NORTH-WEST UNIVERSITY
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Optimisation of Supercritical Carbon Dioxide Derived Soybean Oil

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Optimisation of supercritical carbon dioxide derived soybean oil

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B. Pharm. (NWU 2007)

Dissertation submitted in partial fulfillment of the degree

Magister Scientiae
in
Pharmaceutical Chemistry

School of Pharmacy
North-West University
Potchefstroom Campus

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Potchefstroom
2008

SUMMARY

The objective of the study was to extract soybean oil [Glycine max (L.) Merrill] from seed by supercritical carbon dioxide (sc-CO_2) and to optimise the yield and composition of the oil by varying relevant process parameters.

Extraction runs were conducted with a commercially available laboratory size supercritical fluid extractor (LECO TFE 2000) capable of operating in a static, dynamic or combined static/dynamic mode.

Density was shown to play a major role in extraction efficiency as it determines solvent strength of sc-CO_2. It was found to be optimum at liquid-like densities (0.8 – 1 g/mL), with an almost exponential increase in extracted oil yield on changing from gas-like to liquid-like densities. The solubility of soybean oil in sc-CO_2 was measured utilising the static mode of the supercritical extractor and found to be 0.00198 g oil / 1 g of CO_2 at 300 atm and 40 °C. The oil content of the seed was established by utilising the dynamic extraction mode and found to be 18.7%, in agreement with a corresponding value in the literature.

The sc-CO_2 extracted soybean oil was analysed by several chromatographic techniques including GC-MS, GCxGC/TOF-MS and GC-FID. The extracts were found to be component-rich and up to 113 compounds could be successfully identified by GCxGC/TOF-MS. The results compare favourably to those of oil extracted by other methods (soxhlet, cold press).

The quality of the sc-CO_2 extracted oil was benchmarked against a commercially available standard.

OPSOMMING

Die doel met hierdie studie was om sojaboonolie [Glycine max (L.) Merrill] uit saad met superkritieke koolstofdioksied (sc-CO_2) te ekstraheer en die opbrengs en samestelling van die olie te optimaliseer deur tersaaklike prosesparameters te varieer.

Ekstraksielopies is uitgevoer met 'n laboratoriumgrootte superkritieke-fluïed-ekstraktor (LECO TFE 2000) wat in die handel beskikbaar is en wat instaat is om ekstraksies in 'n statiese, dinamiese of 'n gekombineerde statiese-dinamiese modus uit te voer.

Dit kon aangetoon word dat digtheid 'n beslissende rol in ekstraksiedoeltreffendheid speel omdat dit die oplosmiddelsterkte van sc-CO_2 bepaal. Dit bereik 'n maksimum by tipiese vloeistofdigthede (0.8 – 1 g/mL) en 'n bykans eksponensiële toename in die hoeveelheid geëkstraheerde olie word waargeneem soos wat die digtheid vanaf tipiese waardes van gasse na dié van vloeistowwe toeneem. Die oplosbaarheid van sojaboonolie in sc-CO_2 is gemeet deur van die statiese ekstraksiemodus van die ekstraktor gebruik te maak en is as 0.00198 g olie / 1 g CO_2 by 300 bar en 40 °C bepaal. Die olieinhoud van die saad is vasgestel deur die dinamiese ekstraksiemodus van die ekstraktor te gebruik en is as 18.7% bepaal. Die waarde vergelyk goed met 'n ooreenstemmende waarde wat in die literatuur gerapporteer word.

Die sc-CO_2 geëkstraheerde olie is geanaliseer deur van verskeie chromatografiese tegnieke waaronder GC-MS, GCxGC/TOF-MS en GC-FID gebruik te maak. Die resultate vergelyk goed met dié vir olie wat met ander metodes (soxhlet, kouepers) geëkstraheer is Die ekstrakte het geblyk komponentryk te wees en tot 113 verbindings is suksesvol met GCxGC/TOF-MS geïdentifiseer.

Die gehalte van die sc-CO_2 geëkstraheerde olie is getoets deur die chemiese samestelling met dié van 'n kommersiële standaard te vergelyk.

ACKNOWLEDGEMENTS

I thank God for wisdom and strength throughout the study.

I thank Prof. Breet for his expertise and guidance throughout the study; offering continual support and specialist advice.

I thank Pieter van Zyl for assistance with training on the extractor and skilled advice.

I thank Derik van der Westhuizen for providing insight into problem areas and an innovative approach.

I thank Bryson Neizel for lending a helping hand in performing additional experiments in order to diversify my project.

I thank Sylvia Odendal for friendly and timely assistance at Nola Industries.

I thank Dr. Peter Gorst-Allman for training and advice on the GCxGC/TOF-MS.

I thank Deorette Maartens and my parents for their love, support and guidance.

I thank NRF for the financial support throughout the study.

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CHAPTER 1

Actuality, Objectives and Strategy

I, Frikkie van Deventer, completed this research project for the purpose of obtaining an M.Sc. in Pharmaceutical Chemistry as part of my academic internship following the B.Pharm. qualification. It was performed in the supercritical research group accommodated within the newly established Chemical Resource Beneficiation (CRB) focus area of the North-West University (NWU) Potchefstroom Campus. The principal reason for selecting this particular topic is the actuality and diverse application possibilities of soybean oil of which several are relevant to the pharmaceutical industry at large.

1.1 ACTUALITY

The soybean plant [Glycine max (L.) Merrill] is a major crop in the world today. The active ingredients found in soybean seed are phospholipids, soy proteins, protease inhibitors, isoflavonoids and triterpene saponins. The seed therefore has, among others, direct relevance for the food, pharmaceutical and medical industries. It is believed to reduce blood lipid levels. It is also of importance for enhancing liver physiology, for reducing symptoms of menopause and for inhibition of prostatic and breast cancers [1].

Biodiesel is a modern substitute for fuel and consists of alkyl monoesters of fatty acids derived from plant oils such as soybean oil [2]. It is a renewable fuel and it reduces the production of pollutants. The most common form of biodiesel in the United States is

made of methanol and soybean oil and is known as soymethyl ester or methyl soyate [3].

The applications of soybean oil are numerous and that made it relevant to conduct a project in which supercritical carbon dioxide is used to extract soybean oil from seed. Supercritical extraction yields an extract free from hazardous solvent residues. The principal focus of the project was to optimise the extraction process and the composition/quality of the extracted oil.

1.2 OBJECTIVES

The objectives with the envisaged project were

- to extract soybean oil from seed with supercritical carbon dioxide (sc-CO_2);
- to optimise the yield and composition of the extracted oil by varying relevant process variables;
- to identify/develop suitable protocols to analyse the extract qualitatively and quantitatively;
- to refine/beneficiate the extracted oil towards a product having potential applicability.

1.3 STRATEGY

The work plan to achieve these objectives required the researcher to

- acquire soybean seed of suitable quality and representative of available cultivar from a renown source (Agricultural Research Council);
- perform *sc*-CO_2 extraction runs on a laboratory scale supercritical fluid extractor (LECO TFE/M 2000) in order to prove the viability to derive soybean oil from seed with this technology;

- systematically vary different operational conditions (time, temperature, pressure, density, flow rate, moisture content and cosolvent) in order to optimise the developed extraction procedure;
- develop a suitable chromatographic based protocol (GC-FID, GC-MS, GCxGC/TOF-MS) for the analysis of extracted oil in terms of yield and composition;
- develop/utilise standard methods of chemical analysis for both extracted and commercial soybean oil in order to evaluate the acquired oil in terms of quality, composition and properties;
- compare the results (yield of oil, nature of extract) of *sc*-CO_2 extraction with available literature data on similar extractions by traditional techniques;
- cooperate with knowledgeable people in the pharmaceutical, nutritional and chemical engineering sectors to evaluate *sc*-CO_2 extracted soybean oil for health related purposes and biofuel usefulness.

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CHAPTER 2

Soybean

The soybean plant was chosen because of the vast number of possible applications in medicine, nutrition and biofuel.

2.1 Soybean plant

Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Genus:	Glycine
Species:	G. max
Binomial name:	Glycine Max

Figure 2.1 Soybean and its scientific classification [1]

The soybean plant is native to East Asia and has been used for thousands of years as a food source and as medicine. According to some of the earliest references soybean has been consumed in Asia for nearly 5000 years. This came to light as part of a study as to why the incidence of cardiovascular disease is lower among Asian women [2, 3].

The soybean plant may vary in height from 20 cm to 2 m. The leaves are trifoliate and are covered in fine brown or grey hairs. Each seed pod contains 2 to 4 seeds which measure 5 to 11 mm in length. The composition of the oil is ca. 60% protein and 35% carbohydrate [1, 4].

Soybean is cultivated around the world with the main producers being the United States of America and Brazil with 87.7 and 52.4 million metric tons per annum respectively [1, 3].

2.2 Medicinal use of soybean

2.2.1 Cholesterol

In 1999 the American food and drug administration approved food labels on soy products as being protective against coronary heart disease. Studies found that 25 g of soy protein per day lowers the LDL levels of cholesterol [5, 6].

The proposed mechanism of cholesterol lowering effect includes:

- modulation of lipoprotein metabolism by reducing LDL and VLDL and increasing DLL cholesterol
- reduction of LDL oxidation
- reduction of proliferation and migration of smooth muscle cells in the vasculum
- reduction of platelet activation and aggregation
- improvement in vascular reactivity [2].

When animal protein is replaced by soy protein, hypercholesterolemia does not occur. Soy diet contains lower amounts of saturated fat, so patients lose weight, which also lowers the cardiovascular risk in humans [6].

The three main isoflavones found in soybean are genistein, diadzein and glycitein. These phytoestrogens are structurally similar to the human hormone estrogen which protects the female body against cardiovascular disease [2].

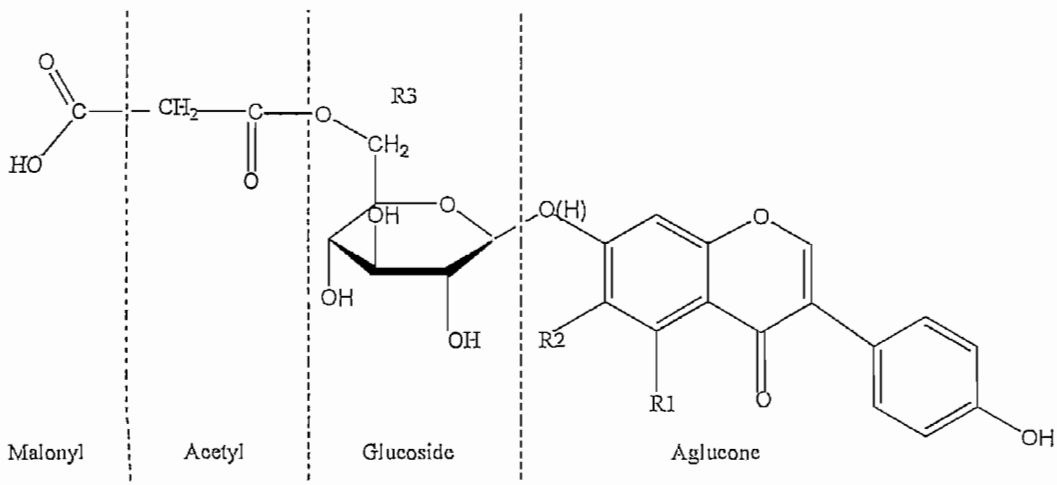


Figure 2.2 Structure of isoflavones found in soybean [7].

