Greuning, C. sc-CO_2 extracted oil from *Jatropha curcas* - directive for the biodiesel industry? M.Sc. Dissertation, School of Physical and Chemical Sciences, North-West University, Potchefstroom, 2009.

[7] Siskin, M. and Katritzky, A.R. Reactivity of organic compounds in superheated water: General background. *Chem. Rev.,* 101 (2001): 825.

[8] Siskin, M. and Katritzky, A.R. A review of the activity of organic compounds with oxygen-containing functionality in superheated water. *J. Anal. Appl. Pyrolysis*, 54 (2000): 193.

[9] Elliott, D.C., Beckman, D., Bridgwater, A.V., Diebold, J.P., Gevert, S.B., and Solantausta, Y. Developments in direct thermochemical liquefaction of biomass: 1983-1990. *Energy & Fuels*, 5 (1991): 399.

[10] Van Eldik, R. and Hubbard, C. D. Chemistry Under Extreme or Non-Classical Conditions. John Wiley & Sons. Inc., 1997: 545.

[11] Nobel, D. *Anal. Chem*., 65 (1993): 693A. See also *Montreol Protocol,* according to which a few traditional solvents were banned since 1995.

[12] Schomburg, G. Two-dimensional gas chromatography: principles, instrumentation, methods. *Journal of Chromatography A*, 703 (1995): 309-325.

[13] Knothe, G., Krahl, J. and Van Gerpen, J. The Biodiesel Handbook. AOCS Press, 2004.

[14] ICS/UNIDO Workshop on *Cleaner Technologies for Sustainable Chemistry*, Cape Town, 9-11 December 2002.

[15] SANS1935:2004. South African National Standard for Automotive Biodiesel Fuel, Standards South Africa (A Division of SABS), 2004.

Chapter 1: Current Status of Global Biofuel Production

World leaders started to discuss biofuel production and investment in research on this topic in view of several important global issues. Climate change, and as a result of that, global warming concerns, energy security and fossil fuel decline are some of the major drivers for biofuel development. It is estimated that carbon emissions need to be reduced by up to 50% by 2050, and consequently biofuel is currently an actively promoted alternative energy source worldwide [1, 2]. The South African Cabinet appointed a Biofuels Task Team in 2005 in order to

- *"stimulate rural development and thereby contribute to government's Accelerated and Shared Growth Initiative (AsgiSA)";*
- *"reduce poverty by creating sustainable income-earning opportunities (Biofuels Industrial Strategy of the Republic of South Africa, December 2007)."*

Biofuels include a wide range of products and production methods. Ethanol and biodiesel are currently the most widely used biofuels [1, 2]. Sugar cane is the primary feedstock for the production of ethanol in Brazil, whereas maize is used in the United States. Vegetable oils and animal fats are used for the production of biodiesel. Although these fuels are considered to be substitutes for fossil fuels in the near future, presently most transportation biofuels are more expensive to produce per unit of energy than oil derived from fossil fuels.

Biofuels can be grouped into two main categories: traditional/classical/first-generation biofuels and second-generation/advanced biofuels [1, 3].

1.1 First-generation biofuels

These biofuels refer to those produced by converting sugar, starch and essential oils, and they are currently limited by high vegetable oil and wheat prices [3].

A few concerns and challenges about first-generation biofuel production are the "food versus fuel" debate, deforestation, sufficient water resources, land requirements, supply chain sustainability, and sustainable fuel generation [1].

First-generation biofuels can be grouped into two main categories: carbohydrate-derived biofuels (ethanol from sugar and starch) and lipid-derived biofuels (straight-chain vegetable oil and biodiesel).
First-generation feed stocks include [4]

- sugar crops: sugar cane, sugar beet, sweet sorghum
- starch crops: corn, wheat, cassava, sorghum grain
- oilseed crops: rapeseed, soybean, palm and diesel tree seed

Other potential oil sources for biodiesel include [4]

- oil seed crops and tree-based oil seed: sunflower, cotton, peanut, mustard, coconut, castor oil, vegetable oil
- micro-algae
- animal fats

Main biofuel crops around the world currently include [2]

- sugar cane crops in Brazil to produce ethanol;
- maize grown and harvested in the United States to produce ethanol;
- rapeseed in Europe to produce biodiesel;
- diesel tree seed in countries like China, India, Kenya and Tanzania to produce biofuels;
- sugar cane and sugar beet, sunflower, canola and soybean for the production of bio-ethanol and biodiesel in South Africa (with sorghum and algae to a lesser extent and maize excluded due to food security concerns).

It is predicted that the crops listed above will continue to provide the bulk biomass supplies for biofuel production (first-generation) over the coming decades. These fuels include bio-ethanol and vegetable oil methyl esters (VOMEs), better known as fatty acid methyl esters (FAMEs) or biodiesel. **Figures 1.1** and **1.2** show the worldwide production during 2006 of these two types of biofuel, respectively. The world currently consumes about 430 exajoules (EJ) of energy per year, and approximately 100 EJ are used in the transport sector. Biomass currently accounts for nearly 40 EJ of this world energy demand [4].

Biofuels produced via biomass grown in tropical regions are cheaper and displace a larger share of petroleum than fuels from more moderate feed stocks. European countries will most

likely import their biofuels rather than attempt to grow their own biomass feed stocks. The United States may produce more local biofuels, but will eventually face a similar situation. Algae have great potential theoretically, and its utilisation is widely researched, but this possible feedstock has not been proven yet to be an economical and viable biomass source.

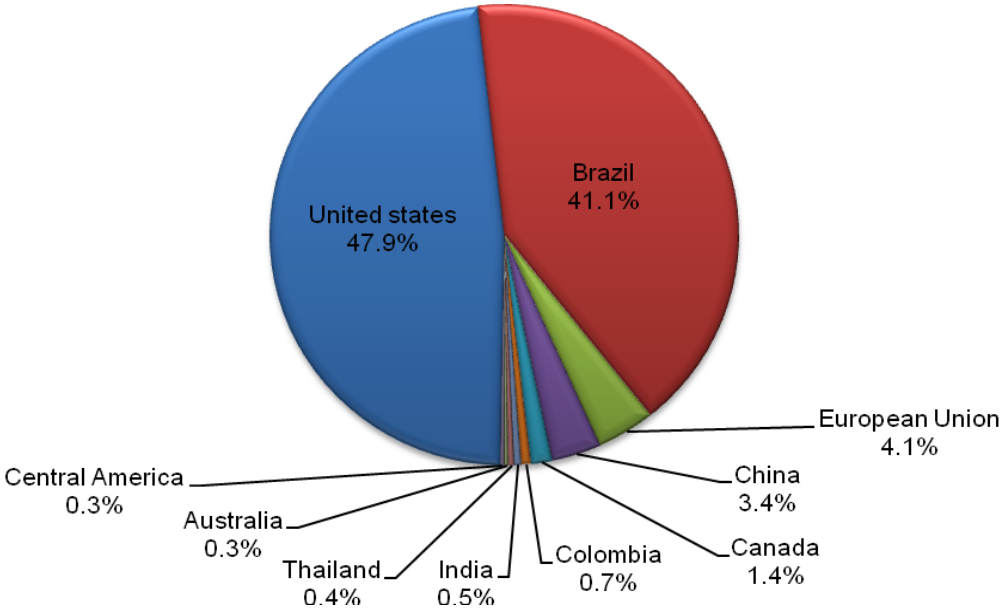

Figure 1.1. : World bio-ethanol production 2006 [4]

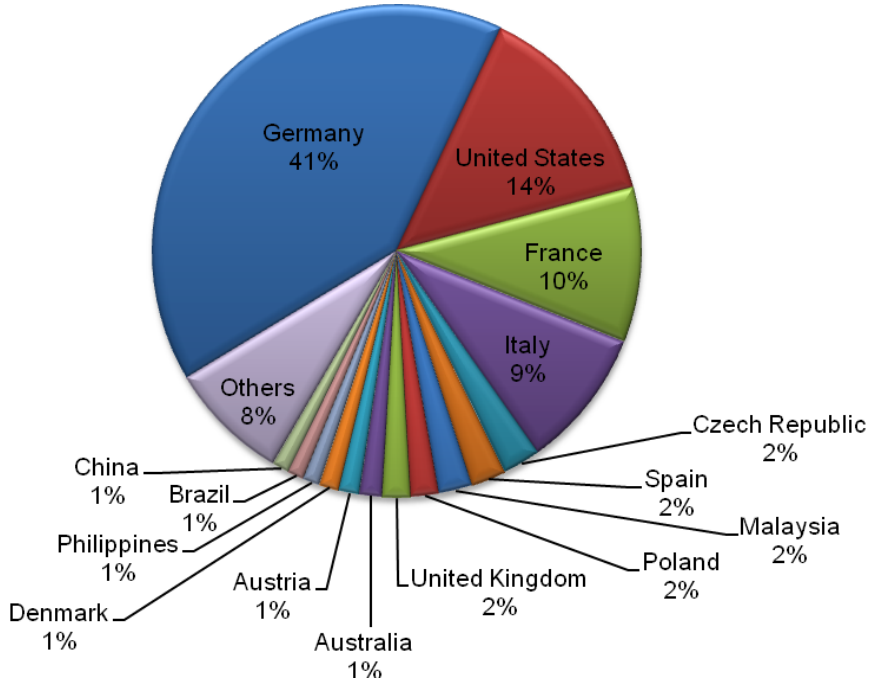

Figure 1.2: World biodiesel production 2006 [4]

1.2 Second-generation biofuels

Second-generation biofuels are those produced from lignocellulosic biomass, i.e. plant material composed of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are carbohydrate polymers which are tightly bound to lignin by covalent and hydrogen bonds [1]. Processes such as hydrolysis, fermentation, gasification or pyrolysis are used to convert this type of biomass to biofuels. Hydro-treatment of vegetable oils or animal fats, and gasification of biomass combined with Fischer-Tropsch feedstocks (hydrocarbon-containing material), yield paraffinic diesel fuels of high quality. If the feedstock is a gas, the process is called GTL (gas-to-liquid), and in the case of a coal feed, a CTL (coal-to-liquid) synthesis is performed [5]. For a biomass feedstock, BTL (biomass-to-liquid) products are obtained. Syngas technologies can also be applied to produce gasoline, methanol and DME (dimethyl ether) [5].

Currently no second-generation biofuels are produced industrially, but research is done worldwide on these fuels, and a limited number of pilot plants exists and are planned for the future. Locations of these plants include North America, Brazil, Japan and Europe. Successful lignocellulosic technologies would allow use of a large variety of feedstocks, as well as agricultural or municipal waste materials and specialised cellulosic crops such as grasses and fast growing trees [4]. Such feedstocks require less water, fertilizer and quality soil, and are less expensive to grow than crops for conventional ethanol production. It is expected that low cost residues and waste sources of cellulosic biomass will provide the first entry of second-generation biofuel feedstocks over the next decade [4].

1.3 Production technologies

Figure 1.3 summarises different known production routes of biofuels (both first-generation and second-generation) from biomass. The methodologies for the different production pathways are well documented, and the challenge rather lies in the development of economically sustainable continuous production plants similar to fossil fuel production facilities.

First-generation biofuels are mainly produced via hydrolysis, fermentation, pressing or esterification technologies. The pathways for conversion of second-generation biomass into liquid biofuels are more difficult to achieve. This is mainly due to natural resistance of cellulosic biomass to be broken down into its elementary components, and although the

costs of cellulosic feedstocks are lower than first-generation feedstocks, conversion technologies are more expensive [4]. The pathways for conversion of cellulosic (second-generation) biomass include gasification and Fischer-Tropsch synthesis, pyrolysis, hydrolysis (acid or enzymatic) and microbial digestion [4]. These production routes can be classified as either thermochemical or biochemical in nature. Thermochemical conversion consists of gasification and pyrolysis. Gasification relates to a catalysed high-temperature conversion of biomass into an intermediate syngas. Pyrolysis is the conversion of biomass in the absence of oxygen to a bio-oil. Biochemical conversion requires complex pre-treatment steps to break down biomass into component sugar molecules. These molecules are then processed by fermentation organisms [4].

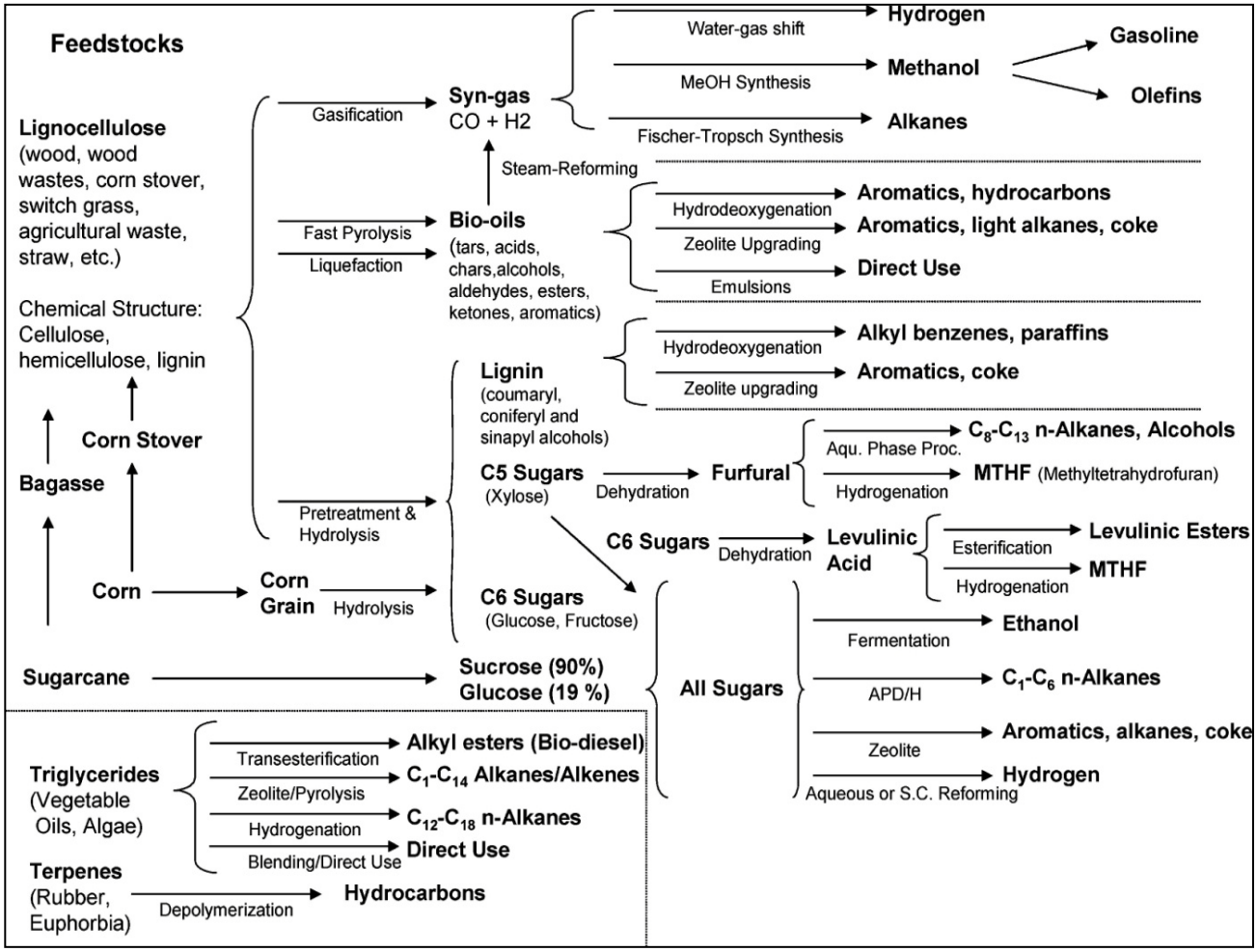

Figure 1.3: Known production routes of first-generation and second-generation liquid fuels from biomass [6].

The production of synthetic type biofuels can be increased by adding to an existing method, e.g. Fischer-Tropsch synthesis, large amounts of biomass feedstock to obtain a combined fossil and biomass derived fuel production facility as shown in **Figure 1.4**. A “bio-jet fuel”

produced this way may reduce greenhouse gas emissions from air transport since there are currently very few alternatives to the petroleum based fuels in this sector [7]. Current biofuel production costs are high but are predicted to decrease as technologies upgrade and experience in conversion processes increases.

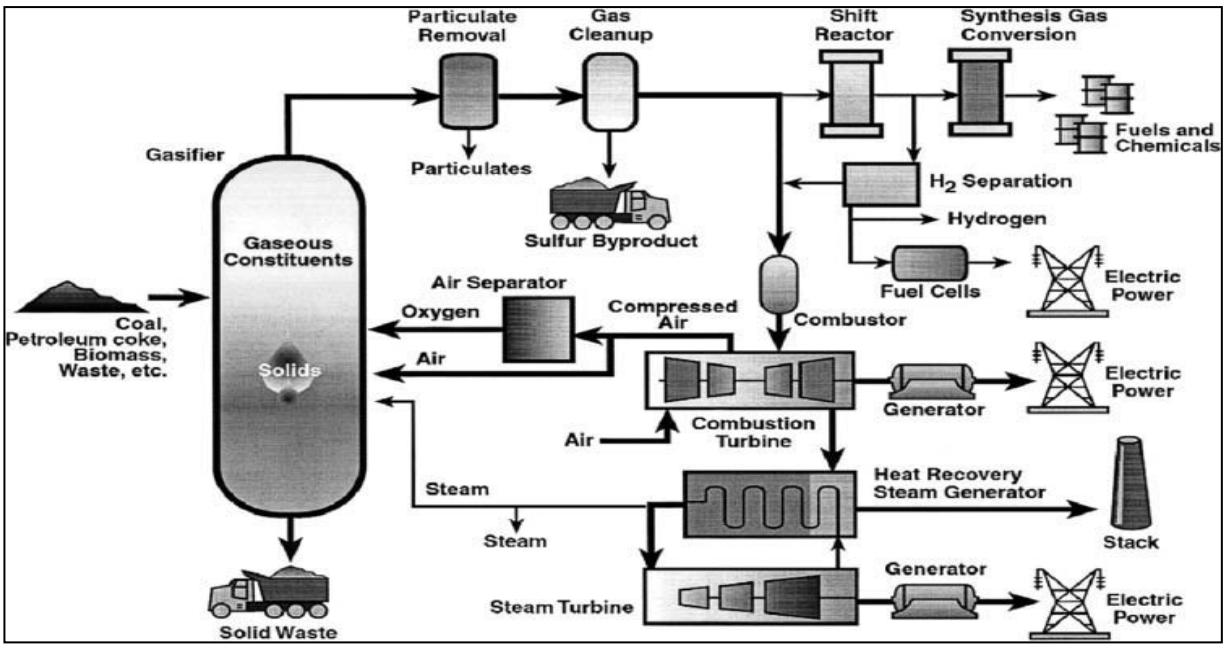

Figure 1.4: Schematic presentation of a plant producing fuels from fossil resources and biomass [6].

1.4 Fossil fuels and biofuels - current scenario

According to the International Energy Agency (IEA) [8] transport fuel demand will rise significantly over the coming few decades. As a result, biofuel production is predicted to increase at a rate of 8.3% per year to reach 7% of the global road-transport fuel demand in 2030 [1,5]. It seems that the world's energy system is at a crossroad. Oil is still the world's largest energy source and this fact will remain true for many years to come. A predicament, however, is that the oil resources needed to meet the rising energy demand, and the oil production costs that consumers will have to pay, are very uncertain and unpredictable at present [8]. If the current level of greenhouse gas emissions continues, the concentration of these gases in the atmosphere may increase to such an extent towards the end of this century that an increase in the average global temperature of up to 6 ^{0}C in the long term can be anticipated [8]. Alternative fuels and energy resources are therefore of cardinal importance for future generations, and currently biofuels seem to be the only viable transportation fuel supplement. **Table 1.1** gives the current status of three important biofuel related issues in South Africa in comparison to that in other countries.

Table 1.1: Current status of some biofuel related issues in South Africa and other countries [2].

	Other countries	South Africa
Labour issues	Brazil: Since the development of the biofuel sector, Brazil has experienced increases in the number of jobs created. The major concern, however, has been the quality of jobs (are they sustainable and will they result in more income for poor families, or merely extend their poverty?). (Memorandum of Understanding between US and Brazil to Advance Cooperation on Biofuels, 2007).	Biofuel has been identified as a key driver in AsgiSA for social and economic development (Biofuels Industrial Strategy of the Republic of South Africa, December 2007).
Environmental issues	The Kyoto protocol obliges industrialised countries to pledge to reduce their greenhouse gas emissions by 2012 (Ruth, 2008). (Memorandum of Understanding between US and Brazil to Advance Cooperation on Biofuels, 2007).	Although the Kyoto protocol does not commit countries like South Africa to any quantifiable emission targets, there is potential for future low-cost emission reduction options. Biofuel projects may apply for carbon emission reduction credits via mechanisms such as fuel switching (Biofuels Industrial Strategy of the Republic of South Africa, December 2007).
Land use and water resources	In some African countries it has been noticed that cultivation of *Jatropha* can reduce soil erosion and increase water retention (Araujo *et al.*, 2007). In the US maize has generally been rotated with soybeans to promote soil quality, and it has been found that corn grown in drier areas will require more water and hence put pressure on already scarce water resources (Memorandum of Understanding between US and Brazil to Advance Cooperation on Biofuels, 2007).	In South Africa a specific requirement for the Biofuels Industrial Strategy was to create a link between the first and second economy. This referred to developing areas such as the former homelands where agriculture was previously undermined to a level that it will compete commercially. Irrigated crops, such as sugar cane, which require a lot of water, will have to compete with other crops for already scarce water resources (Biofuels Industrial Strategy of the Republic of South Africa, December 2007).

1.5 Activities of industrial fuel companies

BP - Beyond Petroleum [9]

BP has been one of the key role-players in the global biofuel industry over the past few years. In 2006 BP blended 3 016 million L of bio-ethanol into gasoline - a 25% increase on the previous year. BP's strategy has involved the formation of a dedicated business unit to pursue opportunities across the value chain, from accessing feedstock through biomass conversion to biofuel, trading and marketing. BP is constantly asking questions such as how much land should be adapted for biofuel production to ensure that greenhouse gas emissions are lowered, or what will be an acceptable level of risk to biodiversity if a field is used to cultivate sugar rather than leaving it as pasture? Such questions are important in

developing sustainable biofuel production units. BP is conducting investigations into the availability of agricultural systems for biomass development which is favourable for the environment as well as the community, especially in the United States and Europe where considerable amounts of bio-products are needed. It is one of the leading petroleum companies to respond to these requirements. The company's biofuel business unit is in its developmental stages, and principles and regulations need to be defined for the biofuel research and production fraternity. The company aims at developing innovative and novel techniques to ensure sustainability in environmental, social and economic terms, as well as competitive pricing in the markets according to three major guidelines:

1. *Understanding current practicalities and future possibilities* [9]

- In 2005 a *Jatropha curcas* production plan was initiated in India. The Energy Research Institute of India planted 8000 ha of *Jatropha* trees (**Figure 1.5**) and, together with BP, investigated the yield and water requirements to better assess the seed as a feedstock for biodiesel. They also evaluated the impact of such a plantation on the ecosystem as *Jatropha* is regarded as a key potential alternative to palm and soy.

Figure 1.5. *Jatropha* nursery, Andhra Pradesh, India [9]

- In 2006 the company announced a partnership with DuPont to manufacture biobutanol.

- In February 2007 BP launched a 10 year US$ 500 million investment project named Energy Biosciences. This project focuses on the conversion of lingo-cellulosic feedstocks such as grasses or waste material from sugars.

2. Creating awareness among those who can shape the industry [9]

- Engaging with customers: Target Neutral is a facility for people, particularly BP customers, to understand their vehicles' GHG emissions and offset them by funding projects which capture carbon.
- Engaging with industry: BP was the first fuel company to become a member of the Round Table on Sustainable Palm Oil.
- Engaging with regulators: BP is actively engaged with governmental bodies in the UK, Germany and the State of California to ensure that practical systems are in place for verification and certification of sustainability of fuels.

3. Having an own "magnetic north" [9]

The company is developing its own principles according to which its business strategy and operational procedures in the value chain are run in order to minimise impact on ecosystems.

Shell [10]

Shell currently buys, trades, stores, blends and distributes more than 600 000 000 L of conventional bio-ethanol and fatty acid methyl esters (FAMEs) biofuels. The company developed a sustainable sourcing policy in 2007 to manage social and environmental issues and to create ways of measuring each biofuel's overall CO_2 emission. Together with non-governmental and governmental organizations, Shell is contributing to determine international standards for biofuels [10].

Shell Global Solutions is a technology division investigating development of next-generation biofuels. As an example, it collaborates with a Canadian company to develop ethanol from wheat straw. A full-scale commercial plant is now being assessed [10]. Various press releases and developmental plans are available on the Shell website.

Sasol [11]

Sasol has been focusing on new energy sources such as nuclear power. A business unit has been established that leads into non-carbon energy technologies. The team developed innovative gas-to-liquid (GTL) technology for the production of environmentally friendly biodiesel. Waste gases which were previously flared are now used to generate electricity for plants. A specialised group is also working on carbon banking and storage. Sasol is investigating the possibility of biodiesel from soybean but needs governmental frameworks and regulation to finalise research and development.

References

[1] WBCSD, Ecosystems, Energy and Climate, Forest Products, Mobility - Press updates and WBCSD news: Issue brief- energy and climate focus area, 16 Nov. 2007.

[2] Public Understanding of Biotechnology (PUB), The South African Agency for Science and Technology Advancement (SAASTA) (Department of Science and Technology: Republic of South Africa), *The current status of the biofuels industry worldwide,* 2009. http://www.pub.ac.za/docs/factfile-biofuesl-status.pdf [Accessed 10 February 2009].

[3] NNFCC, Dr. Grant Evans: International Biofuels Strategy Project, Liquid Transport Biofuels - Technology Status Report, Project 08/017, 2007.

[4] Worldwatch Institute, *Biofuels for transport*, Earthscan UK, 2007, pp 1-38.

[5] Nils-Olof Nylund, Päivi Aakko-Saksa & Kai Sipilä, VTT Research Notes 2426: *Status and outlook for biofuels, other alternative fuels and new vehicles*, (2008):18-20, 44-49.

[6] Huber, G.W., Iborra, S., Corma, A., Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem. Rev.,* (2006), *106*, 4047-4061.

[7] Panorama Technical Report, Innovative, Energy, Environment, Biofuels *Worldwide*, 2005.

[8] International Energy Agency (IEA): *World Energy Outlook (WEO),* 2008*. (Executive Summary)*.

[9] BP Statistical Review, 2006
http://www.bp.com/liveassets/bp_internet/globalbp/globalbp_uk_english/reports_anpublications/statistical_energy_review_2006/ [accessed 12 March 2010]

[10] www.shell.com /About Shell /Our business [accessed 12 March 2010]

[11] www.sasol.com/sasol_internet/frontend/navigation.jsp [accessed 12 March 2010]

Chapter 2: Biomass Sources for Biofuel Production

There is a variety of biomass resources available for biofuel production.

In the USA corn and other edible sources are used for biofuel production, but other nations insist on non-edible feedstocks in view of food security concerns.

One acceptable biomass source for biodiesel production is plant seed containing oils rich in triglycerides. These include sunflower, canola, soybean and diesel tree (*Jatropha curcas* L.) seed. Previous investigations [1-4] showed that such plant oils are readily soluble in sc-CO_2 and can thus be extracted from seed easily and environmentally friendly using this solvent. The most obvious plant seed to use is from the diesel tree as these are unsuitable for human consumption. There is thus no conflict with food resources should this type of biomass be used for biofuel production.

A second promising source of biomass for biofuels is wood fibre. Although wood has been used as a fuel for centuries, the production of transportation fuels from wooden biomass is fairly novel and largely unexplored. It may be possible to cleave wood with superheated water [5-7] into liquid products upgradeable into a biofuel since the solvent (H_2O), oxidising agent (O_2) and organic compound (oxygenate) become increasingly mutually compatible as temperature is increased. Additionally, subcritical and supercritical water could be utilised since the nature of water changes dramatically on going from the superheated through the subcritical to the supercritical state [8], rendering it even more compatible for reaction with oxygen-containing organics. The dissociation constant can, for instance, be three orders of magnitude higher in supercritical than in ambient water (10^{-11} instead of 10^{-14}), while the dielectric constant of 78.5 approaches 10 at near-critical conditions and further decreases with increasing temperature. Under such conditions water acts as an acid or base catalyst because of the high concentrations of H_3O^+ and OH^- involved, and is considered a non-polar solvent with its extensive hydrogen bonding disrupted.

These two biomass sources were investigated in this study for production of crude material that could eventually be converted into biofuel related products.

2.1 *Jatropha curcas* seed

2.1.1 Description

Jatropha curcas, also known as the diesel tree, is a non-edible, seed-bearing tree and belongs to the *Euphorbiaceae* family [4, 9]. Trees grow 5-7 m tall and have a life expectancy of 50 years. The tree originates from Mexico, Central America, Brazil, Bolivia, Peru, Argentina and Paraguay, and has spread to Mozambique, Zimbabwe and into South Africa. *Jatropha* trees can be cultivated without difficulty in soils with low nutrient content and with little water [10]. Oil extracted from the seed is primarily used for the production of biodiesel, but other uses include fertilizer, soaps and cosmetics. The seed reaches maturity 90 days after flowering and are harvested at this stage to ensure a high oil yield [9, 10].

Figure 2.1: Harvested *Jatropha curcas* L. seed used in the laboratory

Figure 2.1 illustrates *Jatropha* seed capped in the fruit as harvested from the tree (left) and after being removed from the core of the fruit (right) for sample preparation and extraction. The quality and composition of the extracted oil vary according to the environment where the trees were grown, and the genetics of the seed. The oil mainly consists of glycerides, free fatty acids and some unsaponifiables. Triglycerides make up about 97% of the *Jatropha* oil composition, whereas monoglycerides and diglycerides are present in small amounts and represent about 2.5% of the oil mass [9, 11, 12]. The glycerides represent the fatty acids that are converted to fatty acid methyl esters (FAMEs) on transesterification of the oil to a biofuel.

2.1.2 Extraction methods

Jatropha oil can be extracted from seed in various ways. The oil is traditionally obtained by the cold press method or by solvent extraction with organic solvents, such as n-hexane.

Clean technology methods have been developed over the past few years to extract botanical oils in a more environmentally friendly way. These methods are termed non-classical (or extreme) [13], since ambient conditions are extended by several orders of magnitude beyond normal conditions to result in novel technology which more closely meet the requirements of green chemistry. In this investigation sc-CO_2 extraction, microwave-assisted superheating and ultrasound-supported extraction were used to extract *Jatropha* oil, and this topic will be discussed in more detail in **Chapter 3**.

2.1.3 Conversion of plant oil to biofuel

Biodiesel is an alternative to fossil derived diesel. Biodiesel comprises mono-alkyl esters of long-chain fatty acids derived from vegetable oils and some animal fats. Vegetable oils, such as *Jatropha* oil, contain complex mixtures of glycerides which are broken down into their resulting fatty acid chains and glycerol. These fatty acids are generally methylated to FAMEs by using an alcohol reagent (methanol) and a reaction catalyst (NaOH). The optimal reagent quantities for the transesterification of *Jatropha* oil are 1% NaOH per mass oil and 20% methanol per mass oil [10]. The optimal reaction time is about 90 minutes at a reaction temperature of 60 °C.

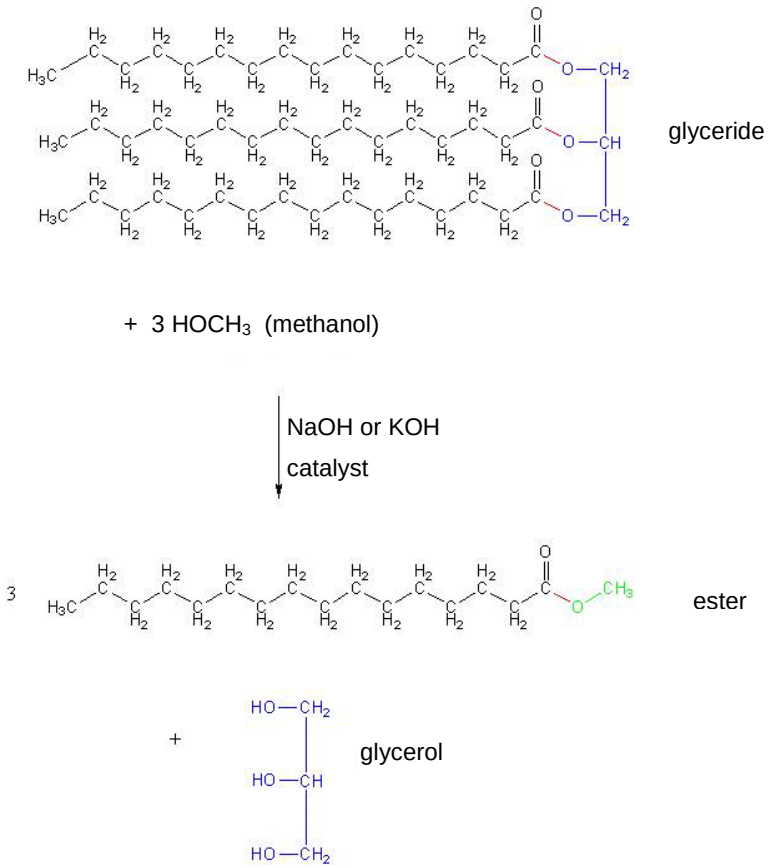

Figure 2.2: Schematic representation of transesterification of triglycerides [10, 14]

Various advanced transesterification processes are explored. These include *in situ* transesterification (extraction step skipped) as well as transesterification with supercritical alcohols. Oil acidity (free fatty acid concentration) needs to be determined before the transesterification step to ensure a neutral reaction environment. If the oil contains free fatty acids in high concentration, pre-treatment is necessary as the fatty acids interfere with the methylation reaction through undesired soap formation and a pH decrease [10, 14].

The fatty acid composition of vegetable oil is dominated by oleic acid (C18:1) and linoleic acid (C18:2), with palmitic acid (C16:0) and stearic acid (C18:0) also present [11, 12, 15]. These acids, and the corresponding methyl esters (FAMEs) contained within *Jatropha* oil derived biodiesel, are listed in **Table 2.1**.

Table 2.1: Major components of pure and converted *Jatropha* oil

Fatty acid	**Structure**	**Acronym**	**Methyl Ester**
Palmitic acid / Hexadecanoic acid	$R\text{-}(CH_2)_{14}\text{-}CH_3$	C16:0	Methyl Palmitate / Methyl hexadecanoate
Stearic acid / Octadecanoic acid	$R\text{-}(CH_2)_{16}\text{-}CH_3$	C18:0	Methyl stearate / Methyl octadecanoate
Oleic acid / 9(Z)-octadecanoic acid	$R\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}CH_3$	C18:1	Methyl oleate / Methyl 9(Z)-octadecenoate
Linoleic acid / 9(Z), 12(Z)-octadecadienoic acid	$R\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{--}(CH_2)_4\text{-}CH_3$	C18:2	Methyl linoleate / Methyl 9(Z), 12(Z)-octadecadienoate
Linolenic acid / 9(Z), 12(Z), 15(Z)-octadecatrienoic acid	$R\text{-}(CH_2)_7\text{-}(CH{=}CH\text{-}CH_2)_3\text{-}CH_3$	C18:3	Methyl linoleate / Methyl 9(Z), 12(Z), 15(Z)-octadecadienoate

The addition of methanol/ethanol reduces the viscosity of the original glyceride mixture, which is favourable to prevent clogging of the injector nozzles of a diesel engine [12, 15, 16]. The nature of biodiesel differs entirely from fossil derived diesel in this regard, but the two types of fuel share basic properties. Biodiesel may be blended into fossil derived diesel provided that certain technical and legislatory requirements are met, such as to reduce harmful gas emissions and to preserve fossil fuel resources.

A diesel/biodiesel blend is referred to as BX, where X denotes the percentage of biodiesel blended into the diesel fuel [4]. The most common blends are B2 and B20. B100 indicates a

pure (100%) biodiesel. The American Society for Testing and Materials (ASTM) has developed a standard for diesel fuels called ASTM D975 [17]. It defines the properties necessary for safe storage, transport and engine usage of a diesel fuel. These properties include flash point, water content and sediment, distillation curve, viscosity, ash content, sulphur, copper strip corrosion, cetane number, cloud point and lubricity. The fatty acid composition of a biodiesel influences these properties. For example, increasing chain length causes an increase in melting point as well as in cetane number [15-17].

During transesterification of vegetable oil intermediates such as monoglycerides and diglycerides are formed. Soap residue due to the alkali catalyst and residual alcohol can also contaminate the final product. The ASTM D6751 standard for biodiesel states limiting values for free glycerol (0.02% by mass) and total glycerol (0.24% by mass), and defines residue testing methods for soap and catalyst. The total glycerol figure includes the amount of free glycerol remaining after transesterification in combination with the three glyceride by-products. Three main analytical methods prescribed for biodiesel include chromatographic, spectroscopic and physical-property based methods. Gas chromatography (GC) forms the basis of measuring total glycerol and amount of methyl esters. The ASTM D6584 describes standard GC methods for biodiesel analysis [17].

Similar to the ASTM methods, the South African Bureau of Standards (SABS) has also created a national biodiesel standard based on the publications of the European Committee for Standardisation (CEN). The South African National Standard is known as SANS 1935:2004 and lists the required specifications for biodiesel in South Africa [18].

2.2 Wooden biomass

2.2.1 Description

Wooden biomass from forests or other sources is of growing interest as a possible energy source. It can be converted into a useful solid, liquid or gas providing energy for industrial, commercial and domestic use [19]. Biomass provides about 10% of the world's primary energy resources, and about 50% of the total of 4 billion m^3 wood used globally per annum is applied towards fuel wood/charcoal for heating and cooking in developing countries [20].

Wooden biomass can be a sustainable and renewable alternative to fossil fuel resources. Biomass production systems generated from conventional forestry arises mainly from the by-products of timber production systems [19, 21]. Harvesting operations for timber wood yield

tops and branches suitable for bio-energy production. In addition, branches and young trees damaged by fires, insects and diseases can also be utilised as biomass sources. These possibilities simply show the economical benefits that development of bio-energy markets can provide without taking environmental and sustainability benefits into account. Bio-energy markets create efficient and profitable treatment of biomass wastes, promote new crops for farmers, especially in developing countries, provide a solution for unused agricultural land and create employment opportunities [19].

When energy crops are well managed and effective, mixing forestry and agricultural ventures become very attractive in the new green energy domain. Greenhouse gas emissions are reduced when fossil fuels derived energy are replaced by bio-energy systems. The IEA showed that the amount of fossil energy consumed is considerably smaller than the amount of bio-energy produced. For every unit of fossil energy consumed, 25 to 50 units of bio-energy can be produced [19]. Liquid bio-energy generally requires 5 units more input energy for every one unit of fossil energy consumed, but the carbon emissions per unit of electricity generated from bio-energy are 10 to 20 times lower than the emissions per unit of electricity from fossil derived energy [19, 21].

All these facts and benefits create the need of expansion of wood energy and use. One such recent expansion is the use of wood pellet fuel to produce heat and electrical power [21]. Wood pellets are produced from sawdust by mechanically compressing the particles into a solid pellet which is easily transported and stored. Other areas of commercial uses of wood include co-firing of wood biomass with coal, or the replacement of fossil fuels with wood residues for energy generation at forestry facilities such as saw mills. Liquid biofuels produced from wood have become a widely researched topic, and a few demonstration scale projects have been underway in the US for some time. These biofuels are of great interest due to the availability of wooden biomass and the large potential of developing renewable transportation fuels and general industrial chemicals.

In this study pine sawdust was used as a wooden biomass source for potential conversion into a biofuel product. A prepared sample for the purpose of extraction by different methods is shown in **Figure 2.3.** The sawdust samples were coarse, jagged particles with an average length of roughly 2-3 mm. The samples were used as received from a local supplier without further grinding or refinement.

Figure 2.3: A sample of pine sawdust after milling as used in this study

2.2.2. Fuels from wood

Biofuels producible from wooden biomass include cellulosic ethanol, diesel, variations of alcohols and large amounts of alkane fuels [20, 21]. Bio-ethanol is produced by fermentation of wood sugars. This method is well known in view of the established production of ethanol from glucose sugars in corn. Production of ethanol from wood may become more economical after optimising the hydrolysis of cellulose and extraction of hemicelluloses. Corn is a major food source in developing countries, rendering wood a more attractive biofuel resource to help ascertain food securities and demand in these countries.

The required properties of wood for conversion to biofuels are high cellulose and/or hemicelluloses content, low lignin content, readily separable lignin and low delivery cost. There are alternative thermochemical pathways for conversion of forest biomass to biofuels and chemicals. These pathways involve gasification into syngas (CO, H_2, etc.) followed by liquefaction of syngas by catalytic reforming or pyrolysis to bio-oils [20]. These are processes similar to Fischer-Tropsch conversions and also generate similar products (mixed alcohols and alkynes with their derivatives). Thermochemical conversions developed from coal-to-liquid technology are very efficient since forest biomass has higher hydrogen content than coal and provides higher conversion ratios to mixed alcohols and alkynes [20, 21]. There are, however, some drawbacks as well, including the formation of tar from lignin and carbon char which inhibits catalytic reactions. Due to lignin causing tar formation, research is devoted to reduce the amount of lignin in wood species and creating more efficient catalysts.

Wood is primarily composed of cellulose, hemicelluloses, lignin and extractives. **Table 2.2** lists the major chemical composition of some wood species.

Table 2.2: Chemical composition of some wood species [20]

Constituent	Scots Pine	Spruce	Eucalyptus	Silver Birch
Cellulose (%)	40	39.5	45	41
Hemicellulose				
-Glucomannan (%)	16	17.2	3.1	2.3
-Glucuronoxylan (%)	8.9	10.4	14.1	27.5
-Other Polysaccharides (%)	3.6	3	2	2.6
Lignin (%)	27.7	27.5	31.3	22
Total Extractives (%)	**3.5**	**2.1**	**2.8**	**3**

Figure 2.4 illustrates the structure of cellulose, which is the major component of wood fibre. It comprises D-glucose molecules linked by β-1,4-glycosidic bonds to create long and straight chains which vary with the degree of polymerisation [20, 22].

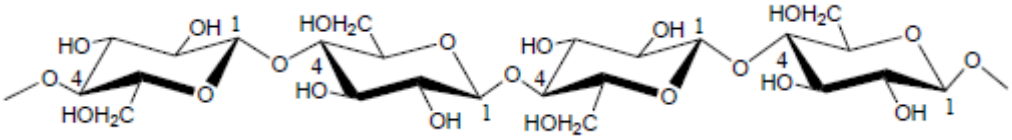

Figure 2.4: Structure of cellulose [20]

Each D-glucose segment contains three hydroxyl groups that can undergo typical primary and secondary alcohol reactions. Bound to the cellulose are hemicellulose chains which have a lower degree of polymerisation and are basically amorphous. These short, branched chains of glucose and other sugar molecules fill the space in the plant wall. They are more soluble in water than cellulose and are often removed during pulping processes. Lignin can be described as a three-dimensional phenolic polymer network and acts as "super glue" that binds the cellulose and hemicellulose fibres together. Extreme chemical processes are necessary to leach out the lignin and break the strong ester linkages without degrading the cellulose fibre. **Figure 2.5** shows the composition of general softwood species such as pine wood [22].