2.2.2 Cancer

The low incidence of breast, endometrial and prostate cancer in Asian countries led to epidemiological studies in these countries to determine the cause of this phenomenon. It is believed that soy isoflavones have an antiandrogenic effect which might explain the low incidence of prostate cancer in Asian men. The Asian diet is mainly vegetarian or semi-vegetarian and the consumption of soy is high compared to that of Western societies [2, 6].

Prostate cancer is the third most diagnosed malignancy in the world according to data from the American Cancer Society. Figure 2.3 shows that incidence of prostate cancer is lower in Asian countries than in Western societies. Soy intake raises antioxidants in the blood without altering testosterone levels [8]

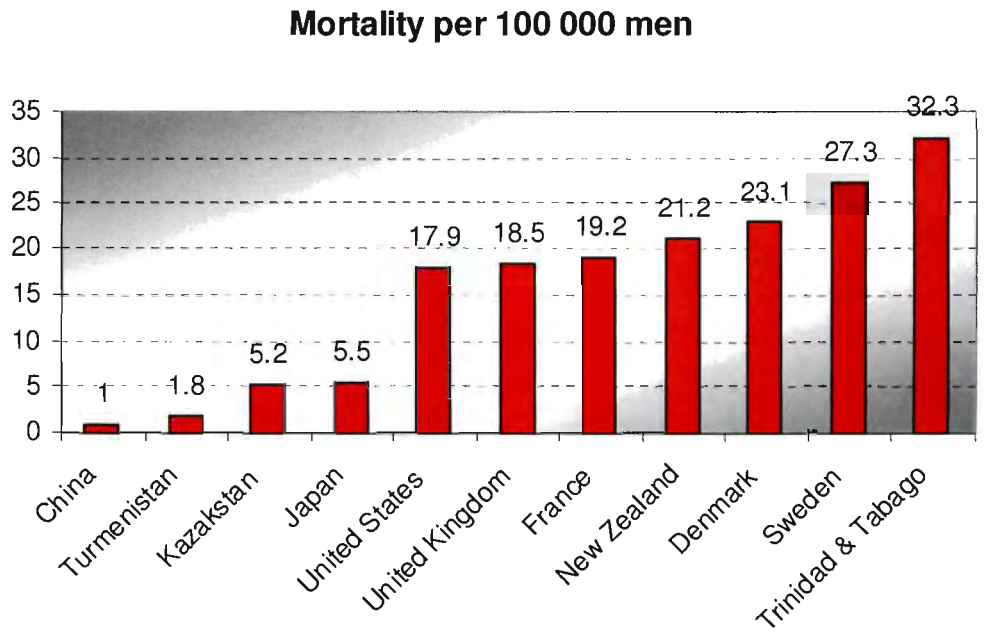


Figure 2.3 Mortality rate per 100 000 men in 2000 for different countries [9]

There is a correlation between phytoestrogens intake and protection against prostate cancer. The studies were done on men with Japanese ancestry and their eating habits were followed and their cancer risk was assessed [10].

2.2.3 Osteoporosis

Soy isoflavones are believed to have a positive impact on bone density and thus on osteoporosis due to the structural similarity to ipriflavone [11].

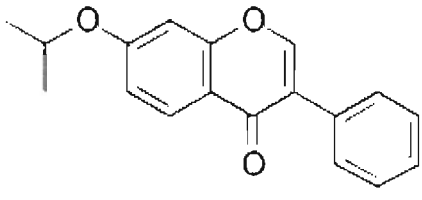


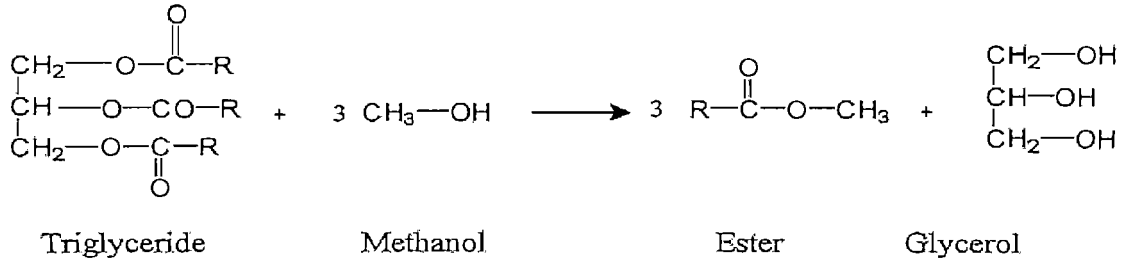
Figure 2.4 Structure of ipriflavone, a synthetic isoflavone [12]

Ipriflavone is used in the maintenance of healthy bones by preventing the buildup of osteoclasts, which erode bone cells. The lower incidence of osteoporosis in postmenopausal Japanese women could be attributed to their high intake of soy protein since it leads to less excretion of calcium [11, 12].

2.4 Biodiesel from soybean oil

Biodiesel is made from renewable resources such as animal fats and vegetable oils. It is produced by transesterification by blending the biological component with methanol to form esters that support combustion [13, 14]. Figure 2.5 shows a schematic representation of the process.

Figure 2.5 Transesterification of soybean oil to produce fatty acid methyl esters [14]



Biodiesel emits less carbon monoxide, sulfur, particle matter, polyaromatics and hydrocarbon emissions than petroleum based diesel [15].

Although the biodiesel industry is still relatively new and only a few people are using biofuel, evaluation of emissions and efficacy of biodiesel has found it to be a viable fuel for use in diesel powered engines [15 & 16].

2.5 Soy products

The list of consumable products made from soy is extensive. It includes products such as soy milk, soy nut butter, soy yogurt and soy cheese. These non-dairy products are mainly consumed by vegans, strict vegetarians and people with allergies to dairy products. Meat alternatives include tofu, miso, tempeh, soy mince, soy chicken nuggets and other meat related products. These are consumed by vegetarians to get the daily protein they need for healthy living.

Lecithin extracted from soybean is used as an emulsifier in fatty foods and as an antioxidant for food preservation. Soybean oil is used in cooking and as a salad dressing. Soy sauce is well known throughout the world and is used in cooking as a flavouring agent.

Other soybean products include body care products, candles, cleaning agents, composite materials, crayons, fabric conditioner, hair conditioner, hair styling aids, hand cleaners, paint, paint removers, pens, polish, shampoos, solvents, animal feed and much more.

Figure 2.6 Soy milk, soy cheese and soy protein bar

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CHAPTER 3

Supercritical Carbon Dioxide

Supercritical carbon dioxide (sc-CO_2) can be described as a highly condensed gas or a highly mobile liquid since it shares properties of the gaseous and liquid phases. It can be adjusted to exhibit gas-like or liquid-like densities by simply selecting appropriate conditions of pressure and temperature. This allows sc-CO_2 to act as a fluid with exceptional transport and solvent characteristics rendering it useful for a variety of novel applications.

3.1 History

The first report on the phenomenon of the supercritical phase of substances appeared in 1822, but its application to industrial processes was only considered in the 1950`s.

A first few studies on supercritical technology by scientists of Max Planck Institute focused on using supercritical fluids as extracting agents in food, petroleum and chemical related topics. They were the first to demonstrate sc-CO_2 extraction of caffeine from green coffee beans. The process was licensed to HAG Corporation, which built the first supercritical based decaffeination plant in 1976. The plant began production in 1978 and was followed by construction of a hops extraction plant in Münchester in 1982 and a tea decaffeination plant shortly thereafter [1].

3.2 Properties of supercritical CO_2

Carbon dioxide in the supercritical phase (the state where liquid and gas phases converge above but close to the critical point of 72.9 bar and 31.1°C) has been shown in numerous cases to be a suitable alternative to organic solvents used in extractions, especially in the food industry, e.g. hops extraction for beer-brewing [2,3].

Since CO_2 is an inert substance, also in the supercritical state, it does not react with food constituents and does not stay behind in extracts as hazardous solvent residue. It is used for flash freezing and has the advantage of being non-toxic and non-flammable. It does not corrode metal when in contact with moisture [3].

CO_2 in the supercritical state saves on the environmental burden, as over 13 billion kilograms of halogenated and other organic solvents annually end up in waste water through solvent extraction [4].

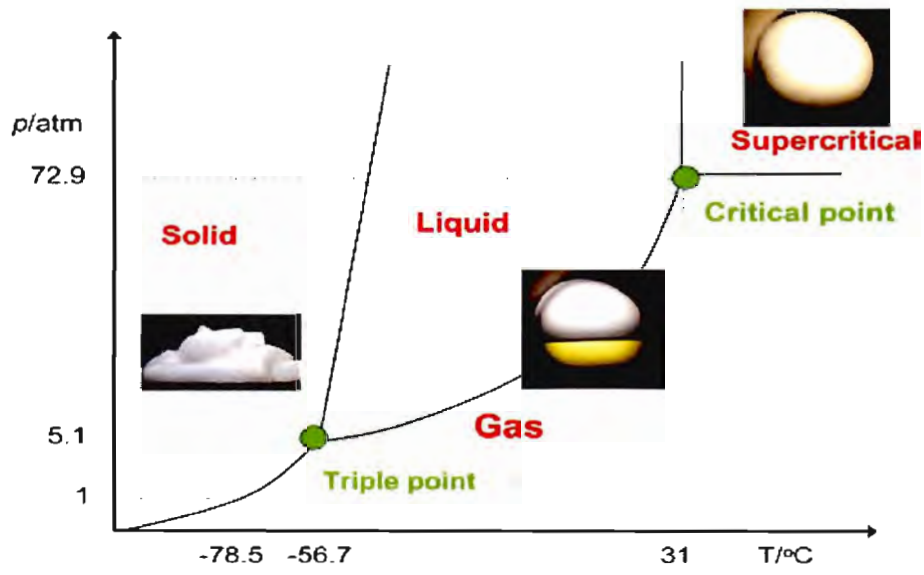

Figure 3.1: Phase diagram of CO_2. (The diagram shows the different phases in which CO_2 can occur, as well as the conditions prevailing at the triple and critical points.) [5].

To achieve the supercritical state, CO_2 is compressed and heated before being transferred to a vessel for extraction. The solute laden sc-CO_2 is then fed into a separator where temperature and pressure are reduced. This lowers the solvent strength of the CO_2 and the product precipitates. Temperature and pressure can be adjusted to optimise extraction and to extract specific components [4].

The extraction with sc-CO_2 is limited, however, by its high degree of non-polarity. It has a dielectric constant of 1.5 versus 2.5 – 4 for organic solvents and 78 for water. The capability of sc-CO_2 to dissolve substances can, however, be improved by addition of small amounts of a co-solvent, modifier or entrainer (e.g. methanol) which improves the polarity of the molecule and its versatility as a solvent on the basis of the principle "like dissolves like" [4].