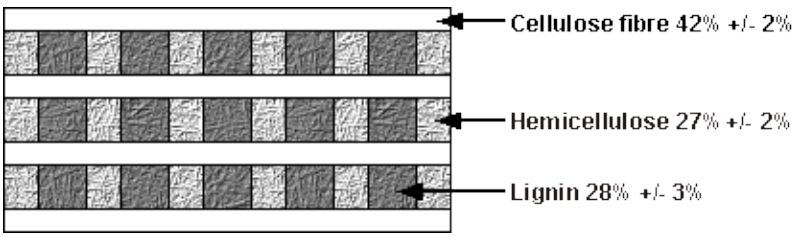

Figure 2.5: Average composition of softwood [22]

In addition to these main components, wood contains a small number of extractable compounds including plant hormones, resin and fatty acids, alkynes, alkenes, monoterpenes and phenolics. Bio-oil is generated from wood by means of pyrolysis. Wood chips or sawdust is heated in the absence of oxygen to create an intermediate bio-gas which is condensed into liquid oil. These oils can then be further developed to a biofuel or used for other bio-chemicals.

Another point of interest is the possibility to break the linkages between cellulose and lignin and cleave the large glucose chains into segments that could be used for the development of biochemicals. By extraction, combined with chemical fractionation, valuable components for enhancement of biofuel development could be obtained.

References

[1] Wessels, A. Extraction of *Helianthus annuus* (sunflower) oil with supercritical carbon dioxide. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2004.

[2] Louw, H.W. Extraction of canola oil with supercritical carbon dioxide. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2006.

[3] Van Deventer, F.J. Optimisation of supercritical carbon dioxide derived soybean oil. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2009.

[4] Van Greuning, C. sc-CO_2 extracted oil from *Jatropha curcas* - directive for the biodiesel industry? M.Sc. Dissertation, Faculty of Natural Sciences, North-West University, Potchefstroom, 2009.

[5] Siskin, M., Katritzky, A.R., A review of the reactivity of organic compounds with oxygen-containing functionality in superheated water. *J. Anal. Appl. Pyrolysis* 2000, *54*, 193.

[6] Elliott, D.C., Beckman, D., Bridgwater, A.V., Diebold, J.P., Gevert, S.B., Solantausta, Y., Developments in direct thermochemical liquefaction of biomass: 1983-1990. *Energy & Fuels* 1991, *5*, 399.

[7] Siskin, M., Katritzky, A.R., Reactivity of organic compounds in superheated water: General background. *Chem. Rev*. 2001, *101*, 825.

[8] Dinjus, E. & Kruse, A. Applications of Supercritical Water. (Van Eldik, R. & Klarner, F-G., High Pressure Chemistry: Synthetic, Mechanistic and Supercritical Applications). London: Wiley, 2002, 422-442.

[9] http://www.jatrophacurcasplantations.com (accessed 13/05/2009)

[10] Achten W.M.J., Verchot L., Franken Y.J., Mathijs E., Singh V.P., Aerts R. & Muys B. *Jatropha* biodiesel production and use. *Biomass and Bioenergy*, 32 (2008): 1063-1084.

[11] Heller, J. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilised and neglected crops. Ph.D. Dissertation, Institute of Plant Genetic and Crop Plant Research, Gatersleben, Germany & International Plant Genetic Resource Institute, Rome, Italy, 1996. (http://www.ipgri.cgiar.org/Publications/pdf/161.pdf)

[12] Azam, M.M., Waris, A., Nahar, N.M. Prospect and potential of fatty acid, ethyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass and Bioenergy*, 29 (2005) : 293-302.

[13 Van Eldik, R., Hubbard, C.D. Chemistry Under Extreme or Non-Classical Conditions. John Wiley & Sons. 1997.

[14] Knothe, G., Krahl, J., Van Gerpen, J. The Biodiesel Handbook, AOCS Press, 2004.

[15] Foidl, N., Foidl, G., Sanchez, M., Mittelbach, M., Hackel, S. *Jatropha curcas* L. as a source for the production of biofuel in Nicaragua. *Bioresource Technology*, 58 (1996) : 77-82.

[16] Pramanik, K. Properties and use of *Jatropha curcas* oil and diesel fuel blends in compression ignition engine. *Renewable Energy*, 28 (2003) : 239-248.

[17] Van Gerpen, J., Shanks, B. & Pruszko, R. (Iowa State University), Clements, D. (Renewable Products Development Laboratory), Knothe, G. (USDA/NCAUR), *Biodiesel Analytical Methods August 2002 - January 2004*, (2004) : NREL/SR-510-36240.

[18] SANS1935:2004. South African National Standard for Automotive Biodiesel Fuel, Standards South Africa (A Division of SABS), 2004.

[19] IEA (International Energy Agency) Bioenergy. Sustainable production of woody biomass for energy, *A Position Paper Prepared by IEA Bioenergy*, 2002:03, also available on http://www.ieabioenergy.com [accessed June 2010]

[20] Sjostrom, E. Wood Chemistry. Fundamentals and Applications, 2nd Edition. Academic Press: San Diego, 1993, 292.

[21] Wegner, T., Skog, K.E., Ince, P.J. & Michler, C.J. Uses and desirable properties of wood in the 21st century. *Journal of Forestry*, June 2010: 165-173.

[22] Reach for Unbleached Foundation, *components of wood,* http://www.ruf.com [accessed June 2010]

Chapter 3: Non-Classical Extraction Methods

During the past few decades new, innovative chemical methods and processes have been introduced in view of the current global energy crisis. Green chemistry has taken the forefront when new chemical processes are discussed, and research is devoted to replacement of current energy resources and fuel production processes to cleaner and more environmentally friendly systems. In view of this, chemistry under non-classical conditions has become a discipline of great significance. The term “non-classical” refers to extension of ambient conditions to more extreme conditions where novel chemistry not accessible under classical conditions becomes viable [1].

Chemistry under extreme conditions includes, among others, high-temperature chemistry, high-pressure chemistry, supercritical fluid technology, ultrasound chemistry, plasma chemistry and microwave chemistry. A short outline of specifically supercritical fluid technology, microwave superheating and ultrasound-supported chemistry is given in the paragraphs below since these have been adopted as principal methods in this study to extract crude material from biomass sources for eventual conversion to biofuels.

Traditionally, two types of extraction are used to obtain oil from biomatrices, viz. cold press and solvent extraction, as shown in **Figure 3.1**.

Figure 3.1: Cold press (left) and solvent extraction (right)

Soxhlet extraction with n-hexane as solvent, for instance, is hazardous due to waste water generation, volatile organic compound emission and carcinogenic solvent residues. It is also time-consuming and thus energy demanding. Mechanical extractions are labour-intensive, time-consuming and energy-demanding. Various purification and refinement steps are necessary, which in turn increase labour costs and environmental impacts due to organic solvents and waste. The development of the non-classical extraction methods pursued in this study has been a logical consequence of these considerations.

3.1 Supercritical fluid technology

There has been considerable development over the past 25 years in the use of supercritical fluids (SCF) as non-classical solvents and reaction media in clean and environmentally friendly processes, and for novel methods of extraction, conversion and renewal of energy resources. Supercritical fluids can be applied in various ways, and the role played by these fluids determines the reaction outcome and products obtained. In the section below, a few typical applications of supercritical fluids are briefly summarised [2]:

- Supercritical fluid as a solvent: extraction of compounds from different matrices; particle formation by rapid depressurisation of a supercritical solution through a nozzle (RESS: Rapid Expansion of Supercritical Solution); reaction medium, such as for transesterification of plant oil in supercritical methanol;
- Supercritical fluid as an anti-solvent: a solute dissolved in a solvent precipitates on mixing with a supercritical fluid in which it is immiscible (SAS: Supercritical Anti-Solvent);
- Supercritical fluid or pressurised gas as a solute (dispersing agent): depressurisation of a highly saturated solution of a molten solute in a pressurised gas/supercritical fluid to form solute particles (PGSS: Particles from Gas Saturated Solution);
- Supercritical fluid as extractant/impregnant: newly developed processes include Supercritical Fluid Extraction from Emulsions (SFEE), Supercritical Assisted Atomisation (SAA), Supercritical Fluid Chemical Deposition (SFCD), SCF impregnation and SCF infusion.

In this study supercritical carbon dioxide (sc-CO_2) was used as a solvent for the extraction of components from the biomass matrices mentioned in **Chapter 2**. In a few experiments

superheated water was used to extract substances from wood at elevated temperatures approaching the subcritical state of water.

3.1.1 Supercritical carbon dioxide

Every substance has a critical point (critical temperature T_c and critical pressure p_c), as shown by the phase diagram of CO_2 in **Figure 3.2**, above which the substance exists as a supercritical fluid. Such fluid exhibits both gas-like and liquid-like properties and can be described as either a highly condensed gas with excellent transport capability or a highly mobile liquid with excellent solvent strength characteristic.

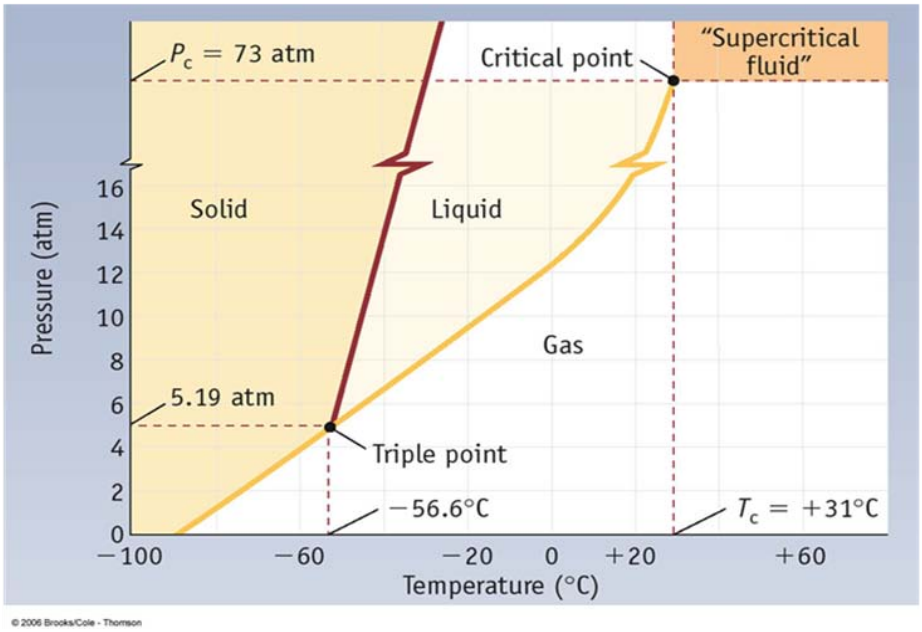

Figure 3.2: Phase diagram of carbon dioxide [3]

sc-CO_2 is obtained by increasing the temperature and the pressure of ambient CO_2 gas to the critical point (31°C, 73 atm), above which the density of the resulting fluid can be varied between 0 and 1 kg/m^3 simply by varying the temperature and/or the pressure. An increase in density causes liquid-like behaviour in combination with gas-like viscosities, whereas a decrease in density causes an increase in diffusivity by orders of magnitude as viscosity further decreases and surface tension becomes absent. The gaseous nature of a supercritical fluid allows it to penetrate microporous materials at a higher rate than a liquid and to exhibit exceptional transport properties within such materials to carry in impregnating agents, for instance, while its liquid nature implies high solvent strength enabling it to dissolve non-volatile, stable compounds from different kinds of matrices, as in this investigation.

The extraction of essential oils, colourants and fragrances by sc-CO_2 can be explained in terms of either desorption or chemical dissolution of these substances from the plant vacuoles [4], depending on their intimate chemical nature. The non-polar character of sc-CO_2 causes polar substances to be less soluble in the fluid according to the general principle of like dissolves like, but its polarity can be adjusted by adding small amounts of a modifier or cosolvent, such as methanol, to the fluid. The extracted material is collected once ambient conditions are restored by depressurisation, and the resulting gas is released to the surroundings. The extract is left behind as a pure, dry product with no further refinement or separation required.

CO_2 is a by-product of fermentation, combustion and other chemical processes and can therefore be obtained easily and affordably. It is a green solvent and a very competitive alternative to harmful organic solvents used in classical extraction.

3.1.2 Supercritical water

Another widely used supercritical fluid is supercritical water (sc-H_2O) with critical parameters T_c = 374 ^{0}C and p_c = 22.1 MPa [5, 6]. The physical properties of water change significantly at such extreme conditions. The density, dielectric constant and ionic product at 24 MPa vary with temperature as shown in **Figure 3.3** [6]. The decrease in dielectric constant causes non-polar compounds to become soluble in sc-H_2O, and especially oxygen dissolves to such an extent that sc-H_2O becomes highly oxidative.

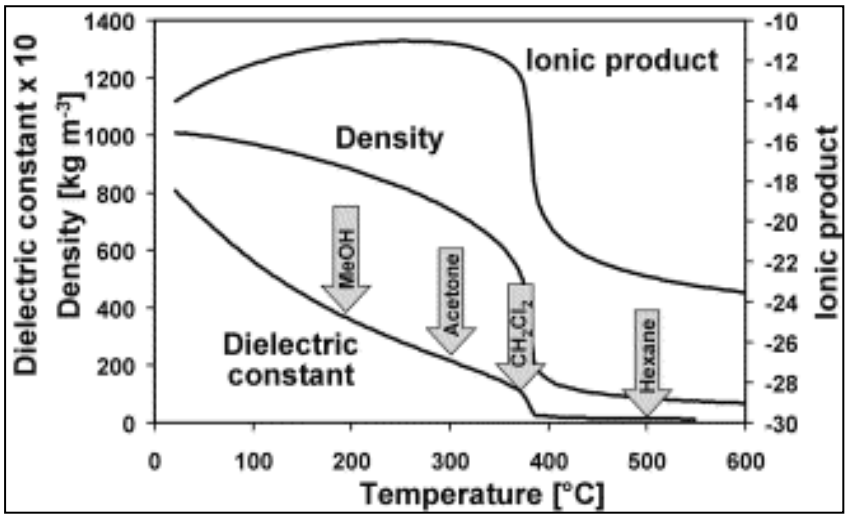

Figure 3.3: Physical properties of water at a pressure of 24 MPa versus temperature. Dielectric constants of some organic solvents at room temperature are indicated [6]

During supercritical water oxidation (SCWO) organic matter is rapidly oxidised to CO_2 and H_2O, with H_2 and CO also present. The hetero-atoms in the organic matter are transformed into mineral acids such as HCl and H_2SO_4. The reaction conditions for SCWO are typically 500-700 ^{0}C and 24-50 MPa. Although these conditions require a large energy input, reaction times are in the order of a few minutes.

Another application of the vast decrease in dielectric constant (i.e. polar nature) of H_2O while approaching its critical point is that highly soluble salts become insoluble and lead to salt formation. This was shown to be advantageous to recover valuable metal salts from waste water of a petroleum industry and to simultaneously purify the effluent for potential re-use [7]. SCWO, although an efficient waste treatment method, suffers disadvantages such as salt deposition, corrosion of vessels/reactors and high energy input while approaching the supercritical state [8].

3.2 Microwave-enhanced chemistry

Domestic microwaves produce wavelengths of 12.4 cm and industrial microwave systems utilise microwave wavelengths of 33.3 cm. These long-wavelength, low-energy waves provide an indirect mechanism for heating of substances by one of two pathways, viz. dipole rotation and ionic conductance [9].

Polar molecules rotate rapidly during microwave radiation in an attempt to align its poles with the alternating maxima of the electromagnetic field. The rotation/oscillation causes the molecules to collide and to generate friction which results in heating of the solution. Ions in solution move towards or away from the maxima/minima of the amplitudes of the electromagnetic waves depending on their charges. This movement results in a conducting current and heating of the solution.

Oscillation of species in solution causes higher temperatures than those obtained by conventional heating. Temperatures well above the normal boiling points of solvents are reached, and these elevated temperatures favour processes such as synthesis and extraction in comparison to ambient/classical conditions. This phenomenon is known as superheating [9, 10]. Water is frequently used as solvent for microwave-assisted reactions, and when the temperature of ambient water is increased under a small applied pressure, superheated water can reach temperatures well above the normal boiling point of 100 ^{0}C.

Superheated water relates to the condensed phase region between 100 ^{0}C and the critical point, often denoted subcritical water rather than superheated water [11].

The permittivity of water becomes comparable to that of common organic solvents when heated. It decreases at a pressure of 129 bar from about 55 at 100 ^{0}C to about 30 at 220 ^{0}C, and close to 0 at 400 ^{0}C, allowing water to dissolve a wide range of compounds with medium or low polarities due to this property [11, 12]. Superheated water has been used as a cleaning agent, to extract PAH's from soil [12, 13] and to extract oils from plant material as was done in this study.

3.3 Ultrasound Chemistry

Ultrasound waves have frequencies between 20 kHz and 2 MHz, and these are applied to enhance chemical reactions by virtue of acoustic cavitation that occurs throughout a solution [1]. Dissolved air in the solution are compressed by the continuing sound waves to form air bubbles. **Figure 3.4** illustrates bubble formation in a solution as a result of emitted ultrasound waves [14].

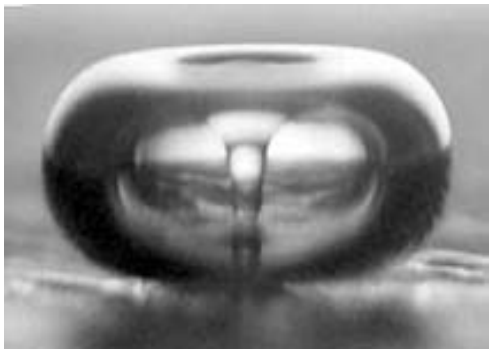

Figure 3.4: Cavitation in a solution due to ultrasound radiation

Bubble formation is initially stable, but bubbles start to oscillate in diameter as a result of pressure from the sound waves on the outside and pressure by the air on the inside. A bubble eventually collapses by either exploding or imploding, and large shear stresses spread throughout the solution. These forces cause breakage of bonds and tearing of long polymeric chains, resulting in the formation of free radicals and activated molecules. Extreme temperatures and pressures (typically 3 000 K and 10 000 bar) are reached during bubble collapse, and localised water molecules become supercritical and extremely oxidising. The inside of the bubble turns into a complex microplasma which is released into

solution on bubble collapse. The nett effect is a highly active molecular environment in which any existing chemical process is extremely enhanced and accelerated.

Continuous radiation of ultrasound waves is needed for activation/enhancement of chemical reactions or extractions, whereas pulsed radiation is required for relaxation of chemical processes [14, 15]. These two mechanistic routes are applied according to the desired outcome of a chemical process. A few applications include production of metal and metal oxide nanopowders [16], synthesis of polymeric and organometallic complexes and synthesis of *in situ* catalysts [17] and rate and yield improvements [18]. During this study ultrasound waves were generated by a soundwave emitter in an ultrasonic bath to assist in the extraction of *Jatropha* oil using water as solvent.

References

[1] Van Eldik, R. and Hubbard, C. D. Chemistry Under Extreme or Non-Classical Conditions. John Wiley & Sons. Inc. 1997.

[2] Badens, E. Dug processing using supercritical fluids. *Proceedings of the 9th Conference on Supercritical Fluids and Their Applications, Sorrento, Italy, 05-08 September 2010.*

[3] Kotz J.C., Treichel, P.M. and Townsend, J.R. Chemistry and Chemical Reactivity, 7th Edition. Thompson Brooks/Cole, 2009, 609.

[4] Van Wyk M. Supercritical fluid extraction - alternative for classical extraction of volatile oils and fragrances. M.Sc. Thesis, Potchefstroom University for Christian Higher Education, Potchefstroom, 2000, 83 pages.

[5] Dongsheng, W., Jiang, H. and Zhang, K. Supercritical fluids for clean biofuel production. *D. Wen et al. / Progress in Natural Science*, 19 (2009): 273-284.

[6] Kritzer, P. and Eckhard D. An assessment of supercritical water oxidation (SCWO). Existing problems, possible solutions and new reactor concepts. *Chemical Engineering Journal*, 83 (2001): 207-214.

[7] Oosthuizen, L. New methods for recovery of inorganic salts from waste water in the petroleum industry. M.Sc. Thesis, North-West University, Potchefstroom, 2005, 84 pages.

[8] Ekin Kıpçak, Onur Ömer Söğüt and Mesut Akgün. Hydrothermal gasification of biomass in supercritical water. *Proceedings of the 9th Conference on Supercritical Fluids and Their Applications, Sorrento, Italy, 05-08 September 2010.*

[9] Kingston, H.M. and Haswell, S.J. Microwave-Enhanced Chemistry. Fundamentals, Sample Preparation and Applications. American Chemical Society: Washington, 1997, 572.

[10] Smith, R.M. Extractions with superheated water. *Journal of Chromatography A*, 975 (2002): 31-46.

[11] Brunner, G. Near and supercritical water. Part II: Oxidative processes. *The Journal of Supercritical Fluids*, 47 (2009): 382-390.

[12] King, J.W. and Srinivas, K. Multiple unit processing using sub- and supercritical fluids. *The Journal of Supercritical Fluids*, 47 (2009): 598-610.

[13] Onuska, F.I. and Terry, K.A. Extractions of pesticides from sediments ising a microwave technique. *Chromatographia*, 36 (1993): 191-194.

[14] Rold´an-Guti´errez, Jannat M., Ruiz-Jim´enez, J. and Luque de Castro, M.D. Ultrasound-assisted dynamic extraction of valuable compounds from aromatic plants and flowers compared with steam distillation and superheated liquid extraction. *Talanta*, 75 (2008): 1369-1375.

[15] Londoño-Londoño, J., Rodrigues de Lima, V., Lara, O., Gil, A., Crecsynski Pasa, T.B., Arango, G.J. and Ramirez Pineda, J.R. Clean recovery of antioxidant flavonoids from citrus peel: Optimising an aqueous ultrasound-assisted extraction method. *Food Chemistry*, 119 (2010): 81-87.

[16] Doh, S.G., Kim, E.B., Lee, B.H. and Oh, J.H. Characteristics and synthesis of Cu-Ni ferrite nanopowders by coprecipitation method with ultrasound irradiation. *Journal of Magnetism and Magnetic Materials*, 272-276 (2004): 2238-2240.

[17] Pirola, C., Bianchi, C.L., Did Michele, A., Diodat, D. and Ragaini, V. Ultrasound and microwave-assisted synthesis of high loading Fe-supported Fischer-Tropsch catalysts. *Ultrasonics Sonochemistry*, 17 (2010): 610-616.

[18] Jime´nez, A., Beltra´n, G. and Uceda, M. High-power ultrasound in olive paste pre-treatment. Effect on process yield and virgin olive oil characteristics. *Ultrasonics Sonochemistry*, 14 (2007): 725-731.

Chapter 4: The Toolbox

This chapter deals with the technical aspects of this study and gives an overview of the chemical, physical/mechanical, procedural and analytical tools employed to achieve the predetermined goals of the investigation. The information is presented in the chronological order in which the study was executed.

Jatropha curcas L. seed and *Pinus taeda* L. sawdust were selected as sources of biomass from which crude material for subsequent conversion to a biofuel type product was extracted by different non-classical methods. These methods included supercritical fluid extraction, microwave-assisted superheating and ultrasound-supported extraction, and instrumentation and procedures associated with these methods are described in this chapter.

Jatropha extracts were analysed by high-temperature GC-MS (gas chromatography with mass spectrometric detection), and pine extracts, as well as biodiesel samples (both prepared and commercial), were analysed by advanced GCxGC-TOFMS (two-dimensional gas chromatography with time-of-flight mass spectrometric detection). The tools for these analyses are covered in this chapter.

4.1 Chemical tools

Jatropha curcas seed (1 to 2 years old) was obtained from Dr. John Morris of the Syringa Institute in Centurion. Several factors influence the oil content of *Jatropha* seed, such as area and climate of cultivation, soil quality and time of harvesting [1]. According to the Syringa Institute, long-term storage of harvested seed does not affect the oil content, but exposure to moisture and heat may damage the seed.

Pine sawdust was obtained from a local wood supplier. The primary logs originated from Mozambique. The material was selected as a source of biomass for non-classical extraction in view of its general availability and its known composition [2, 3].

Technical grade (99 %) liquid CO_2 from a siphon-tube cylinder supplied by Afrox was used for sc-CO_2 extractions. The bench-top supercritical extractor features a pneumatically operated system and compressed dry air (moisture < 5 v/m) also purchased from Afrox was used to drive it.

Deionised water (conductivity < 2 x 10^{-6} S cm^{-1}) produced in our laboratory (Millipore MilliQ+ Ultrapure System) was used as solvent for microwave-assisted extraction. Organic solvents, including n-hexane (for *Jatropha* oil), and dichloromethane and acetone (for pine extracts), were supplied by Merck Chemicals and Labchem, respectively, and were of analytical grade. Pyridine was used for dilution of glyceride standards (mono-olein, di-olein, tri-olein) supplied by Sigma-Aldrich. Methanol, supplied by Labchem, was used for methylation of *Jatropha* oil, and the resulting fatty acid methyl esters (FAMEs) were dissolved in dichloromethane. Sodium hydroxide pellets used to catalyse the transesterification reaction were supplied by Merck Chemicals. N-methyl-N-trimethyl-silyltrifuoracetamide (MSTFA), supplied by Restek, was used as silyating agent.

High-purity helium was used as a carrier gas for all gas chromatographic analyses, and liquid nitrogen and compressed air were utilised for two-dimensional gas chromatographic work. These gases were supplied by Afrox. A fused silica Rtx Biodiesel TG column was installed for high-temperature one-dimensional analysis, a Stabilwax primary column coupled to an Rxi-5Sil MS secondary column and an Rxi-5Sil MS primary column coupled to an Rxi-17Sil MS secondary column was used for two-dimensional analysis. The three columns were supplied by Restek.

4.2 Physical, procedural and instrumental tools

4.2.1 Sample preparation

- ***Extractions***

- *Jatropha* fruit were de-hulled by using a nutcracker to allow manual removal of the black shell, and de-hulled seed were crushed with a commercial coffee grinder to ensure larger surface area exposure for more efficient extraction, as shown in **Figure 4.1.** The particle size of the milled seed was determined as 300-600 µm by using laboratory sieves.

Figure 4.1: *Jatropha curcas* fruit (left), seed with shells (centre) and crushed seed (right)

- The particle size of the pine sawdust obtained from a local supplier was sufficient (1-5 mm length with sub-rectangular shape) to provide a large surface area for efficient extraction. The sawdust was stored between layers of towel paper to prevent contamination from the plastic material of the container. Extracts were stored in acetone (AR) for subsequent analysis. **Figure 4.2** illustrates a prepared sample and subsequently acquired extract of pine sawdust.

Figure 4.2: Pine sawdust sample (left) and subsequently acquired extract (right)

➢ ***Crude product analysis***

- Extracted *Jatropha* oil was dissolved in n-hexane for one-dimensional GC-MS analysis, and sample concentrations of about 100 µg/mL were prepared for this purpose. Samples were derivatised by adding to each sample 100 µL MSTFA silyating agent, shaking the sample thoroughly and leaving it at room temperature for 30 min before analysis.
- Five different dilutions (10 µg/mL, 30 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL) of triolein standard (5000 µg/mL dissolved in pyridine) were prepared in an attempt to quantify the triglycerides in the oil samples by constructing a calibration curve with the instrumental software.
- Wood extracts were dissolved in acetone (AR) to final concentrations of 100 µg/mL. Derivatisation was performed similarly to *Jatropha* oil samples.

➢ ***Converted product and commercial biodiesel analysis***

- Converted *Jatropha* oil was qualitatively analysed for FAMEs after appropriate dilution (50x, 100x and 1000x) with n-hexane.
- The commercial biodiesel samples (pure B100 blend) obtained from the Syringa Institute in Centurion were diluted similarly to the botanical samples converted in the laboratory.

4.2.2 Extraction of biomass samples

Extraction was done with a selection of both classical and non-classical methods in order to mutually compare the nature of the products and the efficiency of the different methods, and to broaden the scope of crude material from which a biofuel could eventually be derived.

➢ ***Soxhlet extraction***

A standard soxhlet extraction setup shown in **Figure 4.3** was used to either extract approximately 500 g of crushed *Jatropha* seed with 2.5 L of n-hexane for 5 days at 65 ^{0}C and continuous stirring, or 5 g of pine sawdust with 1 L of acetone for 4 hours at the same

conditions. In both cases the solvent was evaporated and the extracted oil stored in a refrigerator for subsequent analysis.

Figure 4.3: Soxhlet extraction setup for extraction of *Jatropha* seed and pine sawdust

- ***Supercritical carbon dioxide extraction***

The LECO TFE2000^{TM} supercritical extractor in **Figure 4.4** was used to perform bench-top extractions of prepared *Jatropha* seed and pine sawdust samples.

The instrument is designed to extract oil and fats from biomatrices without use of hazardous organic solvents [4]. It is equipped with a single flow line splitting into three separate flow lines, each having an extraction thimble, a heated variable restrictor (HVR), and a collection vial. Three different extractions can thus be done simultaneously or a single extraction can be performed in triplicate. The HVRs revert the fluid to the gaseous phase and release it at a preselected rate to the surroundings at restored ambient conditions. The extract is precipitated and accumulated in collection vials. The restrictors are heated to prevent blockage of the flow lines by viscous and fatty materials extracted, or cooled to prevent loss of volatile components from the extract.

The instrument can be operated in both static and dynamic extraction modes. During a dynamic run a continuous flow of sc-CO_2 through the sample thimble is maintained at a pre-set flow rate of 1-3 L/min. This allows for exhaustive extraction of the sample and is thus used to determine the oil content of a botanical sample.

Figure 4.4: LECO TFE2000^{TM} supercritical extractor.

The extraction time is limited to a maximum of 90 min, though, and therefore several consecutive extractions need to be performed to determine the oil content of the sample. The oil content can also be determined by extrapolating a plot of oil yield versus time to its maximum after performing several dynamic extractions of different duration.

For the static mode, a sample resides in a fixed aliquot of sc-CO_2 in the extraction thimble for a pre-selected time interval to reach equilibrium at the conditions concerned. The material dissolved in the supercritical solvent is then recovered at the HVR and captured in a collection vial. This mode can be utilised for solubility studies by determining the yield of oil extracted during static runs of different duration and plotting these yields as a function of time. The maximum or plateau of such a plot signifies the solubility of the oil in sc-CO_2 at the prevailing conditions as it represents the point where the solvent is saturated with the extracted oil.

The static and dynamic extraction modes may be combined to find the most efficient operational mode for a given application. However, in this investigation these modes were used separately to determine the oil content of the biomatrix and the solubility of the material in sc-CO_2, respectively.

Pre-prepared samples of 1 g of *Jatropa* seed or pine sawdust were loaded into the stainless steel extraction thimbles. The mass of the loaded thimbles as well as that of the collection

vials was recorded prior to and after extraction in order to perform a mass balance by comparing the mass loss during extraction to the mass of extract collected. The yield of oil was determined as a mass percentage of the sample of material by the equation

$$Yield(\%) = \frac{Mass(extract)/g}{Mass(material)/g} \times 100$$

The mass loss of the thimble and its contents after extraction should equal the mass gain of the collection vial (mass of extracted material) to render reliable extraction/solubility data. The extraction conditions for the wood samples (85 ^{0}C, 60.8 MPa, 0.9 g mL^{-1}) were slightly harder than those for the *Jatropha* samples (40 ^{0}C, 35.5 MPa, 0.9 g mL^{-1}) in view of the hemicelluloses and cellulose chains that are bound together by strong lignin linkages.

- ***Superheated Water Extraction***

Water is considered the least harmful solvent to the environment. Even superheated water is considered a green solvent, since it does not destroy any biomass components required for biofuel conversion. *Jatropha* seed and pine sawdust prepared in the same way as before were extracted with superheated water (demineralised) by heating samples in closed vessels for a selected time interval with a MARS 5 industrial microwave system. **Figure 4.5** shows the architecture of the microwave system. It allows ten extractions to be performed simultaneously.

Figure 4.5: MARS 5 microwave system with loaded control vessel (left) and in operation (right)

A control vessel monitors the pressure and temperature by means of a temperature probe inserted into the vessel and a pressure probe connected to a nozzle on the vessel. These

two probes are connected to sensors on the inside of the microwave and communicate the measured temperature and pressure inside the control vessel to nine other vessels accommodated within the instrument. The latest models are equipped with an infrared control mechanism which shifts control among the vessels to maintain constant temperature throughout.

The vapour pressure of water increases with increasing temperature inside the extraction vessels as a result of solvent changing into the vapour phase as predicted by the Clausius-Clapeyron equation [5, 6]

$$P_2 = \frac{P_1}{(\exp(\Delta H_{vap} / R)) * (1/T_2 - 1/T_1)}$$

where T_1 = 373 K, P_1 = 1.013 x 10^5 N m^{-2} and ΔH_{vap} = 40.7 kJ mol^{-1}

A slight pressure increase is necessary for water to become superheated. This pressure increase is generated by heating liquid water in closed vessels, causing the temperature to rise above the normal boiling point of 100 ^{0}C.

Approximately 0.5 g of crushed seed or wood sawdust was weighed off into the extraction vessels (XP1500), and 20 mL demineralised water was added to each. A common heating program was created with the instrumental software, featuring a ramp time of 10 min at 1200 W to a final temperature of 150 ^{0}C. Higher temperatures resulted in vessel failure, although a few runs at 180 ^{0}C and 200 ^{0}C could be achieved and allowed for analysis of additional compounds that might have been extracted at such elevated temperatures.

The extracts, suspended in water, was separated from the solid waste by decanting the solution through a sieve into pre-weighed glass beakers. The liquid product was left in a fumehood overnight to evaporate to dryness, and the dry extract was re-dissolved in either n-hexane or acetone after reweighing the beakers to determine the mass of material extracted. The main focus was to obtain a maximum amount of extract and to analyse the products qualitatively for comparison to those obtained by other extraction methods.

- ***Ultrasound-assisted extraction***

Ultrasound chemistry is applied in various chemical processes including extraction and synthesis. The collapse of bubbles formed during ultrasound excitation generates energy which can be utilised for chemical and mechanical processes. One such process is extraction of oil from *Jatropha* seed. For ultrasound-assisted extraction 0.2 kg *Jatropha* seed and 600 mL n-hexane were mixed and placed in a FINNSONIC ultrasonic bath with soundwave emitter for 60 min.

The slightly heated mixture (40°C for n-hexane and 85^0C for water as solvent) was irradiated at an ultrasonic frequency of 40 kHz and nominal/peak ultrasonic power of 80/160 W as shown in **Figure 4.6.** After completion of the extraction, the solvent was distilled off and the extracted product stored for analysis.

Figure 4.6: Ultrasound-assisted extraction of *Jatropha* oil using water as solvent

4.2.3 Conversion of crude products

sc-CO_2 extracted *Jatropha* oil was converted to FAMEs by base-catalysed transesterification [7] outlined in **Figure 4.7**. Due to the limited capacity of the supercritical extractor, very little oil (approximately 0.3 g) was obtained from a single extraction run, and yields of several identical runs had to be combined to perform a small-scale transesterification by adding to 2 g of oil a mixture of 0.2 g of NaOH and 20-30 mL of methanol.

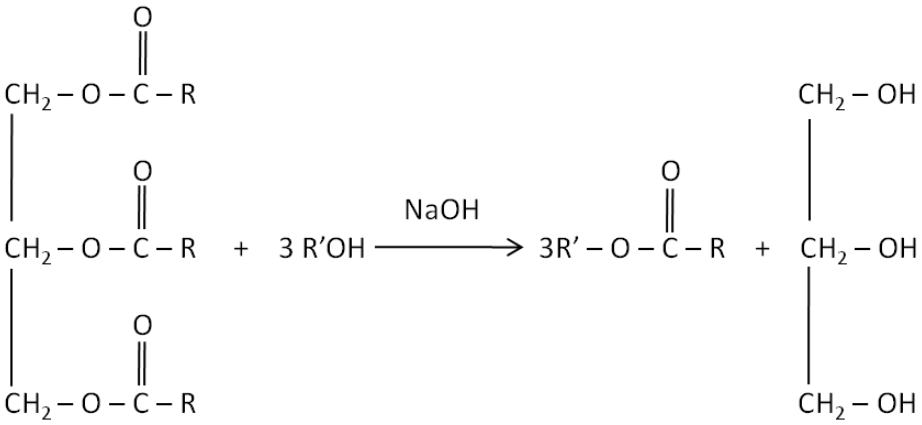

Figure 4.7: Reaction scheme for transesterification of *Jatropha* oil [7]

The mixture was heated and the temperature controlled between 40 and 50 ^{0}C with continuous stirring for 3 hours. The products were left for three days to separate into a biodiesel layer on top and a glycerol layer at the bottom. The two layers were separated by removing the top layer with a Pasteur pipette. The separated layers were stored in a refrigerator for subsequent analysis.

An *in situ* biodiesel conversion was also performed by premixing 0.2 kg seed, 0.4 g NaOH, 10 mL methanol and 550 mL n-hexane. The mixture was irradiated at peak power of 160 W for 2 hours. The mixture was left overnight to separate and the converted oil was dissolved in dichloromethane for analysis.

Figure 4.8: Biodiesel conversion reactor at Syringa Institute, Centurion

A larger scale conversion within an industrial reactor is done at the Syringa Institute at Centurion. **Figure 4.8** shows the pilot plant used to produce a biodiesel currently at test to propel lawnmowers. This type of reactor is equipped with an ultrasonic probe that reduces the amount of glycerol by-product as the glycerol molecules are mostly emulsified with the FAME molecules. As a result, no washing of the biodiesel is required.

4.3 Analytical tools

4.3.1 Instrumentation

All extracts and converted samples were analysed using a LECO Pegasus® 4D GCxGC-TOFMS shown in **Figure 4.9**. This powerful instrument is capable of separating components in two-dimensional gas chromatographic fashion and analysing the time-of-flight mass spectrometric detected components by using an equally powerful on-board database.

Figure 4.9: LECO Pegasus® 4D GCxGC-TOFMS

Gas chromatography is an efficient technique for separating a sample into its basic components as a result of the difference in affinity of the components for the stationary phase through which these are transported by an inert mobile phase immiscible with the stationary phase. The latter is packed either within a column (gas chromatography) or onto a flat surface (liquid/thin layer chromatography). In gas chromatographic applications the detector plays an important role in identifying the compounds separated. For complex samples containing large numbers of compounds that can easily overlap due to small

differences in retention times, a mass spectrometer is considered to be the preferred detector due to fragmentation of molecular compounds into their unique mass constituents. Time-of-flight mass spectrometry is applied for gas chromatographic detection in view of its high-speed fragmentation and sampling capabilities (up to 500 spectra/second) for fast eluting compounds [8].

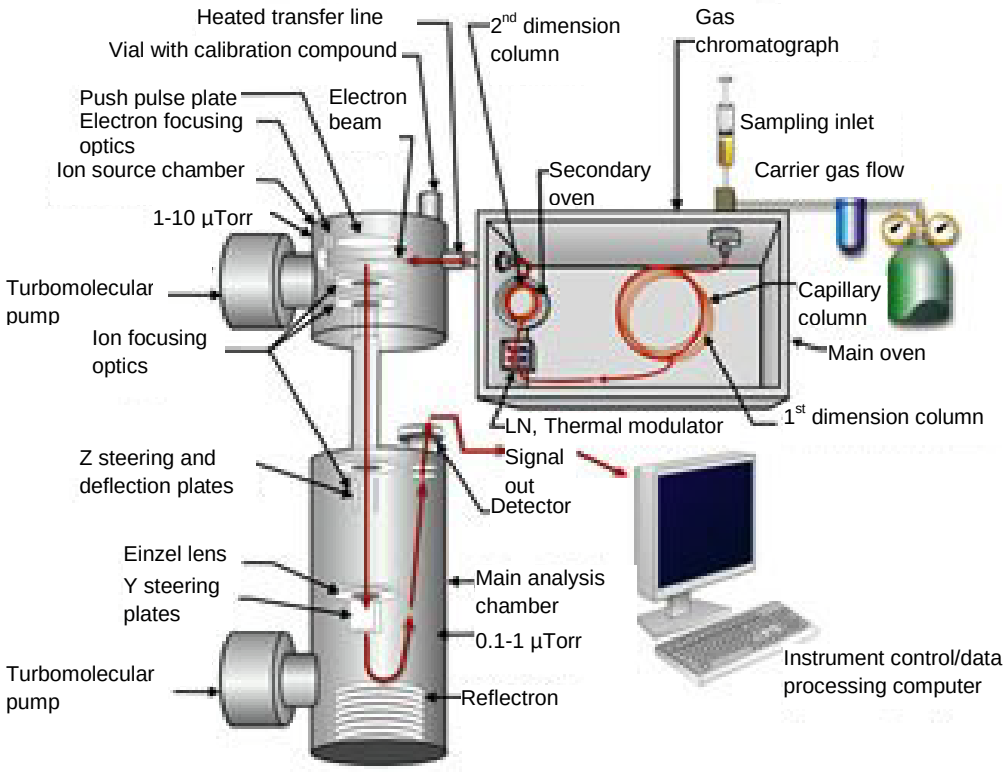

Figure 4.10: Architecture of Pegasus 4D instrument

Figure 4.10 shows the basic architecture of the instrument [8]. A sample is injected into a heated injection port (the Pegasus 4D has two injection ports: front or back inlet) where it is vaporised and carried onto a capillary (primary) column. The analyte interacts with the stationary phase packed on the inside of the column, and this causes retention of the analyte on the specific stationary phase. The time from retention to desorption is influenced by the packing material, type of analyte, type of carrier gas, temperature of the column and flow through the column. Separation of the sample is achieved by multiple retention and desorption phases as the vaporized sample moves through the heated column.

The analytes elute from the column one at a time after separation. When using an ordinary gas chromatographic detector, analytes are identified by their individual retention times. During separation of complex mixtures containing more than 150 components, co-elution of some of the analytes generally occurs. This decreases the resolution and efficiency of

separation, and valuable components are often not detected due to overlap of neighbouring peaks. In order to overcome this problem, a second column is introduced in a two-dimensional configuration. The primary column typically contains a non-polar stationary phase, has a length of about 30 m and internal diameter of 0.25-0.32 µm, and separates components on the basis of a difference in boiling point. The secondary column is much shorter (1-2 m) and narrower (0.18 µm), and generally has a polar stationary phase to provide fast separation and elution based on a difference in polarity [8, 9].