3.3 Apparatus for SFE

The basic setup for supercritical fluid extraction (SFE) is shown in Figure 3.2

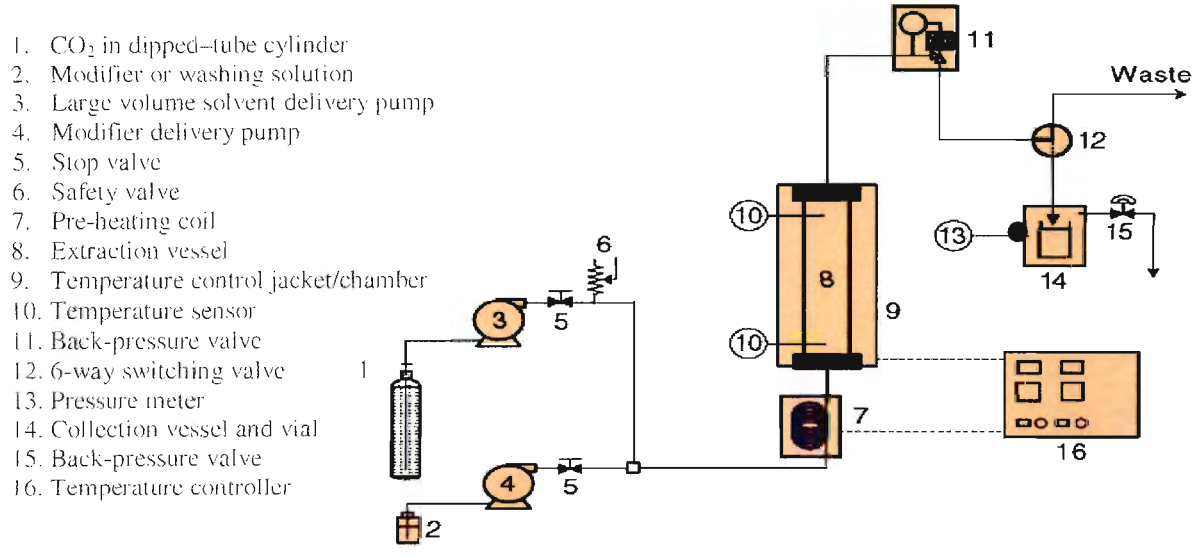

Figure 3.2: Basic layout of supercritical fluid extractor

The pump is initially filled with the gas of choice cooled to its liquid state. It is then activated to pressurise the gas to above its critical pressure (which for CO_2 is 72.9 bar) and to pump it through tubing to a temperature controlled jacket or chamber where it is heated to above its critical temperature (which is 31.3 °C for CO_2).

Inside the chamber is an extraction vessel which contains the sample, which may be plant material from which an ingredient, e.g. an essential oil, needs to be extracted. The matrix and supercritical fluid are allowed to interact for a given period of time, either in static mode if zero flow is maintained for the entire period of exposure, or in dynamic mode if the supercritical fluid is allowed to continuously flow through the vessel during exposure. Extraction may sometimes call for both static and dynamic modes, depending on whether partial or exhaustive extraction is desired.

Once the supercritical fluid and the solubilised solute have left the extraction vessel, separation is needed. The pressure is reduced, which reverts the sc-CO_2 to the gas phase. The CO_2 loses its solubilising capability and the solute precipitates [1].

3.4 Uses of supercritical CO_2

In addition to SFE, a few other uses of sc-CO_2 include

- thin-layer deposition of materials on surfaces, such as in the preparation of ceramic filtration membranes;
- separation of components in supercritical fluid chromatography (SFC), by using the fluid as the mobile phase;
- particle formation by RESS (rapid expansion of supercritical solvent) and SAS (supercritical antisolvent) where homogeneous particle size is required;
- impregnation of strengtheners, colourants and preserving agents into matrices such as wood or paper.

Although sc-CO_2 is a non-polar solvent, which limits the solubility of polar substances, high flow rates and simple recovery renders sc-CO_2 ideal for extraction of non-polar analytes. sc-CO_2 is the mostly used supercritical fluid because of its liquid-like density at moderate conditions to warrant good solubility for extraction and its gas-like viscosity to penetrate material for impregnation of matrices [6,7].

Some of the advantages of SFE over other extraction methods include:

- pressure and temperature can be varied to optimize extraction efficiency and selectivity;
- solvent properties can be fine-tuned to meet special requirements;
- product recovery can be achieved simply by depressurizing the system;
- heat and mass transfer are efficient in the liquid state;
- conditions are optimum for certain types of reaction (e.g. free-radical) [8].

3.5 Comparison between soxhlet and SFE

Soxhlet is a traditional extraction technique for which an experimental setup as shown in Figure 3.3 is required. The solvent is heated to its evaporisation point and the vapour condenses in the Liebig condenser and drips into the sample placed into a special sock. It dissolves components from the sample and flows into the flat-bottom flask.

The disadvantages of soxhlet extraction are

- large volumes of hazardous and flammable liquid organic solvents;
- potential toxic emissions during extraction;
- need for expensive, high-purity solvents;
- non-selective;
- a labour intensive procedure;
- a time-consuming procedure.

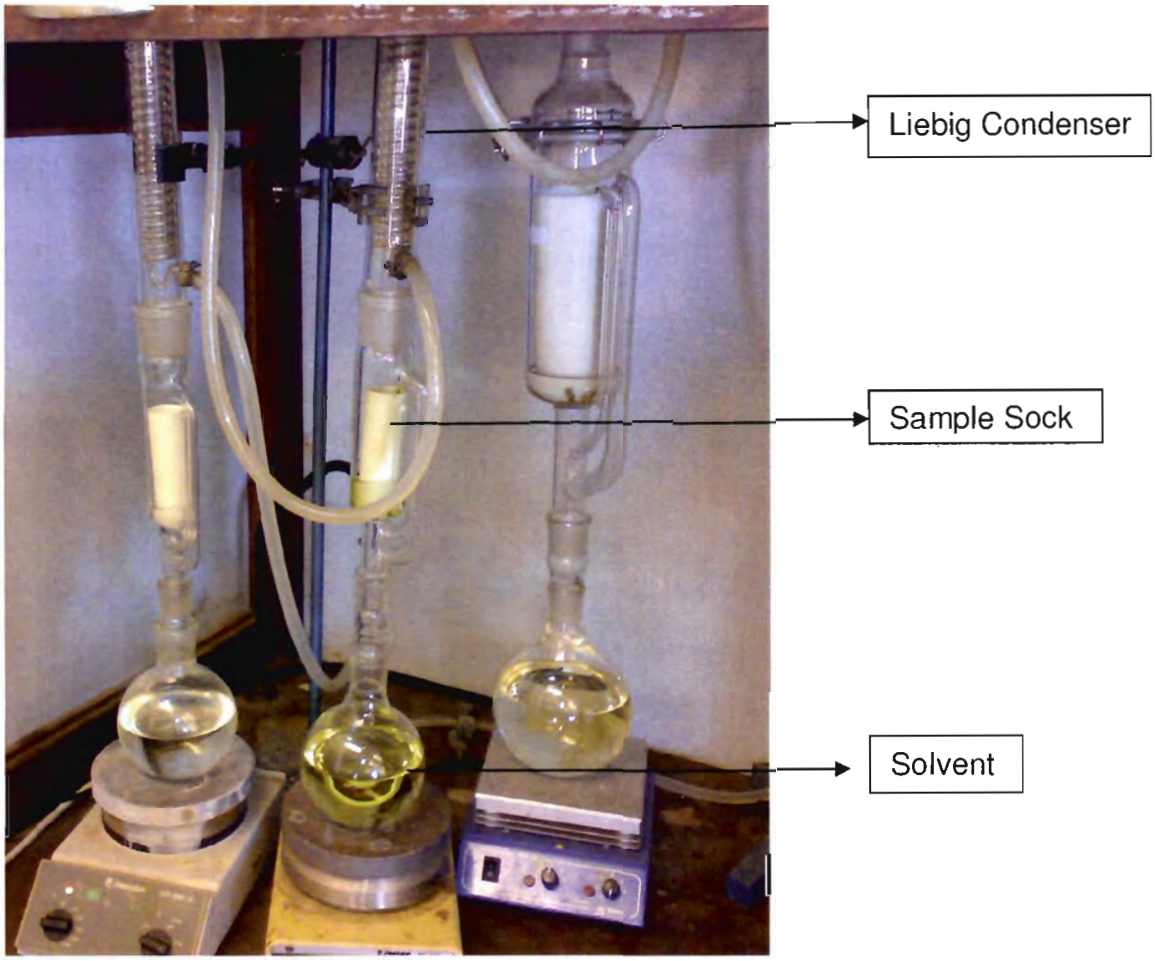

Figure 3.3 Setup for soxhlet extraction

Soxhlet extraction is not environmentally friendly as it adds to pollution because it generates large volumes of contaminated, hazardous solvents and toxic fumes. CO_2, to the contrary, is readily available at low cost and non-toxic, and a clean alternative to conventional solvents for liquid/solid extraction in the supercritical state. The initial cost of a full-scale supercritical carbon dioxide extraction plant is high, but in the long run it is partly offset by lower operating costs [6, 9].

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CHAPTER 4

Experimental Procedures

This chapter describes the methodology and technology used in this investigation and which determined the experimental procedures adopted or developed.

4.1 Materials

The soybean seed was supplied by Dr. A Nel, Agricultural Research Council, Private Bag X804, Potchefstroom 2520.

The compressed air used to power the pneumatics of the supercritical extractor was supplied by Afrox®. UN number: 1002, Item number: 340011, Composition: O_2/N_2. CO_2, also supplied by AFROX®, was of technical grade, UN: number 1013, Item number: 340101.

4.2 Apparatus

4.2.1 Extractor

The supercritical fluid extractor used was a LECO® TFE 2000 complete with M2000 modifier module. The different facilities of this laboratory scale extractor are shown in Figure 4.1.

The unit is ideal for botanical oil extraction in small quantities. The density of the CO_2 can be varied by selecting appropriate values of temperature and pressure of the gas.

The material to be extracted is placed in a thimble. Compressed air is used to activate the pneumatic closing of the thimble chamber once the thimbles are inserted. The extraction parameters are set to the required values, the limits of which are given in Table 4.1. The pump is switched on to compress the CO_2 entered from a separate dipped tube cylinder. The pump cooler pre-cools the CO_2 to 0°C to increase the pump flow capacity. The flow of CO_2 is controlled by heated variable restrictors (HVR). The extracted oil is collected in collection vials after the fluid has been depressurized and released as gas into the atmosphere [1 & 2].