A thermal or flow modulator is positioned between the two columns to capture the large number of components separated within the primary column and to properly focus each of these components onto the secondary column. The latter column is accommodated in a secondary oven situated inside the primary oven. The temperature programming of the two columns is done individually, and separations from each of them occur independently. Two chromatograms are obtained and plotted perpendicular to each other with advanced built-in software. All compounds can be identified in this way since analytes co-eluting from one column behave differently on the second column and peak overlap is eliminated. The secondary column can also be bypassed to analyse in normal one-dimensional fashion by feeding the primary column directly to the detector.

Advanced detection, such as TOFMS, is used to assist in identification of multiple analytes eluting from the columns. The outlet of the secondary column connects to the electron impact ionisation source of the mass spectrometer via an independently heated transfer line. The vacuum system of the mass spectrometer allows for high flow rates of carrier gas (up to 10 mL/min). The molecules entering the source are bombarded with a beam of electrons of approximately 70 eV. The electron beam disperses the molecules into molecular ions, which may break into lower mass fragment ions if sufficient energy is acquired during ionisation. Fragmentation is unique to each individual molecule and is represented in the form of a mass spectrum.

The mass spectrum can be compared to those in a spectral library to identify each component accurately. The travel time of the ions from the ion source to the detector is measured. A data processing method embedded in the instrumental software processes the signals from the detector and identifies the compounds by comparison to an on-board database.

4.3.2 Analysis of crude and converted products

- ***Jatropha* oil**

Samples were analysed using high-temperature gas chromatography with programming and conditions summarised in **Table 4.1**. Uni-liners were used in the inlet to create a pseudo on-column injection [10] where the sample flows directly from the injector needle through the liner onto the column. These injections are generally done splitless, but can also be done at a given split ratio if necessary. An Agilent auto sampler with a 1-10 µL syringe was used for injections.

Table 4.1: Conditions for high-temperature 1-D analysis

GC	Modified Hewlett Packard 6890N
Carrier gas	Helium
Inlet type	Front inlet split / splitless
Sample size injected (µL)	1
Constant pressure / flow	Constant flow
Flow rate (mL/min)	5
Inlet temperature (°C)	280
Column 1	Rtx Biodiesel, 10 m x 0.32 mm ID, 0.18 µm film
Column 1 oven temperature	80 °C for 1 min, to 350 °C at 20 °C/min, hold for 8 min.
Transfer line temperature (°C)	350
Detector	LECO Pegasus 4D time-of-flight mass spectrometer
Acquisition delay (seconds)	180
Mass range (u)	50-1000
Acquisition rate (spectra/second)	100
Detector voltage (volts)	-1700
Electron energy (volts)	70
Ion source temperature (°C)	240

- ***Pine* extract**

Initially wood extracts were analysed chromatographically using exactly the same high-temperature column at the same conditions as for *Jatropha* oil samples, except that acetone was used as solvent, but subsequently samples were also analysed in two-dimensional mode in an attempt to improve separation of the complex mixtures. This was made possible by implementing special high-temperature secondary columns supplied by Restek. The two-dimensional setup for the analysis of pine extracts with these columns is listed in **Table 4.2.**

Table 4.2: Conditions for 2-D wood analysis

GC	Modified Hewlett Packard 6890N
Carrier gas	Helium
Inlet type	Front inlet split / splitless
Sample size injected (µL)	1
Constant pressure / flow	Constant flow
Flow rate (mL/min)	1.20
Inlet temperature (°C)	250
Column 1	Rxi-5Sil MS, 30 m x 0.25 mm ID, 0.25 µm film thickness.
Column 1 oven temperature	50 °C for 1 min, to 300 °C at 20 °C/min, hold for 8 min.
Column 2	Rxi-17Sil MS, 1.5 m x 0.1 mm ID, 0.10 µm film thickness
Column 2 oven temperature	60 °C for 1 min, to 310 °C at 20 °C/min, hold for 8 min.
Second dimension separation time (seconds)	5
Transfer line temperature (°C)	240
Detector	LECO Pegasus 4D time-of-flight mass spectrometer
Acquisition delay (seconds)	180
Mass range (u)	50-800
Acquisition rate (spectra/second)	100
Detector voltage (volts)	-1700
Electron energy (volts)	70
Ion source temperature (°C)	240

- **Biodiesel samples (converted and commercial)**

Analysis of biodiesel samples (converted *Jatropha* extracts as well as commercial biodiesel standard) was done according to the program summarised in **Table 4.3**. Two-dimensional chromatography could be used since the temperature required for FAME analysis is much lower than that for triglycerides present in the crude extracts [10, 11]. To determine the total glycerine present in the samples, however, one-dimensional high-temperature gas chromatography was required.

Table 4.3: Conditions for 2-D biodiesel analysis

GC	Modified Hewlett Packard 6890N
Carrier gas	Helium
Inlet type	Front inlet split / splitless
Sample size injected (µL)	1
Constant pressure / flow	Constant flow
Flow rate (mL/min)	1.20
Inlet temperature (°C)	250
Column 1	Stabilwax, 30 m x 0.25 mm ID, 0.25 µm film thickness.
Column 1 oven temperature	50 °C for 1 min, to 250 °C at 5 °C/min, hold for 10 min.
Column 2	Rxi-5Sil MS, 1. m x 0.1 mm ID, 0.10 µm film thickness
Column 2 oven temperature	60 °C for 1 min, to 260 °C at 5 °C/min, hold for 10 min.
Second dimension separation time (seconds)	5
Transfer line temperature (°C)	240
Detector	LECO Pegasus 4D time-of-flight mass spectrometer
Acquisition delay (seconds)	180
Mass range (u)	45-500
Acquisition rate (spectra/second)	100
Detector voltage (volts)	-1700
Electron energy (volts)	70
Ion source temperature (°C)	240

References

[1] Van Greuning, C. sc-CO_2 extracted oil from *Jatropha curcas* - directive for the biodiesel industry? M.Sc. Dissertation, Faculty of Natural Sciences, North-West University, Potchefstroom, 2009.

[2] Sjostrom, E. Wood Chemistry. Fundamentals and Applications, 2nd Edition. Academic Press: San Diego, (1993): 292.

[3] Reach for Unbleached Foundation, *components of wood,* http://www.ruf.com [accessed June 2010]

[4] LECO TFE2000™ Instruction Manual, Copyright LECO® Corporation, 2003.

[5] Atkins, P. and de Paula, J. Atkins' Physical Chemistry, 9th edition. Oxford University Press, (2010) : 149.

[6] Smith, J.M., Van Ness, H.C. and Abott, M.M. Introduction to Chemical Engineering Thermodynamics, 7th edition. McGraw-Hill International Edition, (2005): 22.

[7] Knothe, G., Krahl, J. and Van Gerpen, J. The Biodiesel Handbook, AOCS Press, 2004.

[8] LECO Pegasus® 4D Instruction Manual, Copyright LECO® Corporation, 2003.

[9] Schomburg, G. Two-dimensional gas chromatography: principles, instrumentation, methods. *Journal of Chromatography A*, 703 (1995): 309-325.

[10] Buchgraber, M., Ulberth, F. and Anklam, E. Interlaboratory evaluation of injection techniques for triglyceride analysis of cocoa butter capillary gas chromatography. *Journal of Chromatography A*, 1036 (2004): 197-203.

[11] ASTM Standards D6584, Test Method for the Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography.

Chapter 5: Results and Discussion

In Chapter 5 experimental results obtained in this investigation are presented and discussed. A brief outline of the investigation is given in the next few paragraphs to provide the reader with a bird's eye view before the results are discussed in detail.

Jatropha curcas oil was extracted from seed by three different non-classical methods (supercritical carbon dioxide extraction, microwave-assisted superheating, and ultrasound-supported extraction), and the extracts were compared to that obtained by classical soxhlet extraction to identify the most suitable method of deriving oil for conversion to biodiesel.

Analysis by one-dimensional and two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOFMS) of the extracted oil before and after trans-esterification to fatty acid methyl esters (FAMEs) proved that the composition of the final converted product correlated closely with that of an SABS approved biodiesel standard. In addition, the oil content of the seed and the solubility of the oil in sc-CO_2, in comparison to that of other botanical oils, were studied to further support the viability of *Jatropha* oil as a source for conversion to a strategically important biofuel.

Extractions were extended to wood as another biomass source for biofuel production, and the same non-classical methods and advanced analytical techniques were utilised to assess the capability of pine sawdust as a potential source for biofuels.

5.1 Extraction of *Jatropha* oil

Figures 5.1-5.4 show the one-dimensional chromatograms of a sample of *Jatropha* oil extracted from seed by soxhlet extraction with n-hexane, by sc-CO_2, by superheated water (microwave-assisted extraction) and by ultrasound-supported extraction in water, respectively. The sample preparation and extraction procedures are described in **Chapter 4** and are not repeated here, but the extraction conditions and the parameters used for recording the chromatograms are included in the figure captions to enable direct comparison of the results obtained by the four different methods of extraction.

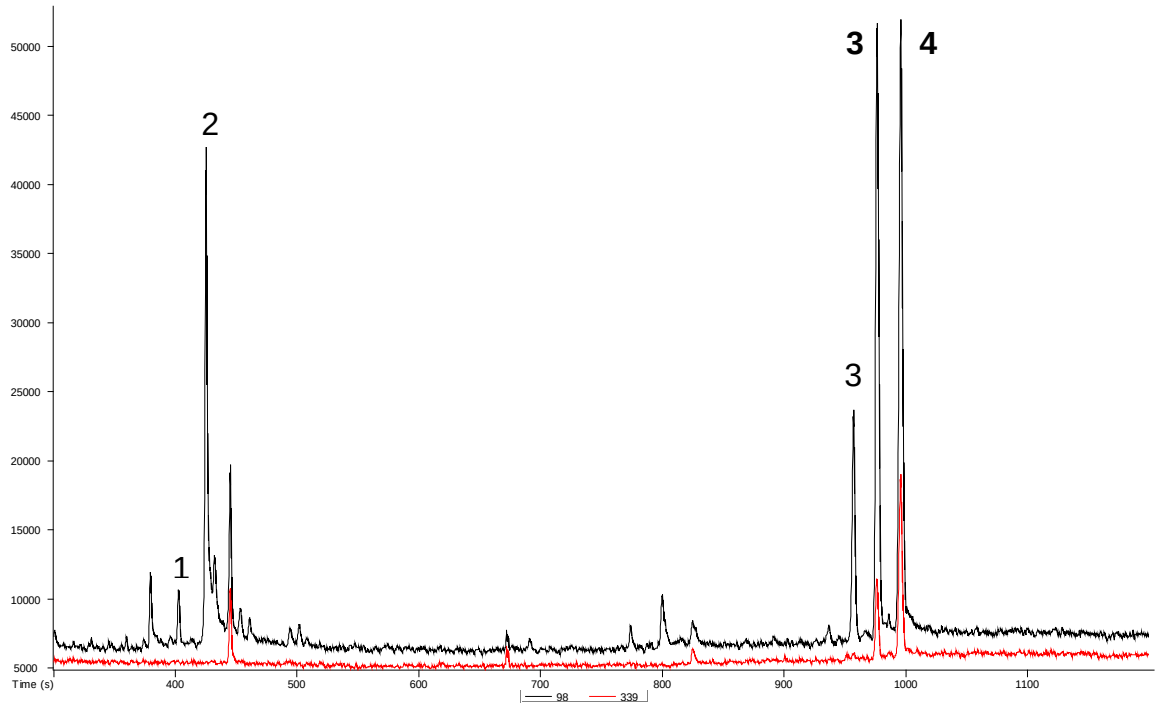

Figure 5.1: High-temperature 1-dimensional gas chromatogram of *Jatropha* oil extracted from seed by n-hexane (soxhlet extraction)

Extraction conditions: t = 5 days, T = 65 ^{0}C, p = 101.3 kPa (1 atm)

Chromatography conditions: Rtx Biodiesel TG column at 20^{0}C/min to 360^{0}C, hold for 5 min

1 = C18 fatty acid methyl ester; 2 = C16-C18 free fatty acids; 3 = C16-C18 triglycerides; 4 = triolein (C18:1)

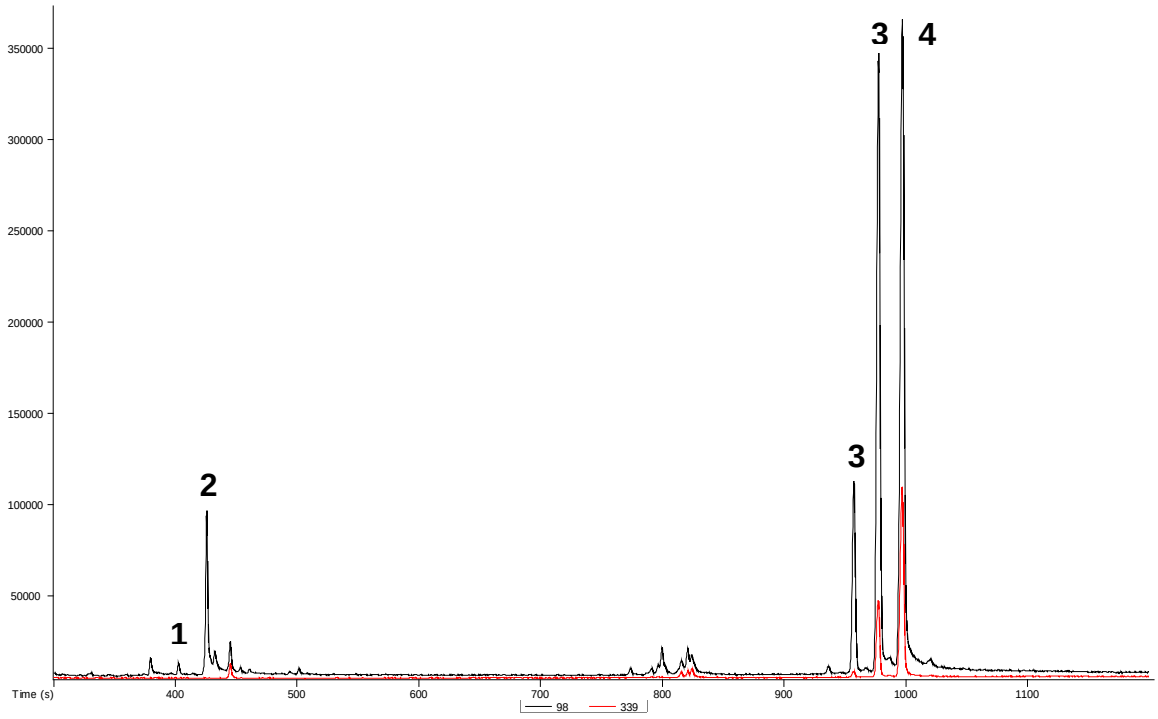

Figure 5.2: High-temperature 1-dimensional gas chromatogram of *Jatropha* oil extracted from seed by sc-CO_2 (bench-top extractor)

Extraction conditions: t = 60 min, T = 40 ^{0}C, p = 35.5 MPa (350 atm), ρ = 0.9 g mL^{-1})

Chromatography conditions: Rtx Biodiesel TG column at 20^{0}C/min to 360^{0}C, hold for 5 min

1 = C18 fatty acid methyl ester; 2 = C16-C18 free fatty acids; 3 = C16-C18 triglycerides; 4 = triolein (C18:1)

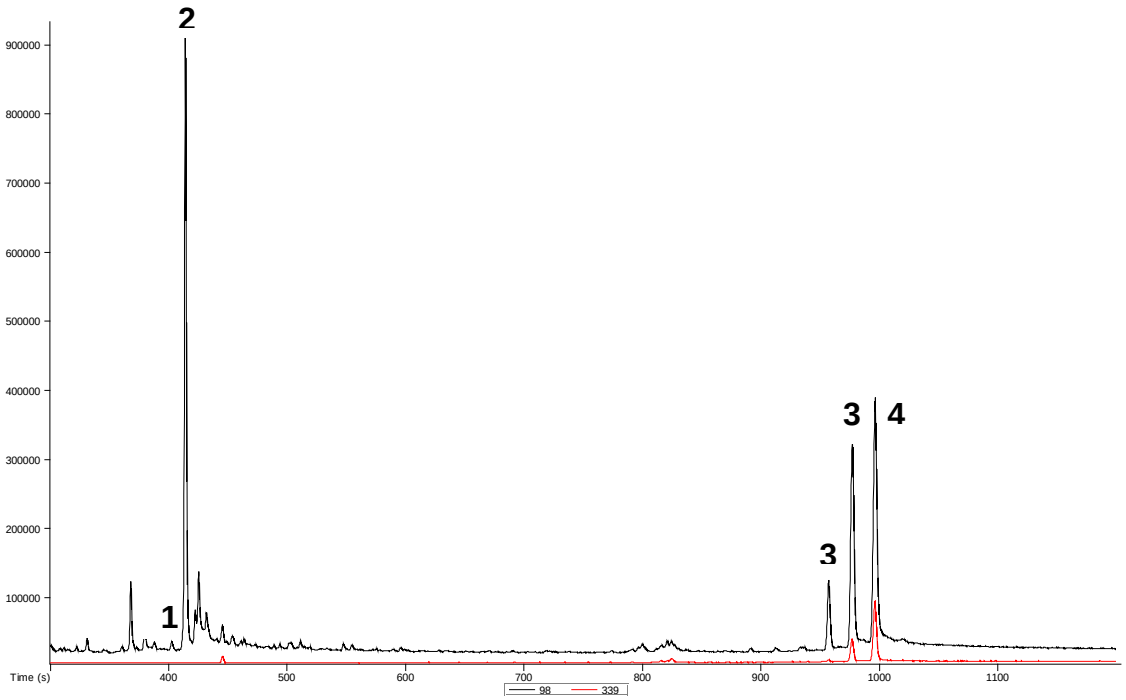

Figure 5.3: High-temperature 1-dimensional gas chromatogram of *Jatropha* oil extracted from seed by superheated H_2O (microwave-assisted extraction)

Extraction conditions: t = 10 min, T = 150 ^{0}C, p = 101.3 kPa (1 atm), power = 1200 W

Chromatography conditions: Rtx Biodiesel TG column at 20^{0}C/min to 360 ^{0}C, hold for 5 min

1 = C18 fatty acid methyl ester; 2 = C16-C18 free fatty acids; 3 = C16-C18 triglycerides; 4 = triolein (C18:1)

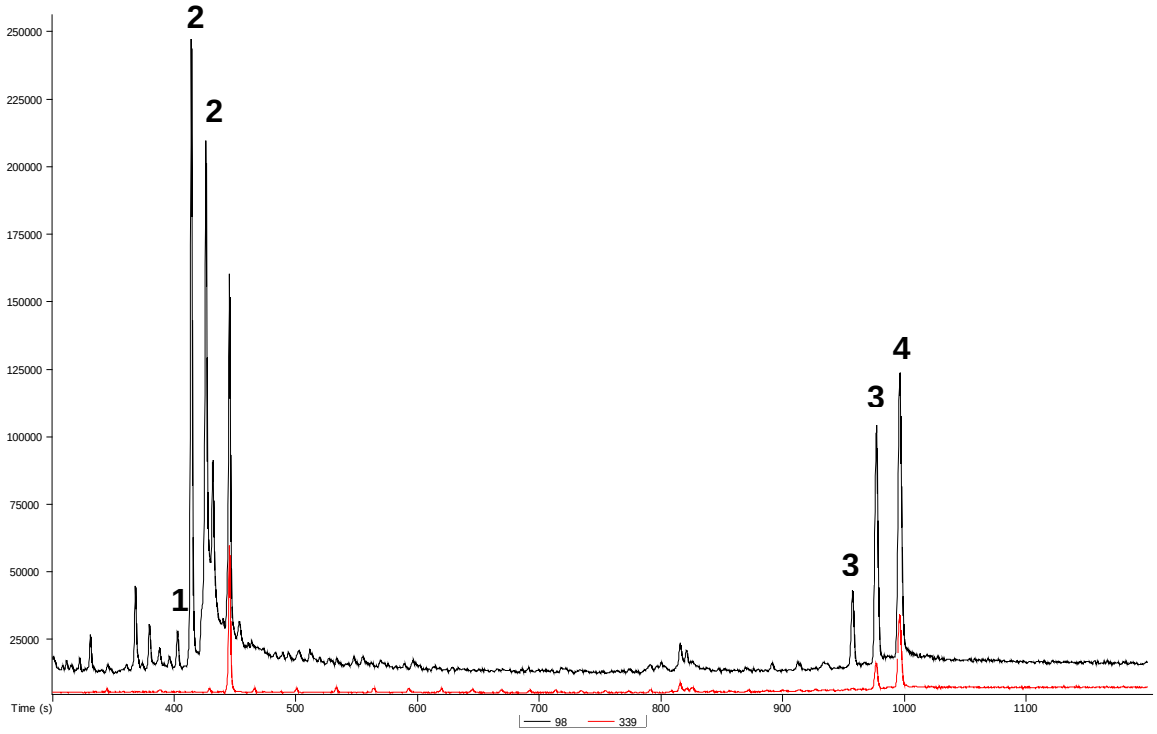

Figure 5.4: High-temperature 1-dimensional gas chromatogram of *Jatropha* oil extracted from seed by ultrasound-supported extraction in n-hexane or H_2O (this chromatogram)

Extraction conditions: t = 60 min, T = 85 ^{0}C, p = 101.3 kPa (1 atm)

Chromatography conditions: Rtx Biodiesel TG column at 20^{0}C/min to 360^{0}C, hold for 5 min

1 = C18 fatty acid methyl ester; 2 = C16-C18 free fatty acids; 3 = C16-C18 triglycerides; 4 = triolein (C18:1)

The gas chromatograph was used in one-dimensional mode with a special high-temperature column to facilitate detection of the less volatile triglycerides. Extracted ion chromatograms showing m/e 98 (fatty acids and triglycerides) in black and m/e 339 (triglycerides) in red have been superimposed to assist in the assignment of the different peaks. A triolein standard solution was used as a reference for the identification of the triglycerides since it is a major component of *Jatropha* oil. The analysis of this compound and the way it was utilised to qualitatively identify the triglycerides present in *Jatropha* oil samples are described later in this chapter.

The chromatograms all show several mixed C16-C18 triglycerides (peaks 3), with triolein (glycerol trioleate) (peak 4) having the highest peak intensity, and varying amounts of free fatty acids (peaks 1 and 2). The fatty acid composition of *Jatropha* oil is reported [1] to be 14% maximum and that of the triglycerides (main components) to be 80-90% as discussed in **Chapter 2**.

There are larger amounts of free fatty acids present in the soxhlet derived extract, possibly as a result of triglycerides being partly broken down to these acids at the higher extraction temperature (65 ^{0}C versus 40 ^{0}C) over prolonged extraction time (5 days versus 1 hour). It is also possible that small amounts of water present in the solvent and seed may lead to hydrolysis of the triglyceride fatty acid chains, leading to the appearance of the free fatty acids in the sample. The presence of very low concentrations of diglycerides in some oil samples may be explained by this process. The evaporation of excess solvent after extraction by n-hexane may also cause high-temperature breakdown. The low concentration of free fatty acids in the sc-CO_2 extract emphasises the less destructive nature of this method.

The most striking feature of extracts derived by superheated water (microwave-assisted extraction) and by ultrasound-supported extraction in water is the presence of more free fatty acids in comparison to triglycerides. These free fatty acids need to be neutralised before converting the triglycerides to fatty acid methyl esters (FAMEs) as they interfere with the transesterification reaction by decreasing the pH and increasing soap formation. Consequently extracts obtained by these two non-classical methods are less useful for conversion to biodiesel. The higher temperature of superheated water (150-200 ^{0}C) and the extreme temperatures (> 3000 K) generated by bubble collapse during sonication may be responsible for increased degradation of triglycerides into free fatty acids by rapid hydrolysis.

The ultrasound-assisted extraction with n-hexane (chromatogram not shown) resulted in triglyceride yields comparable to those obtained with soxhlet extraction (**Figure 5.1**), but with water (**Figure 5.4**) only about half of the soxhlet derived yield could be obtained. The use of water for the extraction thus decreased the amount of triglycerides, probably because of hydrolysis. The extraction time with sonification turned out to be much shorter than with soxhlet extraction, though.

By comparison, sc-CO_2 seems to be the preferred solvent to produce *Jatropha* oil from seed in pure form within short extraction times and at low temperatures. The extract contains minimal free fatty acids and should be free from organic solvent residues. Organic solvents, such as n-hexane, are also harmful to the environment and need to be used responsibly.

5.2 Qualitative chromatographic analysis of *Jatropha* oil

A standard solution of triolein, the most abundant C18 triglyceride in *Jatropha* oil, was used as a reference for the identification of mixed C16-C18 triglycerides present in the oil, as it is to be expected that all triglycerides have similar mass fragment patterns through loss of characteristic functional groups. The analysis was done using the same chromatographic method and column conditions as for the *Jatropha* oil samples. **Figure 5.5** shows the chromatogram of the triolein standard, **Figure 5.6** shows the mass spectrum of the standard and a library spectrum for triolein and **Figure 5.7** shows the mass spectrum of triolein from a *Jatropha* oil sample.

By comparing the mass spectrum of each oil sample to the mass spectrum of triolein, some of the triglycerides in the oil samples could be identified with a satisfactory match factor (70 % or higher) in spite of variation in the length of the fatty acid chains of the mixed triglycerides. As an example of such identification the first mass ion 603 in **Figures 5.6** and **5.7** may be considered. The molar mass of triolein (glycerol + three C18:1 chains) is 884 g/mol and the 603 ion results from loss of a C18:1 fatty acid chain containing one double bond (loss of 281 g/mol). Similarly, tripalmitin (glycerol + three C16:0 chains) has a molar mass of 807 g/mol and loses a saturated C16:0 fatty acid chain (mass loss of 255 g/mol) to give rise to the primary mass ion 552 visible on the mass spectrum of this C16:0 triglyceride (not shown). By calculating the initial mass losses from the mass spectra obtained for the various peaks on the chromatogram, the different possible triglycerides present in the oil sample could be determined. Among these were triolein (C18:1), tripalmitin (C16:0), trilinolein (C18:2) and tristearin (C18:0).

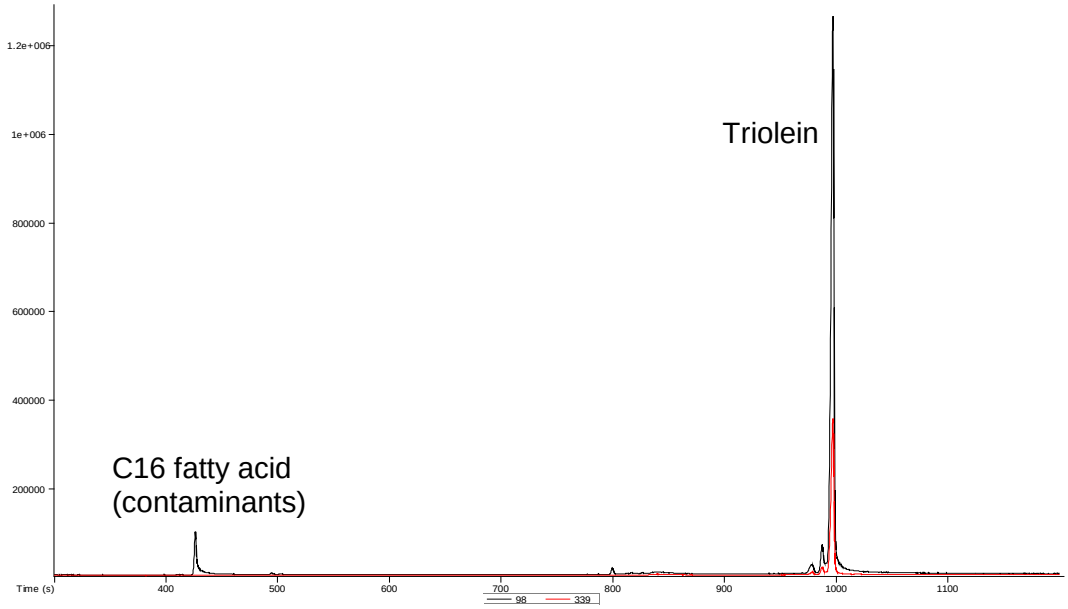

Figure 5.5: One-dimensional chromatogram of triolein standard
Rtx biodiesel TG column at 20°C/min to final temperature of 360°C, hold for 5 min.

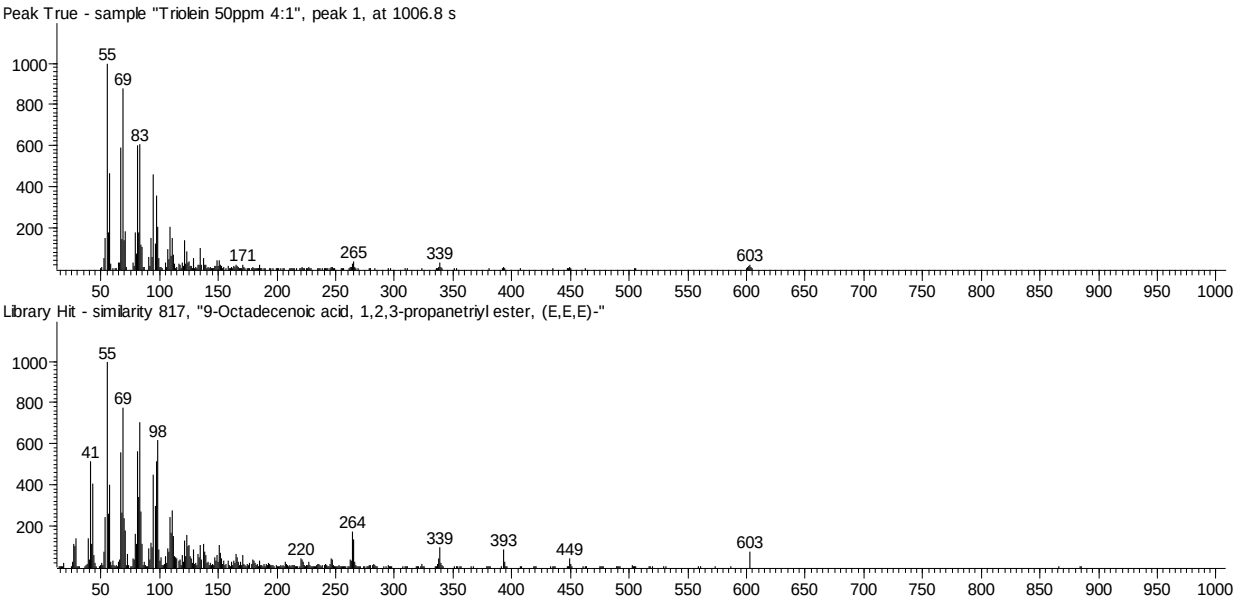

Figure 5.6: Mass spectrum of triolein standard vs. library hit

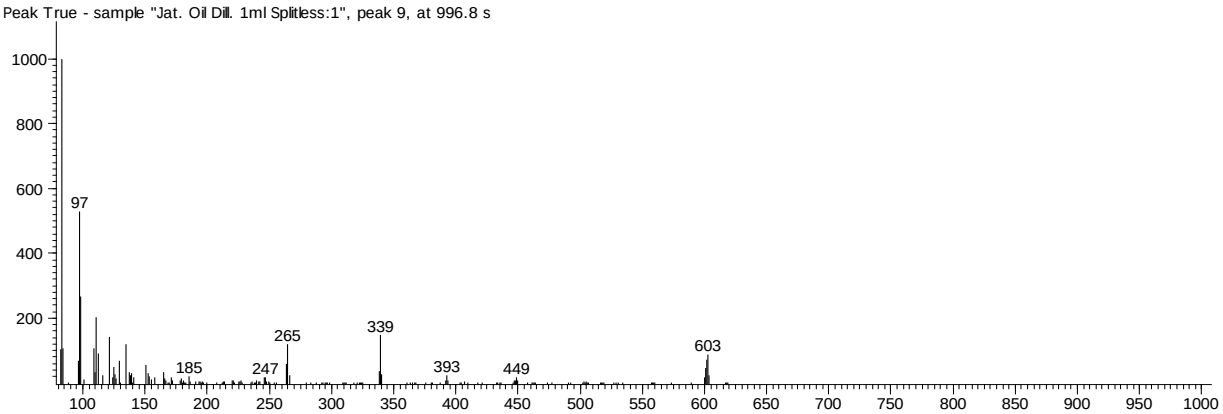

Figure 5.7: Mass spectrum of triolein peak in *Jatropha* oil sample

Further breakdown by loss of, for instance, C18:0 (saturated chain containing 18 carbon atoms), C18:1 (unsaturated 18-carbon chain with one double bond) or C18:2 (unsaturated 18-carbon chain with two double bonds), resulted in mixtures of triglycerides of different chain length. The characterisation of triglycerides in such mixtures became increasingly difficult as a result of co-elution and peak overlap. Chromatographic analysis of triglycerides generally is a complicated matter due to their lack of volatility, which makes it difficult to achieve elution and to prevent clogging of column and inlet, even after only a few injections.

Quantitative analysis of *Jatropha* oil samples obtained by the different extraction methods could not be completed since construction of a calibration curve failed. Despite repeated injections, the triolein standard did not elute at concentrations used for the oil samples, and some modification to the instrument is needed if samples are to be analysed quantitatively. The reader is referred to recommendations in this regard in **Chapter 6**.

5.3 Conversion of *Jatropha* oil to a biodiesel

Figure 5.8 shows a two-dimensional chromatogram of a sample of sc-CO_2 extracted *Jatropha* oil converted to crude biodiesel by transesterification. The sample contained primarily C16-C18 fatty acid methyl esters (FAMEs), though some lower chain-length esters (C13-C15) were also observed. Non-reacted free fatty acids could be detected, but only in low concentration. This chromatogram may be compared to the one in **Figure 5.9** (recorded under identical conditions) of a commercial biodiesel sample produced from used sunflower oil originally extracted by cold press and refined by n-hexane distillation. This sample is an SABS (South African Bureau of Standards) approved biodiesel according to the SANS1935 standard [2], and can be regarded as an appropriate standard against which the *Jatropha* biodiesel produced in our laboratory could be benchmarked. The chromatograms of the two samples compare well. The *Jatropha* sample contains all the FAMEs present in the commercial sample, and these are listed in **Table 5.1**.

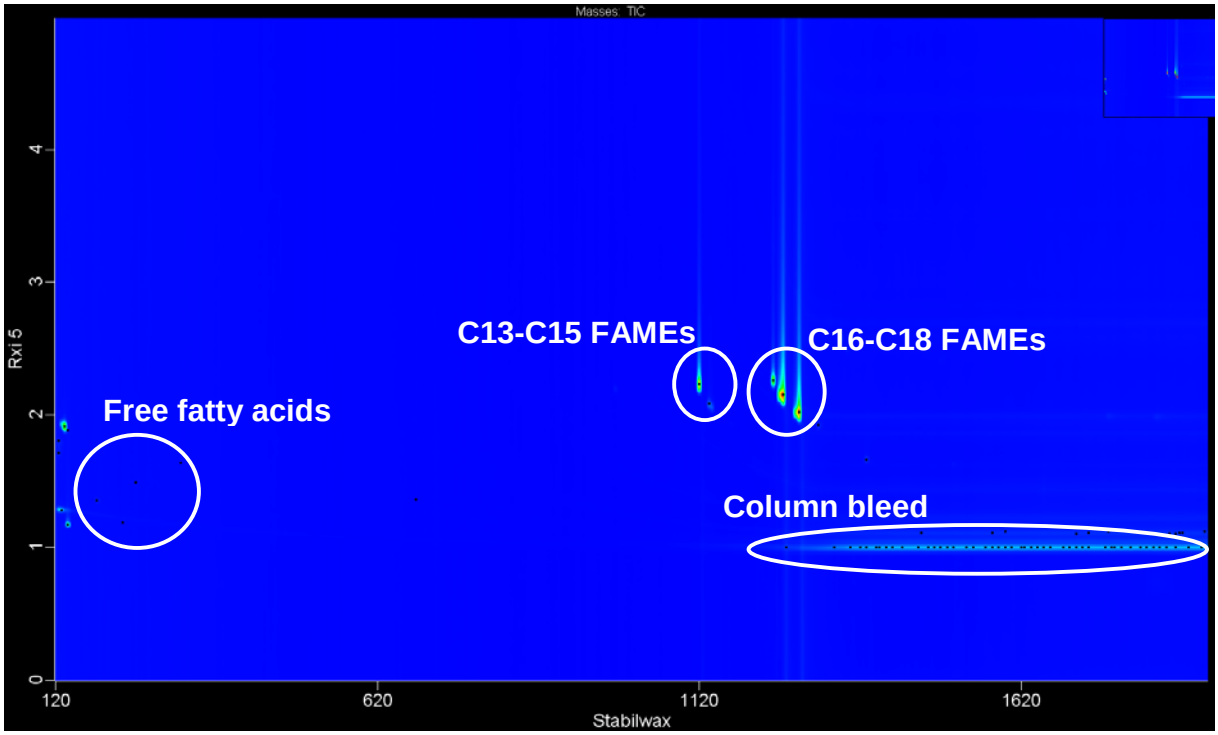

Figure 5.8: 2-Dimensional gas chromatogram (Stabilwax primary column (x-axis) 5 ^{0}C/min to 250 ^{0}C, Rxi-5Sil MS secondary column (y-axis) 5 ^{0}C/min to 260 ^{0}C, hold for 8 min) of crude biodiesel sample derived from sc-CO_2 extracted *Jatropha* oil by transesterification

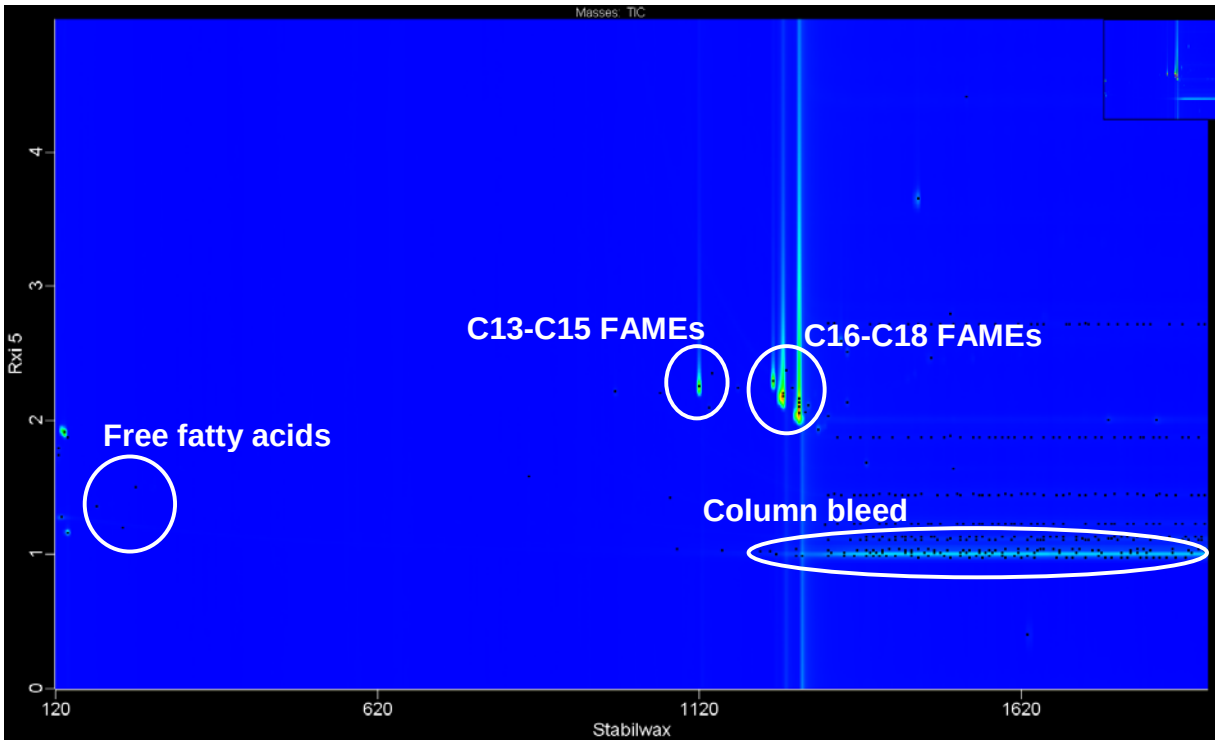

Figure 5.9: 2-Dimensional gas chromatogram (Stabilwax primary column (x-axis) 5 ^{0}C/min to 250 ^{0}C, Rxi-5Sil MS secondary column (y-axis) 5 ^{0}C/min to 260 ^{0}C, hold for 8 min) of commercial biodiesel sample derived from cold press extracted sunflower oil

Table 5.1: FAME composition and respective contributing factors (calculated from mass percentages) of a commercial South African biodiesel [2]

Methyl ester	Factor
Methyl ester of saturated fatty acids	0
Methyl hexadecenoate (Methyl palmitoleate) C16:1	0.950
Methyl octedacenoate (Methyl oleate) C18:1	0.860
Methyl octadecadienoate (Methyl lineolate) C18:2	1.732
Methyl octadecatrienoate (Methyl linolenate) C18:3	2.616
Methyl eicosenoate (Methyl gadoleate) C20:1	0.785
Methyl docosenoate (Methyl erucate) C22:1	0.723

These results once again confirm that sc-CO_2 extraction is the preferred method of deriving *Jatropha* oil for conversion to a biofuel as it yields a pure product with minimal pre-treatment and converted end-products that meet industry standards.

5.4 Other considerations of *Jatropha* as biofuel source

In order to investigate further the suitability of *Jatropha curcas* as a source of biomass for conversion to biofuel, additional sc-CO_2 based extractions were performed to investigate aspects such as the oil content of *Jatropha* seed and the relative solubility of *Jatropha* oil in sc-CO_2 in comparison to the solubility of other botanical oils. The data used to determine these quantities were collected in a previous investigation [3] as well as in this study, but with such mutual agreement that they could be combined and presented jointly on single graphs in this dissertation.

5.4.1 Oil content of *Jatropha* seed

The oil content of *Jatropha* seed was determined by extracting a sample exhaustively with sc-CO_2, employing the dynamic mode of the bench-top supercritical extractor. A continuous flow of 1 dm^3 min^{-1} CO_2 was maintained at a constant pressure and temperature (40 ^{0}C, 30 MPa), and extraction time was varied from 5 to 99 minutes (the maximum time of a dynamic run with the supercritical extractor). **Figure 5.10** shows the yield-time curve constructed from data collected in these extraction runs. The curve did not entirely reach a plateau after 99 minutes, and ideally longer times would be required to determine the exact oil content of the seed. The curve could, however, be extrapolated graphically, and an oil content of approximately 55% m/m could be obtained from the graph. *Jatropha* seed may, according to the literature [1, 2] contain up to 60% m/m oil.