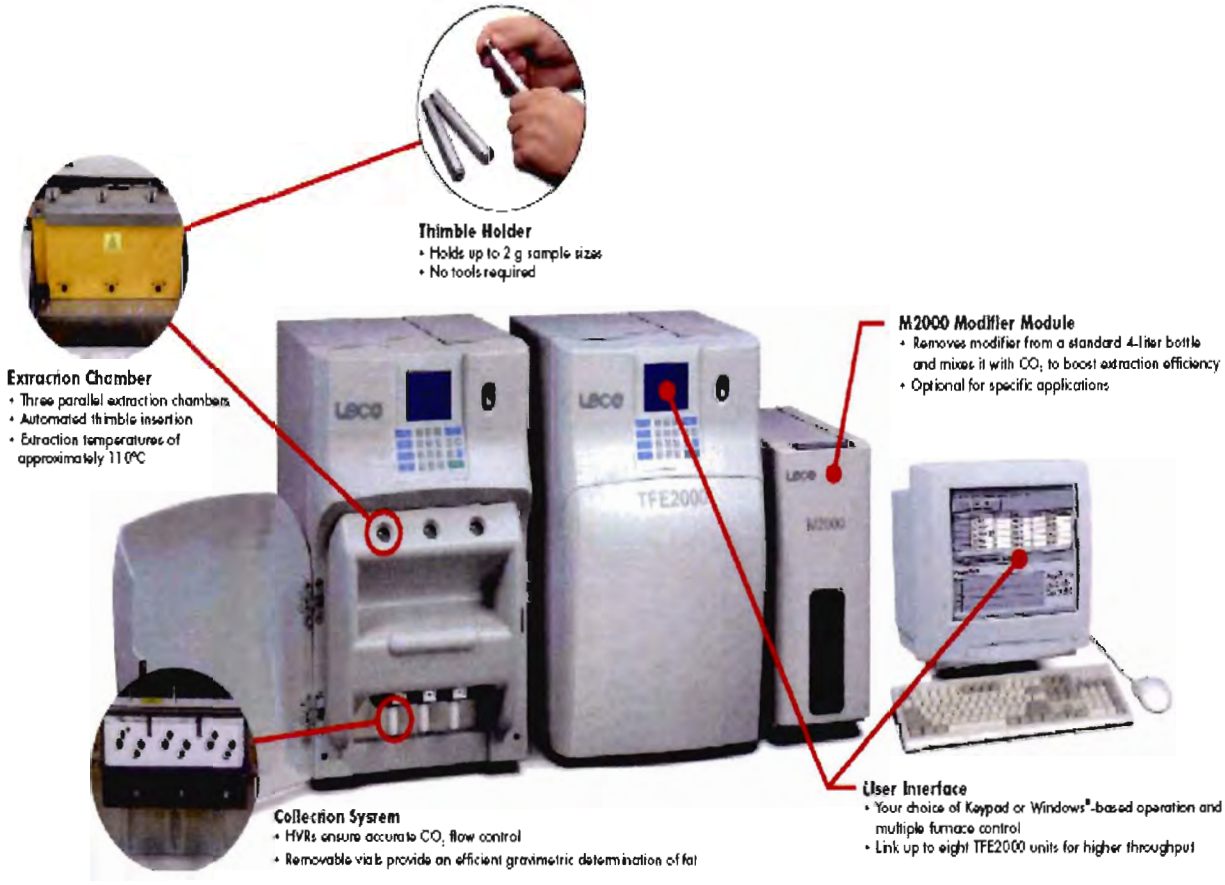

Figure 4.1 Leco TFE 2000® supercritical extractor [1 & 2]

For this study no modifier was used in any of the extractions, since soybean oil is easily extracted with preset parameters and an unnecessary addition of a substance was not desirable in view of the high quality required for the extracted oil.

Table 4.1 Adjustable parameters on LECO TFE 2000 [1 & 2]

Variable	Min Value	Max Value
HVR temperature	0°C	150°C
Sample vessel temperature	0°C	150°C
Pressure	0 atm	680 atm
Static time	0 min	60 min
Dynamic time	0 min	99 min
Flow rate	0.1 L/min	3 L/min

4.2.2 GCxGC/TOF-MS

A LECO Pegasus® 4D GCxGC/TOF-MS was used to identify components in the extracted and commercial soybean oil.

This apparatus is highly suitable for the analysis of complex component rich soybean extracts since it separates components in two different columns with different separation criteria and characteristics. The sample passes through a first column (separation based on boiling point) and then through a thermal modulator into a second column (separation based on polarity). This gives an additional plane in which peaks can be resolved [1 & 3].

Figure 4.2 LECO Pegasus® 4D GCxGC/TOF-MS [1]

4.2.2.1 Modulator

The thermal modulator (which is located between the two columns) creates two trapping zones that focus the sample coming from the first column before it is inserted into the second column. This largely increases analyte detectability [1 & 3].

Figure 4.3 LECO Pegasus® 4D GCxGC/TOF-MS thermal modulator [1]

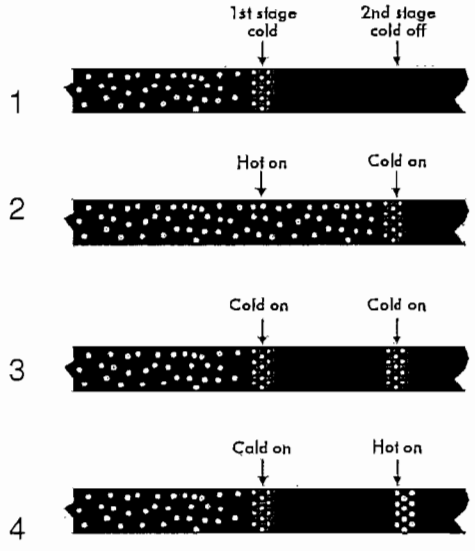

Figure 4.4 LECO two-stage thermal modulator [3]

The 2-stage thermal modulator achieves signal enhancement that cannot be obtained with a normal flow modulator. As can be seen in Figure 4.4 the effluent from the first, longer column (15 - 30 m) enters the thermal modulator and is "trapped" by the first cryo-trap (1). The second cryo-trap holds the sample while the first phase cools again. When the first stage is cooled the second stage releases the effluent by heating up the sample. It is then released into the second phase (2). The cycle samples the "slices" up to 4 times per peak coming from the first dimension. This process focuses the sample to be injected into the second, much shorter column (0.5 - 2m) [1 & 3].

4.2.2.2 Mass spectrometer

The Pegasus 4D® uses TOF-MS (Time of Flight Mass Spectrometry) technology. The main benefits of the technology are drastically reduced analysis time and high data rates (500 spectra/sec) without any quality being lost. The molecules enter the ion source and are fragmented by a beam of bombarding electrons (≈ 70 eV energy). The time of flight (the time it takes to move from the ion source to the detector) is measured. The detection is based on *m/z* ratios so that the heavier the ions the longer the time of flight [1 & 3].

4.2.2.3 Detector

A micro channel plate type electron multiplier detector is used [3].

4.2.2.4 Software

The Pegasus 4D® uses mathematically based True Signal Deconvolution® software that separates overlapping chromatographic peaks as shown in Figures 4.5 and 4.6 [3]. The ion signal plot shows two peaks with apexes at *m/z* = 78 and *m/z* = 119. Because they are only separated by 0,05 s, many GC-MS systems would see them as one component [3].

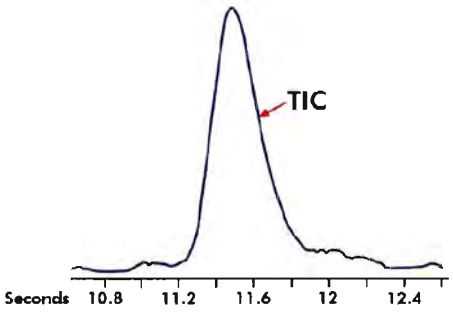

Figure 4.5 Apparently one peak therefore only one component [3].

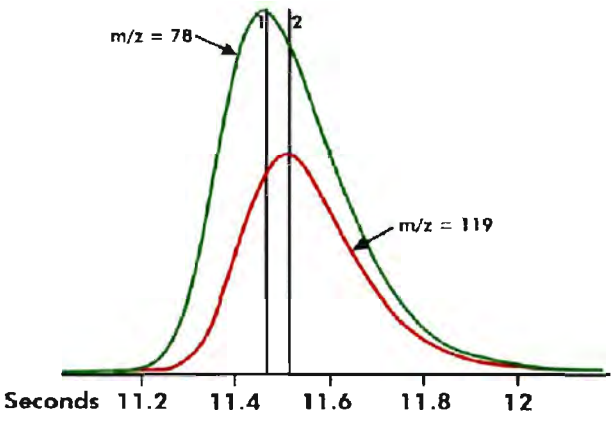

Figure 4.6 Ion signal plot. Two peaks are visible [3].

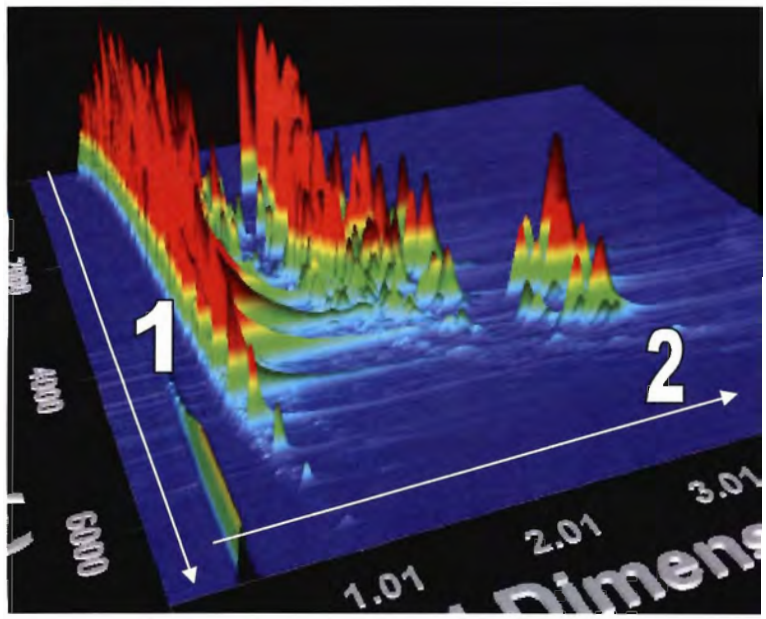

Figure 4.7 Three-dimensional contour plot (arrow 1 shows the direction of the 1st dimension analysis and arrow 2 shows the direction of the 2nd dimension analysis) [1 & 3].

The software displays retention times on the first dimension column as peaks on the X axis and retention times of the second dimension column as peaks on the Y axis as shown in Figure 4.7.

4.3 Methods

4.3.1 Sample preparation

The soybean seed was dehulled by hand and then crushed for improved surface area exposure to sc-CO_2. The particle size was reduced by grinding the soybean seed into a fine powder with a Russell Hobbs® coffee bean grinder to optimise access to the oil by the entering supercritical fluid.

4.3.2 Extractor setup for static runs

The runs in static mode were conducted to measure the solubility of soybean oil in sc-CO_2. All the static runs were done in duplicate to ensure repeatability. The duration of the static runs was varied, and runs were performed with extraction times of 1, 5, 10, 15, 20, 30 and 50 min.

Step by step procedure of static extraction mode:

1. The extractor is switched on and the following parameters are set:
    - Pressure: 300 bar
    - Thimble temperature: 40 °C
    - HVR temperature: 40 °C
    - Dynamic run time: 0 min

2. Both CO_2 and compressed air cylinders are opened. The latter is set to 1800 kPa.
3. An empty collection vial is weighed and the mass noted, and a sample of 1.000 g of finely ground soybean seed is also weighed using a Mettler Toledo® Classic AB204-S mass meter.
4. The sample is placed into a thimble and the thimble cap is inserted to seal off the thimble.
5. The thimble with its contents is inserted into the extraction chamber and the run is started.
6. When the run is completed, the vial with the collected oil is weighed again. The extracted seed is also weighed and both masses are noted.
7. The procedure is repeated for each of the runs of different duration listed above.

4.3.3 Extractor setup for dynamic runs

Extraction in dynamic mode is an exhaustive extraction method to determine the maximum quantity of oil that can be extracted from soybean seed. All the dynamic runs were done in duplicate to test repeatability. The only parameter varied in determining the oil content of the seed was time, and the percentage oil extracted during each run of different duration was noted. Extractions were done for 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 99 min, and the results were plotted onto a graph of percentage oil extracted as a function of time at preselected conditions. A maximum in the concentration-time graph denotes exhaustive extraction and allows the oil content of the seed to be read off from the graph [1 & 2].