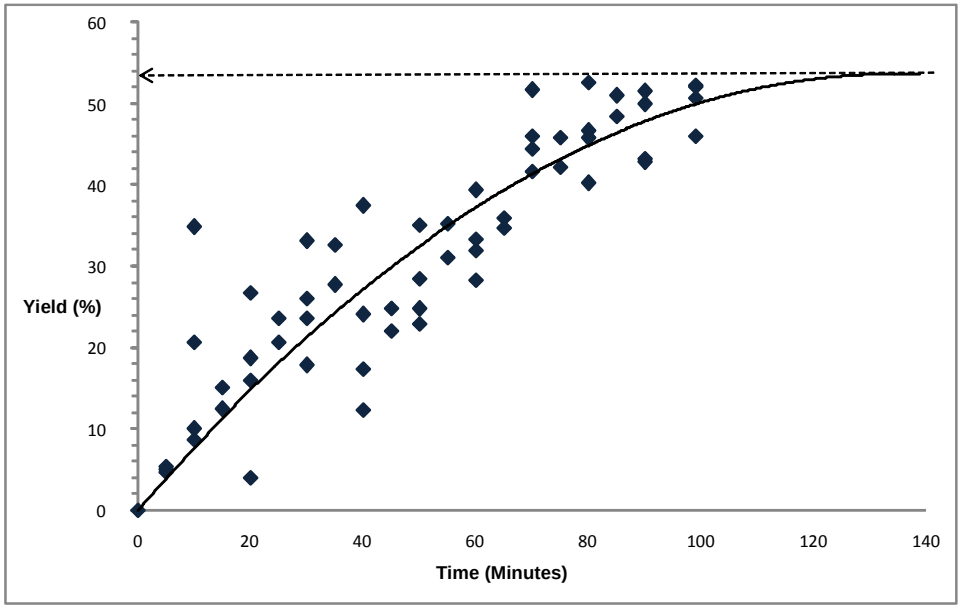

Figure 5.10: Yield-time curve for determination of oil content of *Jatropha* seed

5.4.2 Solubility of *Jatropha* oil in sc-CO_2

The solubility of *Jatropha* oil in sc-CO_2 was measured by performing extraction runs in static mode, i.e. with zero flow rate of sc-CO_2, in order to establish equilibrium between the extractable oil and a fixed aliquot of sc-CO_2 at preselected conditions (40 ^{0}C, 30 MPa, 5-60 min). The resulting amount (measured in grams) of oil dissolved from the seed matrix by a fixed amount of sc-CO_2 (subsequently converted to grams) is plotted as a function of time in **Figure 5.11.**

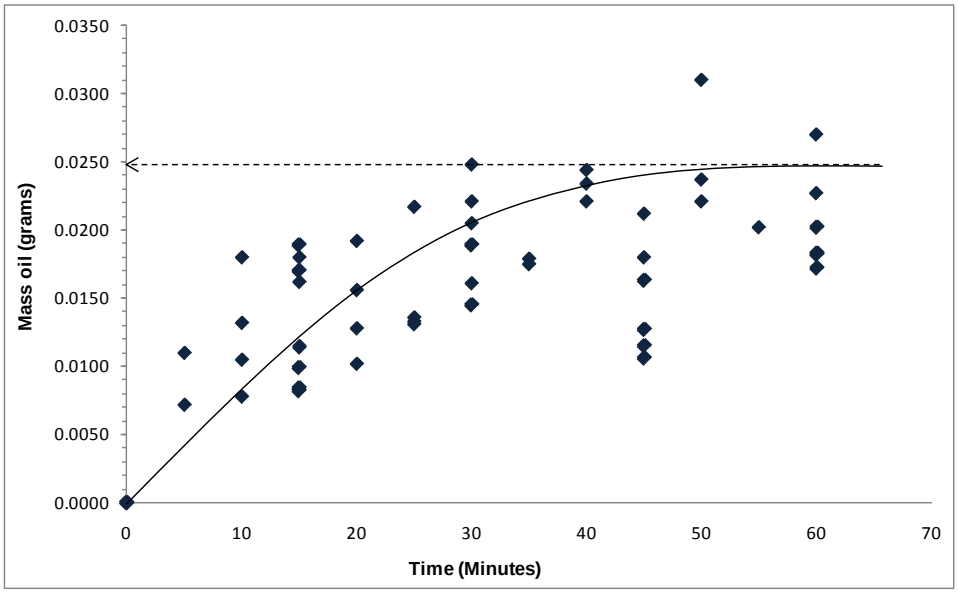

Figure 5.11: Mass-time curve for determination of solubility of *Jatropha* oil in sc-CO_2

The mass of oil extracted from 1 g of seed in static mode equals 0.0250 g as indicated by the arrow in **Figure 5.11**. The density of CO_2 at 40 ^{0}C and 30 MPa is 0.92 g cm^{-3}, which is either read from the display of the supercritical extractor or calculated with SF-Solver [4]. The solubility of the oil, expressed as g of oil per g of sc-CO_2, can then be calculated from the volume of sc-CO_2 as s = 0.00305 g per g CO_2 at the conditions specified, as illustrated below:

Volume CO_2 = (volume of thimble) - (volume of seed)

= (10 - 1.1) cm^3

= 8.9 cm^3

Mass of CO_2 = volume of CO_2 x density of CO_2

= 8.9 cm^3 x 0.92 g cm^{-3}

= 8.19 g

Solubility of oil (gram oil per gram sc-CO_2): s = 0.0250/8.19 = 0.00305 g oil/g CO_2

The scatter of the data points can, among other errors, be attributed to oil remaining in the flow lines and valves of the extractor as a result of zero flow during the extraction, despite proper flushing of the system by CO_2 at ambient conditions after the static run. The loss of extracted oil varied from sample to sample, but a mass balance performed by comparing the decrease in mass of the thimble to the mass of oil collected in the collection vial, showed that on average only a 2-3 % deviation could be attributed to this problem. The oil loss thus impaired reproducibility only marginally, and other factors are to be identified to account for the magnitude of the error involved in determining the solubility by drawing a smooth curve through the average of the data points.

Table 5.2: Solubilities of selected plant oils in sc-CO_2

Plant Oil	Conditions	Solubility/g per g CO_2	Reference
Sunflower	30 MPa, 323 K	6.50 x 10^{-3}	5
Caffeine	28 MPa, 323 K	1.88 x 10^{-3}	6
Soybean	30 MPa, 313 K	1.98 x 10^{-3}	7
Canola	30 MPa, 313 K	3.60 x 10^{-2}	8
Jatropha	30 MPa, 313 K	3.05 x 10^{-3}	This study

In **Table 5.2** the solubility of *Jatropha* oil in sc-CO_2 is compared to the solubilities of a few other plant oils determined at almost identical conditions by other authors. The value is of

the same order of magnitude as that of sunflower, caffeine and soybean oil [5-7], and roughly an order of magnitude lower than that of canola oil [8].

In conclusion, *Jatropha* seed has a high oil content of at least 55% m/m (**Figure 5.10**), which makes it a favourable feedstock for biofuel production. The oil is quite soluble in sc-CO_2 (3.0 x 10^{-3} g per g CO_2 at 313 K and 30 MPa) (**Table 5.2**), rendering sc-CO_2 an efficient solvent to extract it from seed and to replace harsh solvents and prevent unwanted solvent residues in extracts, in agreement with the requirements of green chemistry.

5.5 Extraction of pine sawdust components

Extracts from pine sawdust obtained by traditional soxhlet extraction were initially analysed by one-dimensional chromatography in the same way as the *Jatropha* oil samples, but only a few of the extracted components could be identified. After derivatisation, to enhance volatility and to reduce the reactivity of involatile oxidising components, a large number of compounds co-eluted. **Figure 5.12** shows a one-dimensional chromatogram of pine extract analysed with the high-temperature biodiesel column used for the *Jatropha* oil samples. Broad peaks with some tailing are observed, and mainly hydrocarbons (alkanes, alkenes) and some free fatty acids could be identified.

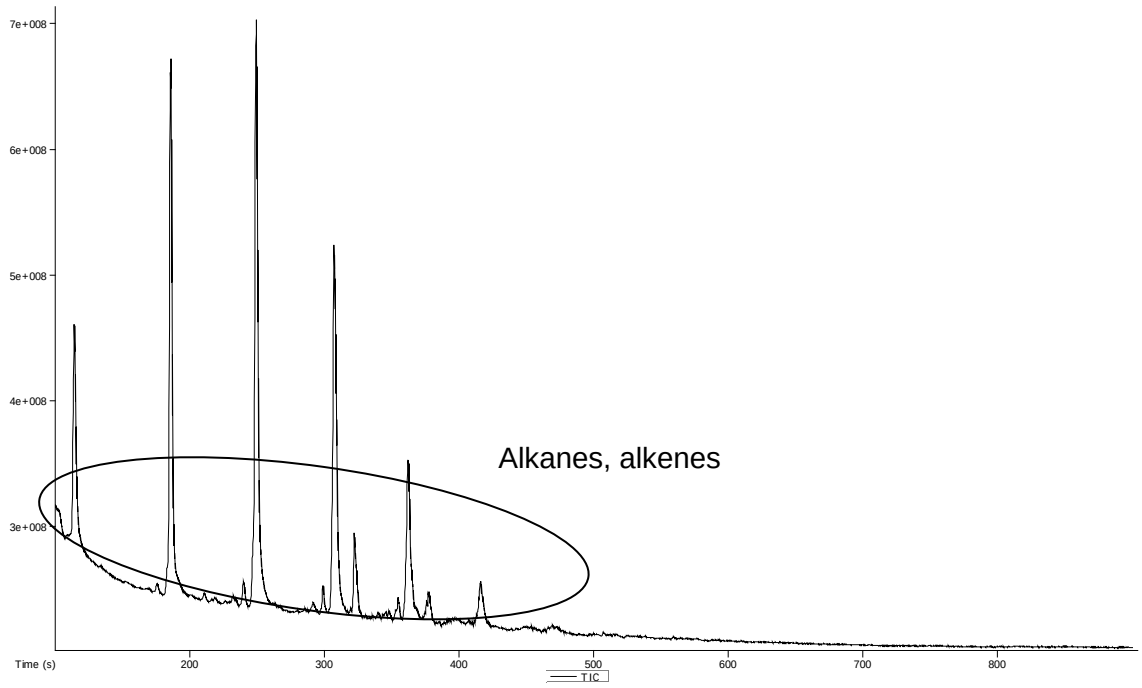

Figure 5.12: One-dimensional chromatogram of pine sawdust sample extracted with acetone (Rtx Biodiesel TG column at 20 ^{0}C/min to final temperature of 360 ^{0}C, hold for 5 min)

Analysis was subsequently performed with two-dimensional GCxGC-TOFMS by implementing a new special high-temperature secondary column from Restek (Rxi-17Sil MS) combined with a primary column (Rxi-5Sil MS) as discussed in **Chapter 4** (not to be confused with the high-temperature primary biodiesel column used for one-dimensional analysis of *Jatropha* oil). The two-dimensional chromatogram of the same sample in **Figure 5.13** shows that all major types of wood components (alkanes, alkenes and branched hydrocarbons (carbon number C8-C36), various terpenoids (such as limonene), flavonoids, abeitic acid and pimaric acid, simple phenolics (such as phenol), vanillin, gallic acid, pinosylvin and various lignans, and some free fatty acids and fatty acid methyl esters) could be identified.

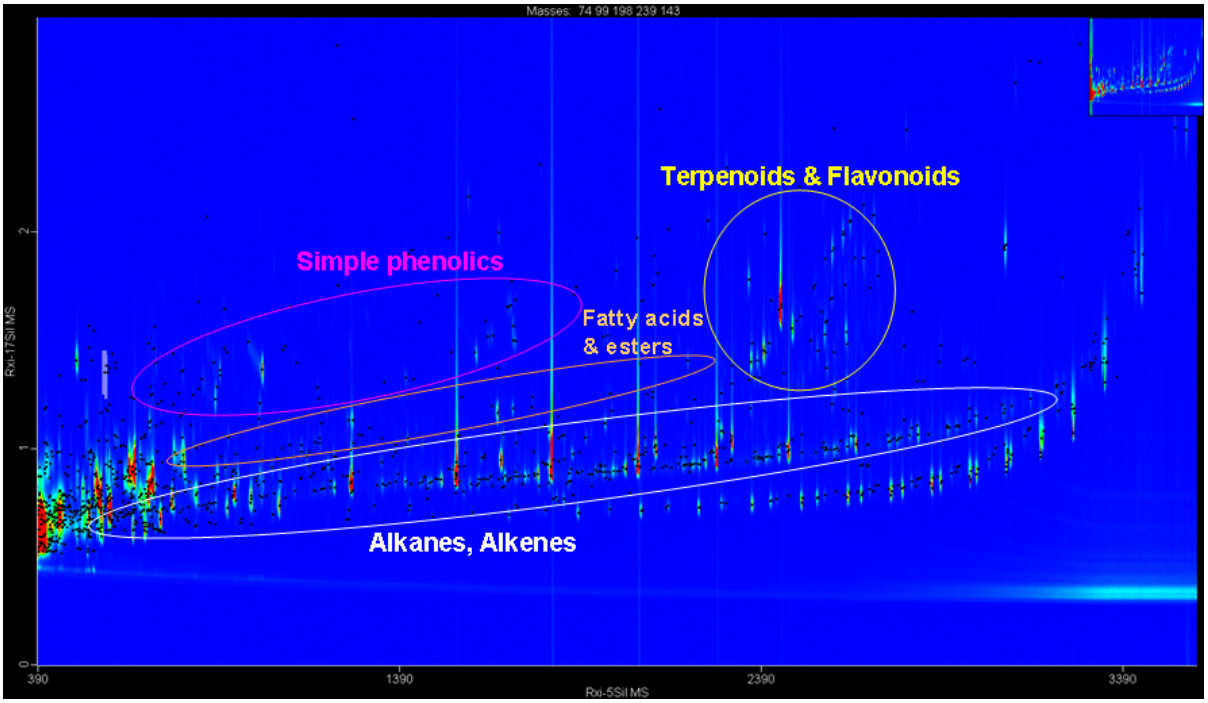

Figure 5.13: Two-dimensional chromatogram of pine sawdust sample extracted with acetone (Rxi-5Sil MS primary column 20 ^{0}C/min to 330 ^{0}C, Rxi-17Sil MS secondary column 20 ^{0}C/min to 340 ^{0}C, hold for 5 min)

Extraction of pine sawdust samples with sc-CO_2 yielded results similar to soxhlet derived samples as shown in **Figure 5.14**. All major types of components were identified, showing that sc-CO_2 is an appropriate solvent for extracting a variety of low molar mass components from wood fibre without causing structural degradation. This confirms that sc-CO_2 is a viable alternative solvent to polar organic solvents, like acetone, which broadens its scope of application even more.

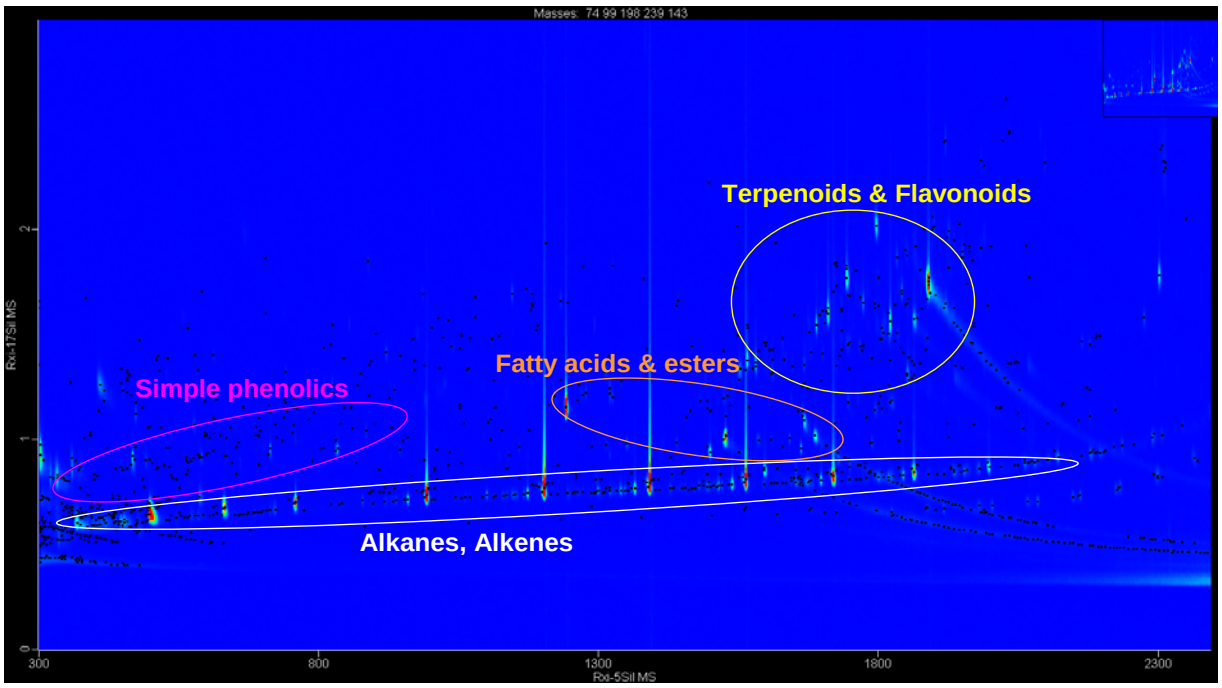

Figure 5.14: Two-dimensional chromatogram of pine sawdust sample extracted with sc-CO_2 (Rxi-5Sil MS primary column 20 ^{0}C/min to 330 ^{0}C, Rxi-17Sil MS secondary column 20 ^{0}C/min to 340 ^{0}C, hold for 5 min)

Table 5.3: Composition of sc-CO_2 extracted pine sample [9]

Extracted components	Group	T_R/min	Match factor/%
Hexadecanoic acid	Fatty acid	23.5	84
Rhodoviolascin	Carotenoid	24.5	54
Heptadecanoic acid	Fatty acid	25.1	84
Linolenic acid	Fatty acid	26	60
Linoleic acid	Fatty acid	26.4	97
Oileic acid	Fatty acid	26.5	80
Pinosylvin monomethyl ether	Phenolic	28.2	96
Primaric acid	Terpenoid	28.4	94
Sandaracopimaric acid	Terpenoid	28.7	94
Pinosylvin	Phenolic	28.9	96
Isopimaric acid	Terpenoid	29.0	90
Diethylstilbestrol	Hormones	29.3	89
Palustric acid	Fatty acid	29.4	92
Dehydroabietic acid	Resin acid	29.9	93
11,14-Eicosadenoic acid	Fatty acid	30.1	40
Abietic acid	Terpenoid	30.6	95
Retinoic acid	Fatty acid	32.7	61
15-Hydroxy-dehydroabietic acid	Resin acid	33.4	89
7-Oxodehydroabietic acid	Resin acid	33.7	96
Behenic acid	Fatty acid	34.3	67
15-Hydroxy-7-oxodehydroabietic acid	Resin	36.9	88
B-Sitosterol	Carotenoid	46.2	81

These results correspond well with those reported in the literature [9] and listed in **Table 5.3**. The table contains the principal types of extractable components discussed in **Chapter 2,** and identified in this study for all pine sawdust samples with match factors above ~ 60% similarity.

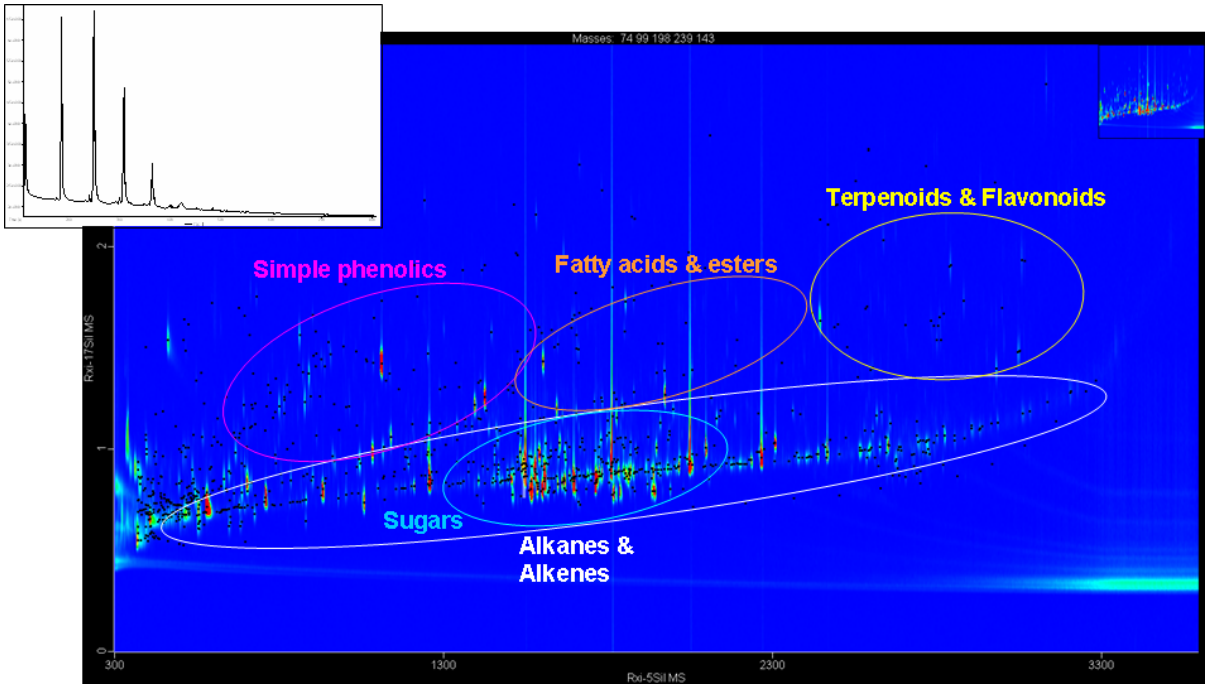

Figure 5.15: Two-dimensional chromatogram of pine sawdust sample extracted with superheated H_2O.
(Rxi-5Sil MS primary column 20 ^{0}C/min to 330 ^{0}C, Rxi-17Sil MS secondary column 20 ^{0}C/min to 340 ^{0}C, hold for 5 min)
Insert: One-dimensional chromatogram of pine sawdust sample extracted with superheated H_2O.
(Rtx Biodiesel TG column at 20 ^{0}C/min to final temperature of 360 ^{0}C, hold for 5 min.)