Step by step procedure of dynamic extraction mode:

1. The extractor is switched on and the following parameters are set:
    - Pressure: 300 bar
    - Cell temperature: 40 °C
    - HVR temperature: 40 °C
    - Flow rate: 1 L/min
    - Static run time: 0 min

2. The dynamic run time is set in the setup menu.
3. Both CO_2 and compressed air cylinders are opened. The latter is set to 1800 kPa.
4. An empty collection vial is weighed and the mass noted, and a sample of 1.000 g of finely ground soybean seed is also weighed using a Mettler Toledo® Classic AB204-S mass meter.
5. The sample is placed into a thimble and the thimble cap is inserted to seal off the thimble.
6. The thimble with its contents is inserted into the extraction chamber and the run is started.

7. When the run is completed, the vial with the collected oil is weighed again. The extracted seed is also weighed and both masses are noted.
8. The procedure is repeated for each of the runs of different duration listed above.

Data processing and graphs for the static and dynamic runs were done on Microsoft Excel spreadsheets.

4.3.4 Extractor setup for density experiments

A major factor determining the yield of oil is the density of the supercritical fluid. It can be varied by changing the temperature or the pressure (or both) of the gas. The density corresponding to a selected combination of temperature and pressure was calculated using a software package called SF-Solver [4].

Extraction runs of fixed duration and in dynamic mode at constant flow rate were conducted to determine the yield of extracted oil at different combinations of pressure and temperature and thus at different densities. The procedures followed for these runs are summarised below:

1. The same sample preparation as described in Paragraph 4.3.1 was used.
2. The desired temperature and pressure were entered into the setup menu.
3. The flow rate was set to 1 L/min and an extraction time of 60 min was selected.
4. Collection of oil and subsequent measurements were performed in the same way as described in Paragraph 4.3.3.
5. Data were processed using Microsoft Excel.

4.3.5 Sample preparation and setup for GCxGC/TOF-MS analysis

The soybean oil from both sc-CO_2 and soxhlet extractions as well as commercial soybean oil derived from a cold press was diluted with n-hexane in a ratio of 1:10. The diluted samples were put into the autosampler of the Pegasus 4D®.

Table 4.2 GCxGC/TOF-MS setup analysis

Setup for GCxGC	
Injection volume	1 µL
Primary column	HP5 (30)
Secondary column	RXI (17)
Carrier gas	Helium
Front inlet mode	Split
Front inlet flow	1 mL/min
Front inlet temperature	200 °C
Modulator temperature offset	20 °C
Second dimension separation time	3 s

Setup for TOF-MS	
Start mass	35 u
End mass	350 u
Spectra per second	10
Detector Voltage	1600 V
Electron energy	- 70 V
Ion source temperature	230°C
Signal to noise ratio	500
Library	Mainlib
	Replib

The temperature for the primary oven was set at 50℃ for 2 min, with a temperature ramp from 10℃ to 300℃ and left for 4 min. The temperature for the secondary oven was set at 60℃ for 2 min, with a 10℃ ramp to 310℃ and left for 4 min.

All analyses were done in both 1-dimensional GC and in 2-dimensional GCxGC modes. The 2-dimensional analysis requires liquid nitrogen in the modulator for cooling of the secondary column. All analyses were done at the same conditions with the only variable being the extraction method. The data analysis was done with ChromaTOF® software.

4.3.6 Soxhlet extraction

1. The sample of soybean seed was prepared in the same way as for the sc-CO_2 extractions.

2. An amount of 30.000 g of ground soybean seed was weighed and put into the sock for extraction.

3. n-Hexane AR was used for extraction.

4. The soxhlet extraction was run for 48 hours.

5. The n-hexane saturated with extract was continuously replaced and transferred to a flat-bottom flask.

6. After 48 hours the mixture of extracted soybean and n-hexane was evaporated using a BÜCHI LABOR TECHNIK Rotavapor II.

7. The extracted oil was left in an oven at 40℃ for another 48 hours to ensure that all n-hexane has evaporated.

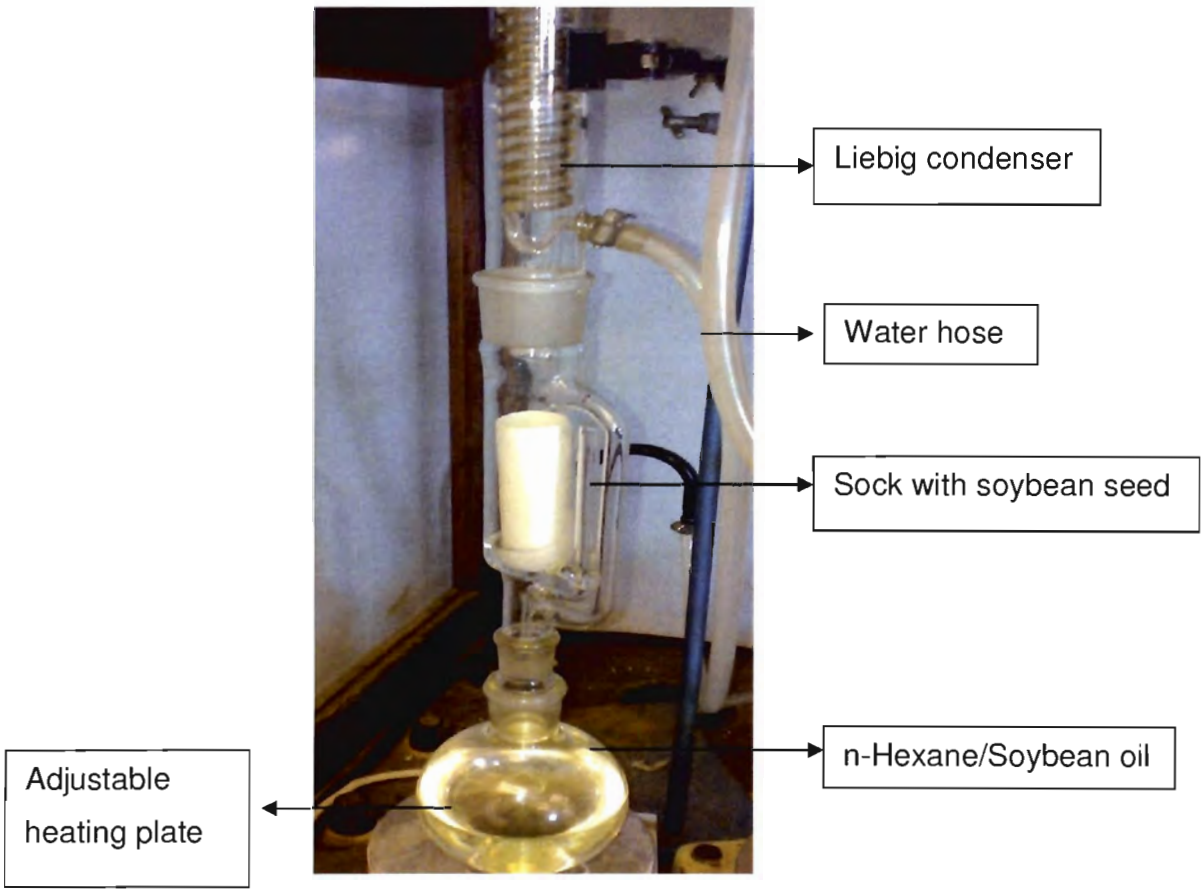

Figure 4.8 Setup for soxhlet extraction

4.3.7 Setup for GC-FID analysis

Soybean oil samples were sent to Sylvia Odendal for GC-FID analysis at Nola® Industries in Randfontein. The method used is outlined in Table 4.4.

Table 4.4 Setup for GC-FID analysis

Sample description	Soya oil
Concentration	1:1
Solvent	*n*-Hexane
Volume injected	1 µL

Column type	Omega wax 320, 30 m, 0.32 ID, 0.25 film thickness (Supelco)
Carrier gas	Helium
Carrier gas flow	2.1 mL/min
Make up gas	N_2
Make up gas flow	25 mL/min
Oven temp. program	Yes
Initial temperature	100 °C
Initial hold	5 min
Program 1 rate	8 °C/min
Program 1 final	220 °C
Program 1 hold	10 min
Program 2 rate	10 °C/min
Program 2 final	320 °C
Program 2 hold	15 min
Detector (Auxiliary) (B)	FID
Hydrogen flow	33 mL/min
Synthetic air flow	460 mL/min
Injection mode	Split
Split flow	200 mL/min

4.3.8 Chemical analysis

The following chemical analyses were done on the soybean oil sample: peroxide value, iodine number and moisture content.

4.3.8.1 Peroxide value

The peroxide value is an indication of the current state of oxidation of the oil. Reagents used [5]:

- Acetic acid/chloroform 2:3
- Saturated KI solution
- 1% starch solution
- 0.01 N thiosulphate

Method [5]:

Weigh 5.000 – 5.050 g of sample in an Erlenmeyer flask.
Add 30 mL of acetic acid/chloroform solution.
Stir flask until sample is dissolved.
Immediately add 0.5 mL saturated KI solution.
Leave the sample for exactly 1 min.
Add 30 mL distilled water.
Use 0.5 mL 1% starch solution as indicator and titrate with 0.01 N thiosulphate. The titration has to be repeated if the titration uses less than 0.5 mL.
Take the reading.

Calculation [5]:

$$\text{Peroxide value (as milli-equivalent)} = \frac{thiosulphate(mL) \times normality \times 1000}{sample(g)}$$

$$= \frac{titre \times 10}{sample(g)}$$

4.3.8.2 Iodine number using Wijs method

This value reflects the unsaturated oil or fat content in terms of the amount of iodine absorbed per centigram. Reagents used [6]:

Stock solution – 100 g monochloride in 315.5 mL glacial acetic acid
Wijs work solution – 100 mL stock solution + 1.830 L glacial acetic acid
Potassium iodide (KI) – 75 g in 250 mL distilled water
Starch solution
Glacial acetic acid
Carbon tetrachloride

Methods [6]:

Accurately weigh 0.18 g - 0.24 g sample in a 250 mL glass flask.
Add 20 mL carbon tetrachloride and 25 mL Wijs solution.
Seal the solution and mix properly.
Leave in dark room for 30 min.
Add 20 mL KI solution and 100 mL distilled water.
Titrate with 0.1 N thiosulphate until yellowish.
Add a few drops of starch solution. Solution becomes black.
Titrate until the solution becomes white.
Take the reading.

Calculation [6]:

$$\text{Iodine value} = \frac{(B-S)\times N\times 12.69}{\text{sample mass (g)}}$$

B – titration of blank
S – titration of sample
N – normality of thiosulphate (0.1 N)

4.3.8.3 Moisture content

This value indicates the moisture content and volatile substances in the oil sample. Method [7]:

Weigh container with lid – *W*1.
Add 5.0 g of sample and weigh it again – *W*2.
Put the open container in an oven at 130°C for one hour.
Close the lid and put the container in a desiccator for one hour to cool down.
Weigh the container again – *W*3.

Calculation [7]:

$$\%Moisture = \frac{(W2 - W3) \times 100}{W2 - W1}$$

References

1. **[Web]**: *Official web site of Leco®*. http://www.leco.com/ [Date of access: 31 October **2008**].

2. DAVIES, R. *TFE 2000 Instruction Manual.* Kempton Park, South Africa, **2002**.

3. Software supplied with LECO® Pegasus 4D GC-GC/TOF-MS instrument.

4. SF-Solver. Software for Supercritical Fluid Analysis, Copyright Isco Inc., 1995.

5. Nola industries, Official Controlled Document Oil 7, Method Cd8-53 A.O.C.S. **2001**, April 19.

6. Nola industries, Official Controlled Document Oil 8, **2001**, April 19.

7. Nola industries, Official Controlled Document Oil 9, **2001**, April 19.

CHAPTER 5

Results and Discussion

Experimental results, processed data, interpretation of data and conclusions on account of observations and measurements are all covered in this chapter. It is the core chapter of this dissertation as it embraces all experiments done to achieve the goals of the research project.

5.1 Solubility of soybean oil in sc-CO_2

The determination of the solubility of soybean oil in sc-CO_2 was done in static mode. In this mode of operation of the supercritical extractor the seed was exposed to a fixed aliquot of sc-CO_2 at specific conditions without fresh fluid entering the thimble. If the duration of exposure is sufficiently long, the fixed amount of sc-CO_2 becomes saturated with dissolved oil.

The optimum time of exposure is best achieved by performing several static runs of different duration, and to plot the corresponding amount of oil dissolved as a function of time as shown in Figure 5.1. The maximum of the resulting graph depicts the highest concentration of oil prevailing in the fixed amount of sc-CO_2 and thus the solubility of the oil in sc-CO_2 at the conditions concerned.

After the static run, the saturated sc-CO_2 is depressurised in the HVR of the apparatus. The fluid is converted back into the gaseous state, as a result of which it loses its solvent strength and causes the dissolved oil to be separated

and collected in the collection vial. The mass of oil collected is the amount of oil soluble in sc-CO_2 at the specific conditions.

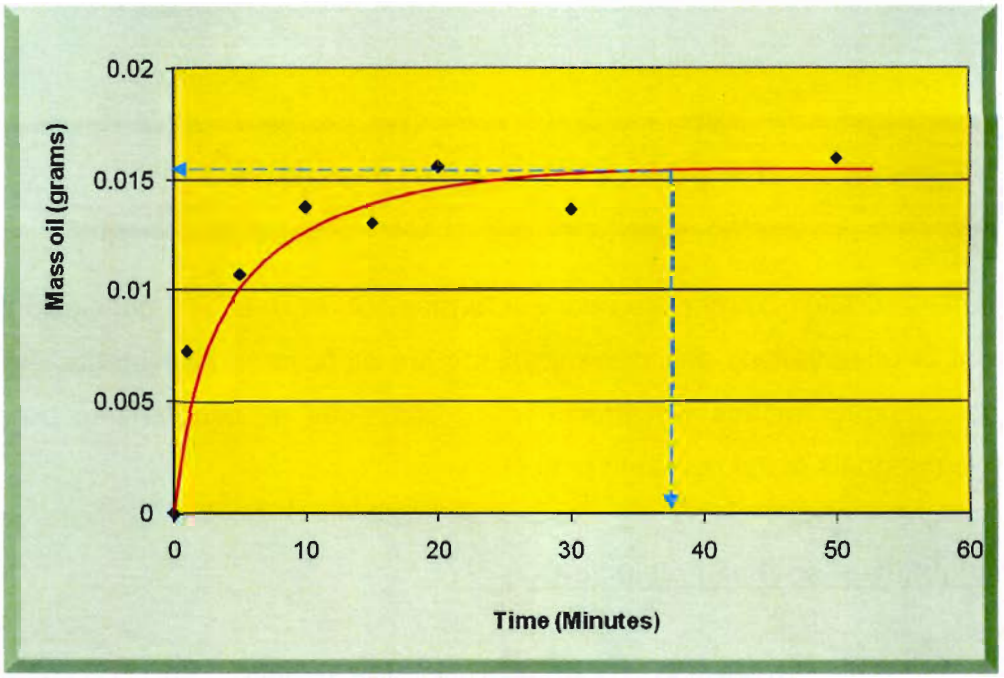

Figure 5.1 Yield-time curve, static runs (300 bar and 40°C)

The mass of oil collected should correlate with the decrease in mass of the plant material in the thimble. The extent to which such mass balance exists, indicates the reliability of the solubility determination.

Mass oil extracted from 1 g of soybean seed by static run = 0.0155 g

Saturation point was reached after 38 min (Figure 5.1).

The solubility of soybean oil in sc-CO_2 is determined as follows:

Density of CO_2 at 300 bar and 40°C is 0.917 g/mL (from SF-Solver)

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

= (10 - 1.5) mL

= 8.5 mL

Mass of the CO_2 = Density of CO_2 x Volume CO_2

= 0.917 g/mL x 8.5 mL

= 7.795 g

Solubility = (0.0155 g oil) / (7.795 g CO_2) = 0.00198 g oil / 1 g CO_2 at 300 bar and 40°C.

Therefore 1 g of sc-CO_2 at a density of 0.917 g/mL is capable of dissolving 0.00198 g of soybean oil.

5.2 Oil content of seed

An exhaustive extraction is needed if the oil content of the soybean seed should be determined. This is best achieved by employing the dynamic mode of extraction, which means that fresh sc-CO_2 is constantly flowing through the thimble and its contents while dissolving the oil.

On restoring ambient conditions after such time that no more oil can be extracted from the seed a maximum in the graph depicts the maximum amount of oil that can be extracted and thus depicts the oil content of the seed.

The best way to achieve this is to perform a number of dynamic runs of different duration and to plot the corresponding yield of oil as a function of extraction time as shown in Figure 5.2.

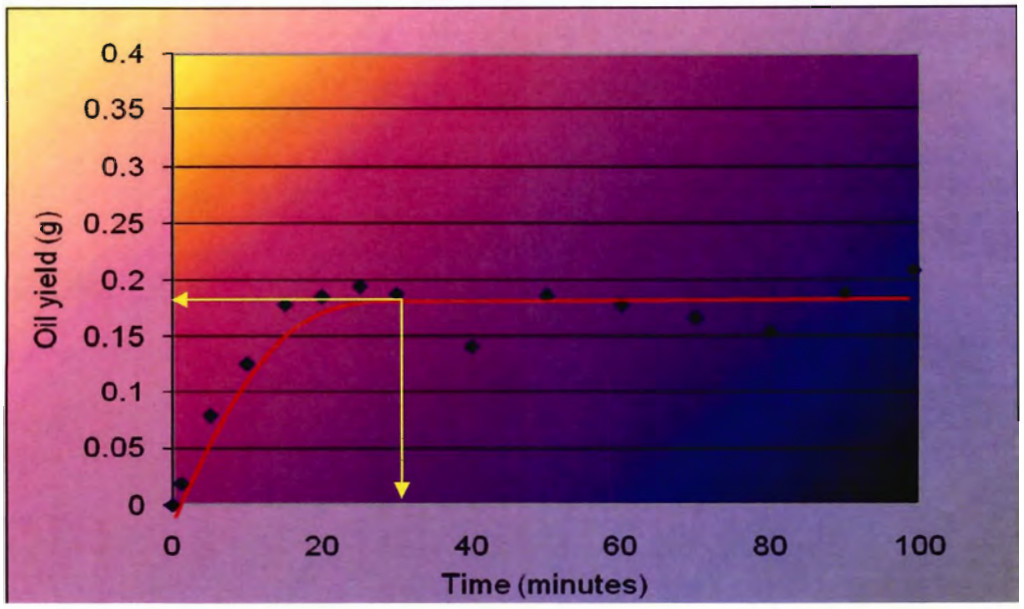

Figure 5.2 Yield-time curve, dynamic runs (300 bar, 40℃, 1 L/min)

It can be seen in Figure 5.2 that all of the oil is extracted after 30 min. The mass of oil collected after 30 min of dynamic extraction is 0.1872 g from a 1 g sample, giving a yield of 18.7%. This correlates quite well with the yield of 18.5% by cold press extraction done by the Agricultural Research Council on the same seed. Yields of 19.6% and 19.9% were obtained in other studies [1, 2] where freshly harvested soybean seed was probably used.

The temperature (40˚C) used in the extraction runs in Figures 5.1 and 5.2 is relatively low compared to typical temperatures at which soxhlet extractions are performed. High temperatures (above 80℃) can damage the proteins and fatty acids in the oil.

5.3 Density as yield determining parameter

The density of sc-CO_2 is crucially important for efficient extraction. For densities between 0 and 0.7 g/mL sc-CO_2 behaves more like a gas and thus has low

solvent strength, but at densities between 0.7 and 1 g/mL sc-CO_2 behaves like a highly mobile liquid with solvent strength comparable to good solvents. This results in an exponential increase in the yield of extracted oil as the density varies from gas-like to liquid-like values. The variation in density is accomplished by varying the pressure and temperature and is one of the most striking features of sc-CO_2 as an extraction agent since solvent strength can be manipulated to turn the fluid into a selective solvent for a variety of substances. The runs in Figure 5.3 were all done in dynamic mode and the effect of density and thus solvent strength shows that the principal mechanism of extraction is chemical dissolution.

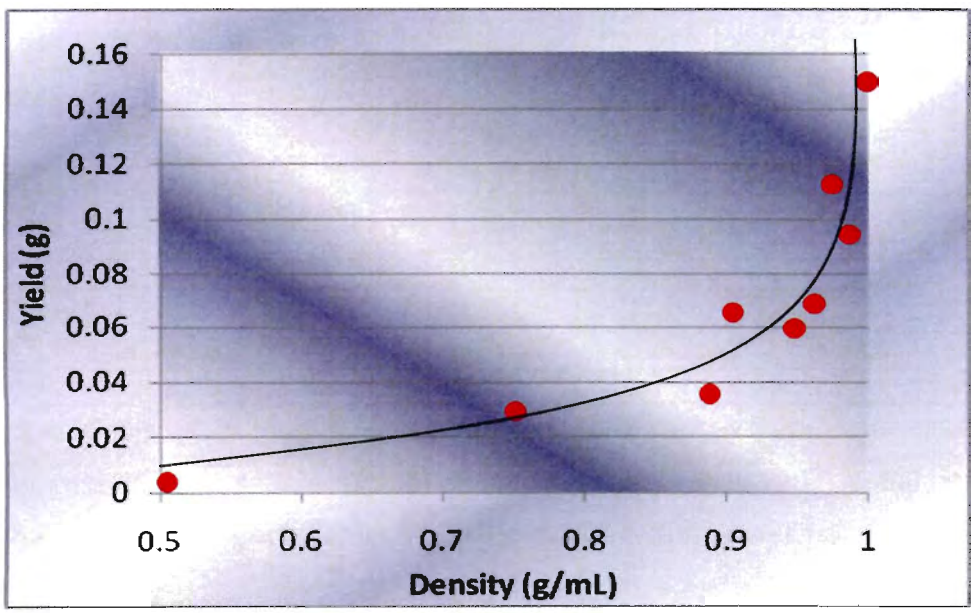

Figure 5.3 Yield-density curve showing exponential increase in oil yield as sc-CO_2 approaches liquid-like densities.

5.4 Analysis of extracted oil

5.4.1 GC-FID analysis

The oil extracted by sc-CO_2 in this investigation was analysed by GC-FID as shown in Figure 5.4, and the corresponding results are listed in Table 5.1 and compared to fatty acid analysis found in the literature [1, 2] in Table 5.2.