Figure 5.15 shows the complex two-dimensional chromatogram obtained by extracting pine sawdust with superheated water. The insert shows a one-dimensional chromatogram of the same sample which shows peaks similar to those observed for the soxhlet extracted sample (**Figure 5.12**). The two-dimensional chromatogram does not only show the hydrocarbons, fatty acids, terpenoids, flavonoids and phenolics of the acetone and sc-CO_2 extracted samples, but also a variety of new components ranging from the C15-alkanes onwards. These components could be identified as a range of sugar entities of large structural variation originating from partial degradation of the cellulose, hemi-cellulose and lignin polymeric chains of the wood fibre backbone by superheated water hydrolysis. No other extraction method, classical or non-classical, was capable of breaking down the structure of wood fibre. Microwave-assisted superheating can therefore create diverse building blocks from which biofuels and other chemical compounds can be developed.

Pine sawdust extractions with water were limited in this study to microwave-assisted methods, since these are more controllable with regard to temperature than ultrasound-supported methods where extreme temperatures of hot spots originating from bubble collapse during sonication cannot be controlled.

5.6 Solubility of pine sawdust components in sc-CO_2 and superheated H_2O

The extraction of material from pine sawdust by sc-CO_2 proceeds by one of two possible mechanisms, viz. physical desorption or chemical dissolution. A clear distinction between desorption and dissolution as two modes of sc-CO_2 extraction could be made in our laboratory by comparing thermodynamic and kinetic quantities for sc-CO_2 regeneration of granular activated carbon (GAC) [10] with those determined for dissolution of $Cu(acac)_2$ in sc-CO_2 [11] studied previously. The numerical values of the enthalpy of activation $\Delta H^{\neq}$, for instance, were clearly different for the two processes having a more physical and chemical nature, respectively. Some of the components of wood fibre have low boiling points (below 100 ^{0}C) and are fairly volatile. The mechanism of sc-CO_2 extraction of wood biomass could therefore be a combination of physical desorption of more volatile components from the ultrastructure of the wood fibre and chemical dissolution of substances soluble in sc-CO_2.

Wood consists of a variety of substances as discussed in **Chapter 2**, but solubilities reported in the literature are usually for specific components present in an extract. In this study, however, a “bulk solubility” of all extractable components is reported in order to compare the two selected biomass sources considered for biofuel production. Solubility of pine sawdust extractables in sc-CO_2 could be determined by performing static extraction runs at 85 ^{0}C and 60 MPa (density = 0.903 g cm^{-3}). **Figure 5.16** shows the solubility curve, which exhibits similar data scatter as that obtained for *Jatropha* oil due to sticky resin from the wood fibre staying behind in the flow lines and valves of the extractor. The maximum mass of wood components dissolved from 1 g of pine sawdust in a fixed amount of sc-CO_2 at the conditions specified could be determined as 0.016 g as indicated by the arrow.

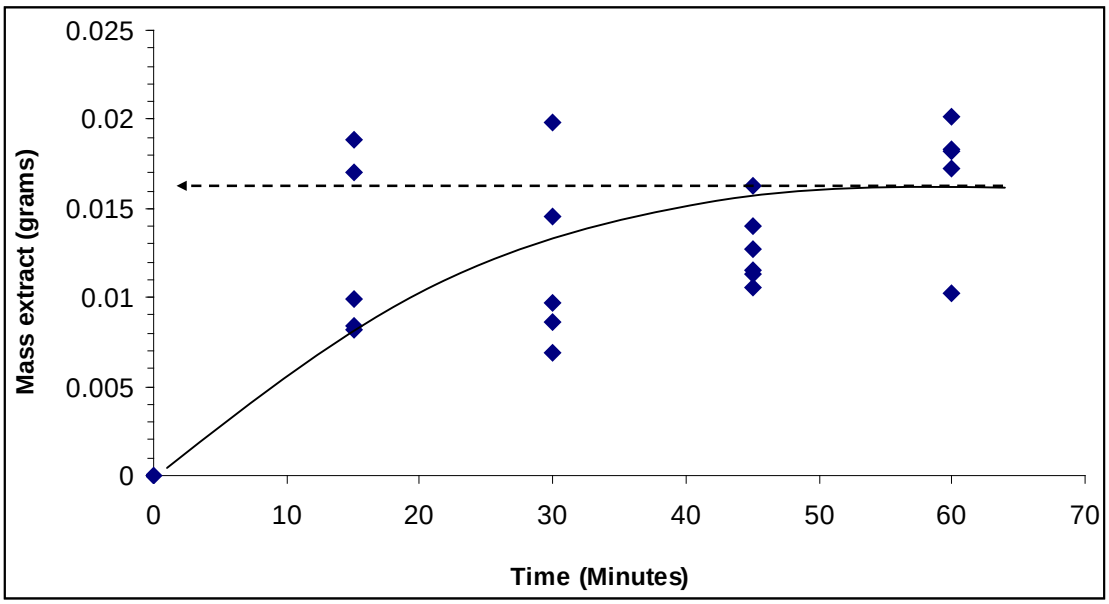

Figure 5.16: Mass-time curve of components "dissolved" in sc-CO_2 by physical desorption and/or chemical dissolution (as explained in the text) using a 1 g sample of pine sawdust

By repeating the calculation previously done for sc-CO_2 extraction of *Jatropha* oil, a "bulk solubility" of pine sawdust components in sc-CO_2 could be determined as follows:

Volume CO_2 = (volume of thimble) - (volume of sawdust)

= (10 - 7.5) cm^3

= 2.5 cm^3

Mass of CO_2 = volume of CO_2 x density of CO_2

= 2.5 cm^3 x 0.903 g cm^{-3}

= 2.257 g

Solubility: s = 0.016/2.257 = 0.00709 g extract / g CO_2

The "bulk solubility" of wood components in sc-CO_2 is of the same order of magnitude as that of most of the botanical oils listed in **Table 5.2**, which means that sc-CO_2 is equally effective in extracting material other than plant oil for biofuel related processes.

Superheated water produces a harsher extraction environment, causing small segments of wood fibre to break away from the chain. Extracts obtained by superheated water therefore have compositions somewhat different from sc-CO_2 extracted samples. The yield-time curve in **Figure 5.17** indicates that 0.037 g pine sawdust material was dissolved in 20 g superheated H_2O, resulting in a "bulk solubility" s = 1.8 x 10^{-3} g extract per g superheated water at 150°C and a vessel pressure reaching 0.5 MPa.

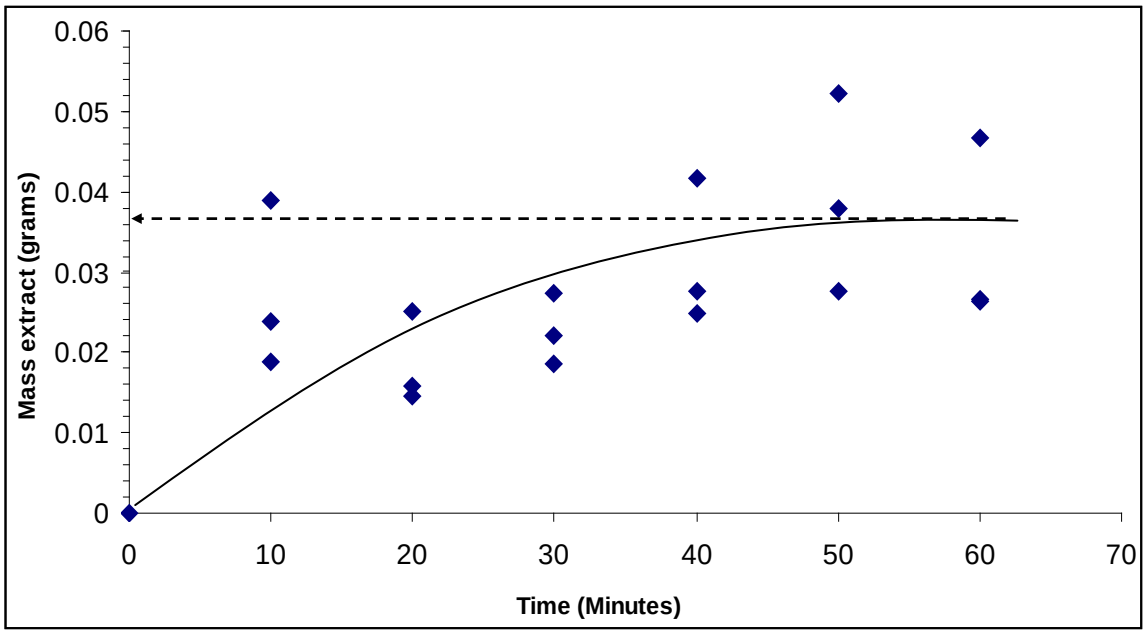

Figure 5.17: Yield-time curve of components "dissolved" in superheated H_2O using a 1 g sample of pine sawdust

The poor reproducibility of the data may be attributed to the incapability of the temperature control vessel of the microwave instrument to maintain exactly the same temperature in all extraction vessels as explained in **Chapter 4**. Temperatures may thus vary from vessel to vessel, depending also on the components being extracted and the extent of insulation of each vessel. The evaporation of water to separate the extracted components could also account for possible loss of volatile components, influencing the dry mass of the final extracts. Despite the data scatter, a trend is observed and an approximate extrapolation could be performed.

References

[1] Tiwari, A.K., Kumar, A. & Raheman, H. Biodiesel production from *Jatropha* oil (*Jatropha curcas*) with high free fatty acids: An optimised process. *Biomass and Bioenergy*, 31 No. 8 (2007): 569-575.

[2] SANS1935:2004. South African National Standard for Automotive Biodiesel Fuel, Standards South Africa (A Division of SABS), 2004.

[3] Van Greuning, C. sc-CO_2 extracted oil from *Jatropha curcas* - directive for the biodiesel industry? M.Sc. Dissertation, School of Physical and Chemical Sciences, North-West University Potchefstroom Campus, South Africa, (2009).

[4] SF-Solver™: Software for Supercritical Fluid Analysis, Copyright© Isco Inc., 1995.

[5] Wessels, A.A. Extraction of *Helianthus annuus* (sunflower) oil with supercritical carbon dioxide. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2004.

[6] De Azevedo, A.B.A., Kieckbusch, T.G., Tashima, A.T., & Mohamed, R.S. Supercritical CO_2 recovery of caffeine from green coffee oil: New experimental solubility data and modeling. *Quim. Nova,* 31 No. 6 (2008): 1319-1323.

[7] Van Deventer, F.J. Optimisation of supercritical carbon dioxide derived soybean oil. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2008.

[8] Louw, H.W. Extraction of canola oil with supercritical carbon dioxide. M.Sc. Dissertation, Faculty of Health Sciences, North-West University, Potchefstroom, 2006.

[9] Ekeberg, D., Flæte, P.O., Eikenes, M.F. & Naess-Andresen, C.F. Qualitative and quantitative determination of extractives in heartwood of Scots pine (*Pinus sylvestris* L.) by gas chromatography. *Journal of Chromatography A*, 1109 (2006): 267-272.

[10] Steenkamp C.J.H. Regeneration of industrially exhausted granular activated carbon by supercritical carbon dioxide, M.Sc. Dissertation, Potchefstroom University, 1998.

[11] F. Van Wyk, Dissolution of $Cu(acac)_2$ in sc-CO_2, M.Sc. Dissertation, Potchefstroom University, 1998.

Chapter 6: Evaluation and Future Perspective

This chapter brings a closure to the dissertation by evaluating the achievements and shortcomings of the study with reference to the project objectives stated in Chapter 0. Recommendations are given for future studies which may complement the work done in this project.

6.1 Achievements and shortcomings

Jatropha oil was investigated as a source of biomass for conversion to a biofuel. Unlike soybean, maize and sunflower oil, which are all edible feedstocks, *Jatropha* oil is not suitable for human consumption and was therefore a preferred choice for biodiesel production attempts.

Several non-classical extraction methods were successfully employed to extract oil as a first step in developing a "green" chemical process to produce an alternative for fossil fuels. *Jatropha* oil could be extracted fairly effectively by n-hexane (traditional solvent extraction), sc-CO_2, superheated water and ultrasound activated water, but with differences with regard to yield and composition of the resulting extracts among these solvents/methods.

sc-CO_2 proved to be the preferred non-classical solvent to extract *Jatropha* oil free of solvent residues and without requiring further refinement. The oil content of the seed (~55% m/m) was determined by extrapolating to infinity a yield-time graph constructed with data from a series of sc-CO_2 extraction runs performed in dynamic (exhaustive) mode. The solubility of the oil in sc-CO_2 (3 x 10^{-3} g per g CO_2 at 313 K and 30 MPa) was obtained by a similar extrapolation to a maximum (plateau) but with data obtained in static (equilibrium) extraction mode. This value turned out to be similar or one order of magnitude lower than the solubilities in sc-CO_2 of other botanical extracts (sunflower oil, caffeine, soybean oil and canola oil) obtained in previous studies. The extent of data scatter prevented proper polynomial regression treatment of the data, but graphical extrapolation, though somewhat arbitrarily, resulted in realistic solubility values.

Superheated water extractions could be performed successfully in closed vessels of an industrial microwave system. The mechanism of extraction is based on a change in polarity of water at high

temperature whereby oxidative O_2 molecules, less polar solvent (water) and non-polar organic matter (plant material) become increasingly mutually compatible and interact more intimately. Yields were less than those obtained with sc-CO_2, though, and triglycerides present in the oil underwent hydrolysis and degradation into their corresponding free fatty acids. This became evident by monitoring high-temperature one-dimensional chromatograms of these extracts, which are less suitable for conversion into a biofuel as the fatty acids reduce the pH of the reaction mixture and result in high levels of soap formation during the base-catalysed conversion with methanol. It could be concluded that superheated water was not as effective as sc-CO_2 to extract *Jatropha* oil for subsequent conversion to biodiesel.

Extraction with water irradiated by an ultrasonic wave emitter led to results very similar to those obtained with superheated water, and hence this method of extraction could also be ranked less suitable for biomass extraction. However, sonication may be employed for *in situ* biodiesel conversion in order to prevent soap settlement in the final product and to eliminate subsequent purification with water.

sc-CO_2 extracted *Jatropha* oil could be derivatised successfully and diluted for chromatographic analysis, and sufficient volumes of oil could be extracted for conversion into a crude biodiesel. This could be benchmarked chromatographically by comparison to an SABS approved commercial biodiesel sample according to the SANS1935 standard.

One of the major achievements of this study was the in-depth qualitative analysis of extracted and converted *Jatropha* oil by both GC-MS and GCxGC-TOFMS methods. Mixtures of C16-C18 triglycerides were identified in the extracted oil, and the corresponding C16-C18 fatty acid methyl esters (FAMEs) obtained by base-catalysed transesterification could be observed in the methylation product.

The quantitative analysis of the triglycerides present in the extracted oil was less successful. These high molar mass substances are very non-volatile and elution could only be achieved if a special high-temperature metal column capable of operating temperatures of 450 ^{0}C or higher were used. These columns are, however, not suitable for use in combination with a mass spectrometer as detector since metal fragments from the column might damage the ion source of the detector. For this reason an Rtx biodiesel column (fused silica) had to employed in this study, which is less effective but compatible with the instrument used.

A second disadvantage was the lack of an on-column injection system where the sample is injected through the injection port directly onto the column. The instrument used in this study was equipped with a normal split/splitless inlet, and a uni-liner was utilised to simulate a pseudo on-column injection. This was not always successful as the inlet temperature could not be increased above 300 ^{0}C, and the triglyceride samples therefore caused clogging of the capillary column and liner in the inlet of the gas chromatograph. As a result of these constraints, repetitive triglyceride injections for the purpose of a calibration curve could not be achieved, and quantitative analysis had to be aborted.

Qualitative analysis of the converted FAME was successfully done by two-dimensional GCxGC-TOFMS. The column combination and temperature programming was retrieved from application notes on the LECO© website, and these proved to be quite suitable for identifying the constituents of the converted biodiesel for comparative purposes (cf. **Paragraph 5.3**).

The two-dimensional configuration was also used to identify components extracted from pine sawdust used as a second source of biomass, and extracts obtained with different extraction techniques could be compared. A newly developed secondary column (Rxi-17Sil MS) in conjuction with an existing primary column (Rxi-5Sil MS) allowed for two-dimensional analysis at higher temperatures, though not high enough for (and also not compatible with) triglyceride analysis. Due to the complex and foreign nature of wood extracts, quantitative analysis could not be performed as that would require extensive standardisation of each type of component in the extracts, which was beyond the scope and objectives of this study.

Wood extracts obtained by sc-CO_2 and soxhlet experiments mutually compared well, with typical components being alkanes, alkenes and branched hydrocarbons (carbon number C8-C36), terpenoids and flavonoids such as limonene, abeitic acid and pimaric acid, simple phenolics such as phenol, vanillin, gallic acid, pinosylvin and various lignans, and some free fatty acids and fatty acid methyl esters. These components can be utilised in various chemical processes such as synthesis of alkane fuels from hydrocarbons. Terpenoids are formed through joining several isoprene base molecules representing the backbone of the terpenoid molecule. The isoprene chain can fold to produce rings and can be functionalised by introduction of heteroatoms. Monoterpenes consist of two isoprene units and are generally used in the flavour and fragrance industries and in the manufacture of turpentine. Diterpenes (abeitic and pimaric acid) consist of four isoprene units and are used as rosins and sizing agents. Flavonoids are used for colouration, flavouring, tanning agents and adhesives. Phenol is the main constituent of other phenolic components and,

depending on the functionality, these molecules have a variety of uses. Phenol itself is widely used in plastics, compact discs and an array of other applications.

In addition to these extractable substances, wood mainly comprises cellulose, hemicellulose and lignin, which are composed of glucose and pentose sub-units and aromatic compounds, respectively. These cellulosic polymer chains degrade into simple sugars which can be used to produce bio-ethanol and methanol. In combination with some other extractables, conversion to biodiesel can also be achieved, though in a different form than from vegetable oils. These sugar units can be observed when a wood sample is extracted with superheated water during closed-vessel, microwave-assisted extractions, confirming the cleavage of cellulose and hemicellulose chains under such conditions. It can be concluded that wood is a crucial alternative energy resource as it may render solid, gaseous and liquid fuel constituents through various conversion processes.

The gross solubility of pine sawdust extractables in sc-CO_2 (7×10^{-3} g per g CO_2 at 358 K and 60 MPa) is of the same order of magnitude than that of most botanical oils, but there is uncertainty as to the mechanism of extraction of all these components, as more polar molecules will resist dissolution and would rather be thermally desorbed at the prevailing extraction conditions (358 K and 60 MPa), depending on their volatility. The bulk solubility of pine extractables in superheated water was determined as 2×10^{-3} g per g superheated water at 423 K (and approximately 0.5 MPa pressure reached within the extraction vessel), but the data used for the yield-time curve suffered bad reproducibility. A more controlled method of evaporation of water or separation of extracted components from water could lead to improved results. Normal air-drying, for instance, was employed to evaporate the water, which could have resulted in loss of some extracted components.

The study proved that non-classical extraction methods are capable of retrieving from biomass sources various materials (vegetable oils, resins, and chemical building blocks such as terpenoids, flavonoids, and pentose/glucose sugars) which are relevant to biofuel production, synthesis and conversion processes. These methods are generally perceived as unsafe in terms of the “extreme” conditions at which they operate and capital demanding as far as equipment is concerned, but proven application possibilities may change the view about them. The biomass sources and non-classical chemical methods of extraction selected for this study together form a point of departure for processes of green biofuel manufacture in future.

6.2 Recommendations for future studies

Future studies should focus on the shortcomings of this study.

The data scatter associated with the extrapolated yield-time graphs for the purpose of solubility measurement of botanical extracts prohibits determination of reliable values. More consistent solvent clean-up (e.g. for water extractions) and equipment handling (e.g. elimination of extract losses) should be adopted. Alternative type equipment (e.g. continuous instead of batch type extractors) should be implemented for commercialisation purposes. Extractors capable of handling large volumes of botanical material are essential for industry-scale implementation of sc-CO_2 extraction.

Superheated water extractions need to be further investigated and refined to improve reproducibility and to attain higher extraction temperatures. Extractions in the subcritical water region may be employed for wood samples in an attempt to achieve more effective degradation of the cellulosic chains without oxidising the sugar units completely as with supercritical water oxidation (SCWO). An effective method of solvent removal, without loss of volatile components, needs to be developed to enhance this extraction method. Ultrasound-assisted extractions should be explored more as it holds great potential for conversion and purification processes.

Quantitative analysis of both extracts and converted products requires urgent attention. Although compounds have been thoroughly analysed qualitatively, exact amounts are unknown and prohibits prediction of yields of converted products. The inlet of the instrument used in this study needs to be modified, and extensive standardisation with various solutions needs to be done to acquire quantitative results. Extensive quantitative analysis may enable wood extracts to be characterised according to the amounts of certain types of components in order to predict the viability of converting these components to other chemicals.

Finally, new and innovative chemical processes for sustained environmental protection should be strived for. The need for alternative fuel resources indeed exists, but fuel producing processes need to be "green" and economical. Much work needs to be done to comply with these requirements and to reduce damage already being done to the environment.