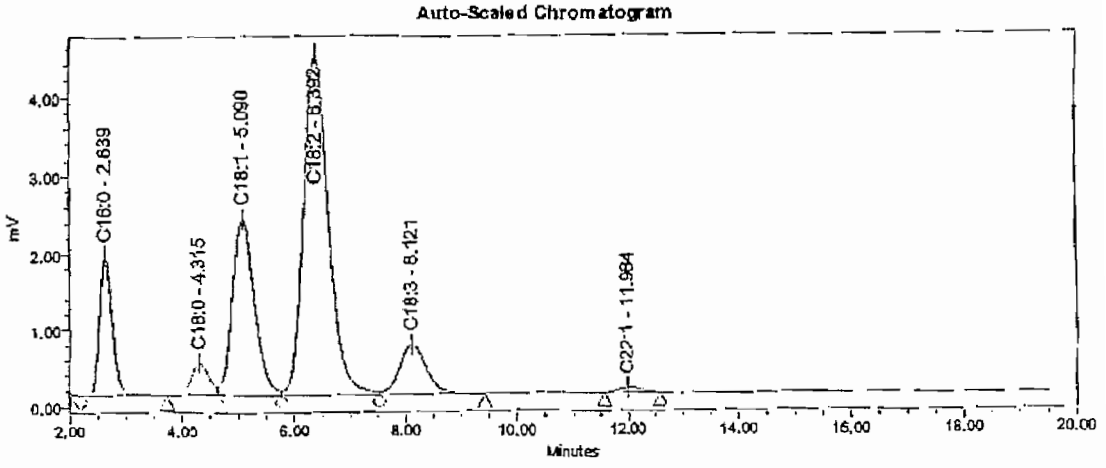

Figure 5.4 Chromatogram of sc-CO_2 extracted soybean oil.

Six peaks are observed in the chromatogram (Figure 5.4) along with their retention times. The substances responsible for these peaks have been identified by their retention times and associated mass data along with their respective percentages present in the extracted oil (Table 5.1).

Tables 5.1 and 5.2 show that the two soybean oil samples are quite similar. The same components are detected in both. The relative amounts of the components in the two cases correspond well. The extent of mutual agreement strengthens confidence in the results obtained in this investigation.

Table 5.1 Fatty acid profile of sc-CO_2 extracted soybean oil in this investigation

No.	Name	Retention time	% Area
1.	C16:0 Palmitic acid	2.639	11.17 %
2.	C18:0 Stearic acid	4.315	3.54 %
3.	C18:1 Oleic acid	5.090	24.05 %
4.	C18:2 Linoleic acid	6.392	52.32 %
5.	C18:3 Linolenic acid	8.121	8.28 %
6.	C22:1 Behenic acid	11.984	0.64 %
Sum			100 %

Table 5.2 Fatty acid profile of sc-CO_2 extracted soybean oil in the literature [1,2]

No.	Name	% Area
1.	C16:0 Palmitic acid	11.0% - 11.8 %
2.	C18:0 Stearic acid	3.5% - 4.2 %
3.	C18:1 Oleic acid	23.7% -27.5%
4.	C18:2 Linoleic acid	51.8 %– 52.0%
5.	C18:3 Linolenic acid	6 % - 6.5%

5.4.2 Chemical analysis

Chemical analysis of the sc-CO_2 extracted oil included determination of the iodine number, peroxide value, free fatty acid content and moisture content. The values are listed in Table 5.3 along with the corresponding values of a soybean oil standard obtained by the expeller pressed method [4].

The iodine number of 132.6 g I_2/100 g oil is acceptable as it falls within the specific range for commercial soybean oil.

The peroxide value of 14.8 meq O_2/kg, is higher than the acceptable value, probably since extraction over a period of three weeks resulted in prolonged exposure of the extracted oil to the atmosphere and thus oxidation of substances within the oil. This value can be reduced by passing an inert gas (e.g. N_2) through the oil and then keeping it in a closed vessel in the dark. The peroxide value of standard soybean oil exposed to the atmosphere for 30 days increased to 4.8 meq O_2/kg indicating that oxidation, however limited, indeed occurs on exposure to air [1]. The vast difference in peroxide value is, however, an aspect to which more attention should be given in future studies.

Table 5.3 Analysis and comparison of sc-CO_2 extracted soybean oil [4]

	sc-CO_2 extracted soybean oil	expeller pressed soybean oil
Iodine number (g I_2 / 100 g oil)	132.6	124 – 139
Peroxide value (meq O_2 / kg)	14.8	2.3
Free fatty acid	0.36	0.04
Moisture content	0.02	-

The free fatty acids in expeller pressed oil are usually neutralised in a subsequent refining step prior to marketing, explaining the low free fatty acid content listed in Table 5.3. The corresponding value for sc-CO_2 derived oil is indeed quite low if taken into account that no neutralisation was done on this oil.

The degree of mutual correspondence of the entries in Table 5.3 broadly reflects the effectiveness of sc-CO_2 extraction as a suitable method for botanical samples when compared to the well established traditional expeller press method.

5.4.3 GCxGC/TOF-MS analysis

GCxGC/TOF-MS analysis was done on three differently obtained soybean oil samples, namely by (1) sc-CO_2 extraction, (2) soxhlet extraction and (3) expeller press (commercial). Although comparisons between different extraction methods are found in the literature [5], specifically GCxGC/TOF-MS analysis is used in this investigation to do such a comparison.

Table 5.4 lists 113 compounds identified by virtue of the database supplied with the instrument when a sample of sc-CO_2 extracted soybean oil was injected. These compounds, however, do not all stem from the oil itself but are silylated compounds either due to partial decomposition of the GC column as seen by the column bleed in the resulting two-dimensional chromatogram or incorrect use of the library which provides no fewer than ten possibilities for each observed peak.

The compounds highlighted (yellow) in the table are those also found in the soxhlet extracted and commercial samples.

Table 5.4 List of compounds identified with GCxGC/TOF-MS

Peak #	Name	R.T. (s)	Similarity	UniqueMass	Quant Masses	S/N	Area
1	Pentane, 3-methyl-	396 , 0.520	843	57	57	9328.9	29384682
*2	Cyclotrisiloxane, hexamethyl-	396 , 0.580	815	207	207	603.54	119617
*3	Cyclopentasiloxane, decamethyl-	399 , 0.860	875	73	73	436.51	825150
*4	Octane	402 , 0.580	765	85	85	3045.1	518925
*5	Cyclohexasiloxane, dodecamethyl-	402 , 1.330	865	73	73	1483	6723389
7	Cyclohexane, 1,3-dimethyl-, cis-	405 , 0.600	832	97	97	356.47	46313
*29	p-Xylene	468 , 0.680	959	106	106	2840.1	407366
33	Cyclohexane, 1,2,3-trimethyl-	474 , 0.610	817	84	84	249.43	32334
*34	Cycloheptanone, 4-methyl-, (R)-	480 , 0.610	846	83	83	700.46	147835
*38	Nonane	489 , 0.630	932	85	85	37971	9305097
39	Cyclohexylmethanol, trifluoroacetate (ester)	489 , 0.650	659	97	97	404.49	94593
47	cis-1-Ethyl-3-methyl-cyclohexane	504 , 0.620	757	97	97	241.94	34887
49	Heptane, 2,4,6-trimethyl-	507 , 0.600	804	85	85	340.44	44776
*52	Octane, 3,5-dimethyl-	513 , 0.600	905	57	57	1161.6	437994
57	Ethanol, 2-butoxy-	525 , 0.680	943	57	57	6012.2	8618015
65	Pentasiloxane, dodecamethyl-	537 , 1.300	672	73	73	603.43	1856690
*68	Decane	546 , 0.610	864	85	85	2716	516965
*69	Decane, 2,5-dimethyl-	549 , 0.610	906	57	57	8933.1	8359127
73	Benzene, 1-ethyl-4-methyl-	555 , 0.710	945	105	105	583.12	184879
74	Tridecane, 7-methyl-	558 , 0.620	916	71	71	7903.8	2534732
84	Cyclodecane	576 , 0.630	823	55	55	528.19	444758
*89	Hexane	585 , 0.500	842	56	56	1778.9	2361710
91	Benzene, 1,2,3-trimethyl-	588 , 0.720	960	105	105	868.11	241690
*104	Undecane	612 , 0.620	843	57	57	364.04	111551
109	1-Octanol, 2-butyl-	618 , 0.620	832	57	57	304.49	182053
117	Indane	630 , 0.770	892	117	117	272.42	42027
*125	Decane, 4-methyl-	642 , 0.630	909	71	71	663.43	205836
126	Benzene, 1-methyl-3-propyl-	642 , 0.720	910	105	105	581.56	186046
135	Decane, 3-methyl-	651 , 0.630	893	71	71	486.12	115895
144	(2-Methylbutyl)cyclohexane	669 , 0.650	811	97	97	289.73	76564
*146	Benzene, 1-ethyl-2,4-dimethyl-	669 , 0.730	908	119	119	762.05	176532
172	Decane, 3,7-dimethyl-	705 , 0.630	890	71	71	534.73	207973
180	Cyclopentane, hexyl-	720 , 0.660	868	83	83	402.93	135662
184	Benzene, 1,3-diethyl-5-methyl-	726 , 0.720	821	119	119	213.78	27770
191	Undecane, 2-methyl-	738 , 0.630	924	71	71	712.01	292907
198	Sulfurous acid, butyl decyl ester	744 , 0.640	891	57	57	849.19	219791
202	Benzene, 1-methyl-4-(1-methylpropyl)-	747 , 0.730	901	119	119	255.8	45202
214	Cyclotetradecane	765 , 0.660	841	97	97	269.77	141904
218	1,3,5-Tris(trimethylsiloxy)benzene	774 , 0.090	528	327	327	300.45	261859
*219	Naphthalene	774 , 0.840	924	128	128	1387.6	231935
221	Benzene, (1,2-dicyclopropyl-2-phenylethyl)-	780 , 0.760	817	131	131	211.92	39081
224	Undecane, 4,6-dimethyl-	783 , 0.630	905	57	57	1136.4	639045
226	Heptadecane, 2,6,10,14-tetramethyl-	789 , 0.640	876	71	71	293.39	42599
*228	Thiophene, tetrahydro-, 1,1-dioxide	789 , 1.060	863	120	120	2115.2	465759

235	4-Tridecene, (Z)-	801 , 0.660	872	69	69	276.88	84436
240	E-2-Octadecadecen-1-ol	810 , 0.670	808	83	83	382.13	79891
243	2,3-Dimethyldecane	816 , 0.640	858	57	57	470.06	238782
246	Pentadecane, 7-methyl-	819 , 0.640	871	71	71	395.93	121129
*250	Dodecane, 2-methyl-	825 , 0.640	896	57	57	548.51	239633
253	Heptadecane, 2,6-dimethyl-	831 , 0.640	892	71	71	1102	221293
*266	Tridecane	855 , 0.640	933	57	57	2835.4	1101189
271	Oxime-, methoxy-phenyl-_	861 , 0.540	844	151	151	173.17	15408
273	Dodecane, 2,6,11-trimethyl-	864 , 0.640	902	57	57	247.86	79250
*277	Naphthalene, 1-methyl-	870 , 0.830	854	142	142	602.66	85224
292	Undecane, 2,3-dimethyl-	897 , 0.640	836	57	57	245.37	120536
293	Heptylcyclohexane	897 , 0.670	812	83	83	257.83	46373
297	Tridecane, 2-methyl-	906 , 0.640	922	57	57	275.93	70254
305	Dodecane, 2,6,10-trimethyl-	915 , 0.640	923	71	71	366.4	74400
307	1,2-Diphenyltetramethyldisilane	915 , 2.750	580	135	135	212.09	158866
315	Silane, trimethylphenyl-	930 , 2.560	545	135	135	343.37	592407
318	Tetradecane	933 , 0.650	931	57	57	571.83	200291
*393	*n*-Hexadecanoic acid	1038 , 1.190	898	60	60	221.09	*129064*
455	Tributyl phosphate	1116 , 0.780	777	99	99	275.61	24920
*484	9,12-Octadecadienoic acid, methyl ester, (E,E)-	1155 , 1.260	822	67	67	202.03	123421
*516	Erucic acid	1191 , 1.110	805	55	55	200.77	218542
517	9,12-Tetradecadien-1-ol, (Z,E)-	1191 , 1.150	755	67	67	252.16	349734
524	9-Octadecynoic acid	1200 , 1.120	770	81	81	243.11	282668
535	Propanephosphonic acid, bis(trimethylsilyl) ester	1215 , 0.780	615	253	253	327.26	51652
*547	Hexadecenoic acid, Z-11-	1227 , 1.030	824	55	55	235.59	489899
*564	Oleic Acid	1245 , 0.990	787	55	55	268.72	737090
*565	ç-Tocopherol	1245 , 2.950	861	151	151	335.49	502911
567	1,E-11,Z-13-Octadecatriene	1248 , 1.010	767	67	67	324.7	814134
575	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	1257 , 0.740	537	253	253	403.59	206226
604	1,3-Dioxolane, 2-heptyl-	1284 , 0.530	615	327	327	153.51	46841
674	Phthalic acid, di(3,4-dimethylphenyl) ester	1350 , 1.400	604	253	253	226.63	41567
696	Phthalic acid, 3,5-dimethylphenyl 4-methoxyphenyl ester	1368 , 1.340	578	253	253	213.47	38823
707	2-n-Propyladamantane	1380 , 0.850	558	327	327	196.87	36545
*708	Mercaptoacetic acid, bis(trimethylsilyl)-	1380 , 0.960	590	221	221	50.273	5183
712	Silane, trimethyl[2-methylene-1-(4-pentenyl)cyclopropyl]-	1383 , 0.520	665	327	327	434.93	45793
737	7-Octylidenebicyclo[4.1.0]heptane	1410 , 0.820	600	209	209	165.15	45818
*746	Tetradecanoic acid	1419 , 0.670	703	60	60	774.16	727898
754	1-Adamantanecarboxylic acid, 2-methylphenyl ester	1428 , 0.800	591	209	209	155.4	48526
794	4-Benzyloxy-3-methoxyphenylacetonitrile	1464 , 1.090	607	253	253	204.6	30702
802	1-Isobutyladamantane	1470 , 0.760	562	415	415	56.44	5980.9
803	Undecenoic acid, trimethylsilyl ester	1470 , 1.140	611	131	131	227.81	119952
813	Quassin	1473 , 1.780	501	373	373	202.72	26245
825	Cyclopropane, 1-(2-methylbutyl)-1-(1-methylpropyl)-	1485 , 0.800	658	116	116	345.98	46552
830	Hexane, 2,5-bis[(trimethylsilyl)oxy]-	1488 , 0.890	640	117	117	211.8	43056
*837	Vitamin E	1491 , 2.380	698	165	165	259.62	68270
840	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	1494 , 1.090	637	131	131	273.75	145937
842	12-Hydroxystearic acid	1497 , 0.690	518	403	403	313.64	37308
849	o-Anisic acid, 3-methylphenyl ester	1503 , 0.680	512	403	403	321.67	37961
*865	Oleic acid, trimethylsilyl ester	1512 , 1.040	608	131	131	295.31	135768
872	1H-Indene, 1-hexadecyl-2,3-dihydro-	1518 , 0.850	609	130	130	199.52	40448
874	9-Tetradecenoic acid, trimethylsilyl ester	1518 , 1.040	612	131	131	381.51	177414
882	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethenyl)-	1527 , 0.610	544	327	327	2477	2308205
904	1-Adamantanecarboxylic acid, 2-tridecyl ester	1545 , 0.660	523	403	403	561.95	116388
910	9,12-Octadecadienoyl chloride, (Z,Z)-	1548 , 0.850	886	262	262	83.828	8308.8
939	Butane, 1-(methylsulfinyl)-	1569 , 0.420	609	64	64	232.95	103153
951	Retinoic acid, 5,6-epoxy-5,6-dihydro-, trimethylsilyl ester	1572 , 1.420	501	179	179	177.08	28866
960	Silane, methylenebis[dimethyl-	1578 , 0.810	609	117	117	371.07	97267
961	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	1578 , 0.960	641	131	131	655.13	294485
983	3-Chloro-2-methylcyclopent-2-enol	1590 , 0.960	622	131	131	497.24	275990
995	2-t-Butyl-3-methyl-1-(toluene-4-sulfonyl)imidazolidin-4-one	1596 , 0.930	576	331	331	87.539	37399
1020	1,2-Benzenedicarboxylic acid, diisooctyl ester	1611 , 0.960	716	149	149	1148.4	172619
1064	Olean-12-ene-3,28-diol, (3á)-	1635 , 0.830	578	204	204	343.75	25659
*1065	4,7-Octadecadiynoic acid, methyl ester	1635 , 0.960	630	131	131	534.53	214228
*1117	Arachidonic acid, trimethylsilyl ester	1668 , 0.960	658	131	131	1070.1	415338
1125	1H-Indole, 3-[[(phenylmethyl)thio]methyl]-	1674 , 0.920	548	130	130	772.73	186335
1140	11,14-Eicosadienoic acid, trimethylsilyl ester	1683 , 0.970	680	117	117	610.02	376161
1194	1-Benzyl-5-nitro-1H-benzoimidazole	1713 , 0.930	565	331	331	175.97	45840
1213	Leucinylglycine hydrazine, N-[2,4-dinitrophnyl]-	1725 , 0.930	582	331	331	174.75	83462

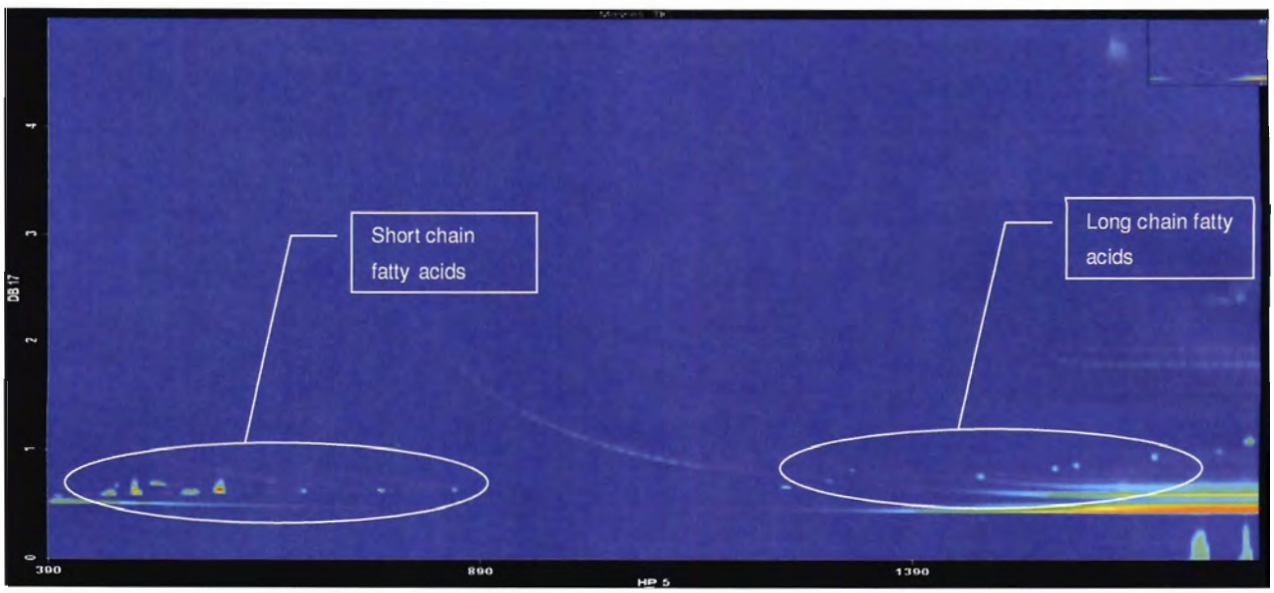

Figure 5.5 Contour plot of sc-CO_2 extracted soybean oil (113 compounds)

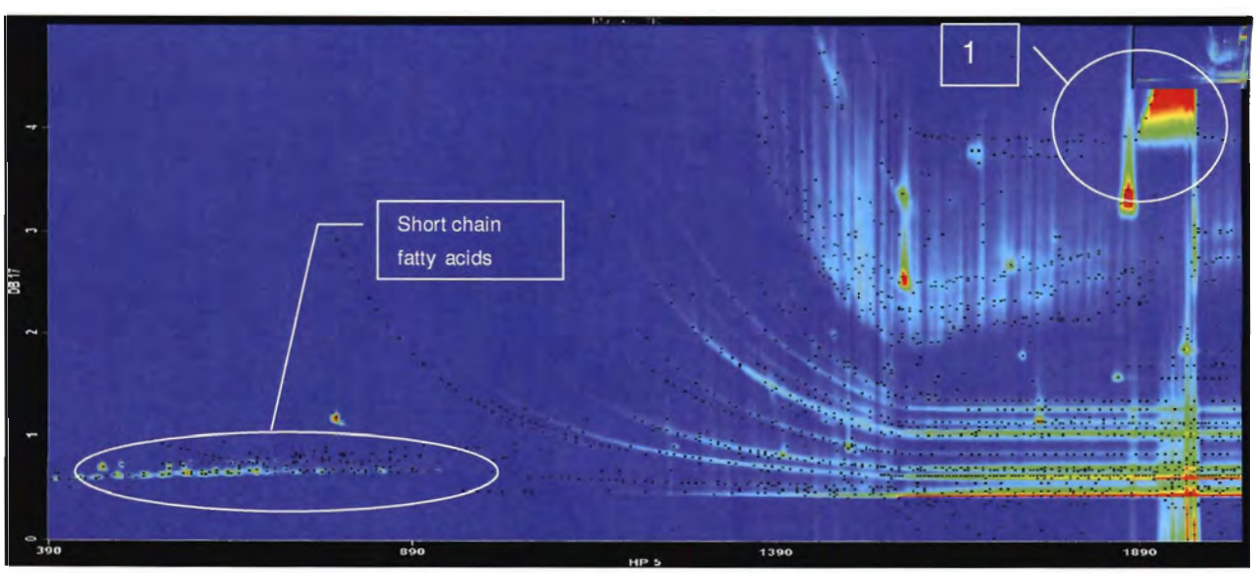

Figure 5.6 Contour plot of soxhlet extracted soybean oil

As shown in Figures 5.5 and 5.6, sc-CO_2 is capable of extracting short and long chain fatty acids as effectively as soxhlet extraction, but with the added advantages of being a faster and environmentally friendlier method. The

highlighted area (1) on the contour plot of the soxhlet extraction shows a fairly large amount of solvent still present in the extracted oil even after the extract was evaporated for 24 hours.

Figure 5.7 shows a comparison of the contour plots of commercial (above) and sc-CO_2 derived soybean oil (below). Some similarity between the two, though the relative number of components is seems to be higher in sc-CO_2 derived oil. The *intensity* of the commercial oil contour plot had to be increased to make the components visible, explaining why the column bleed is much more visible in the first contour plot.

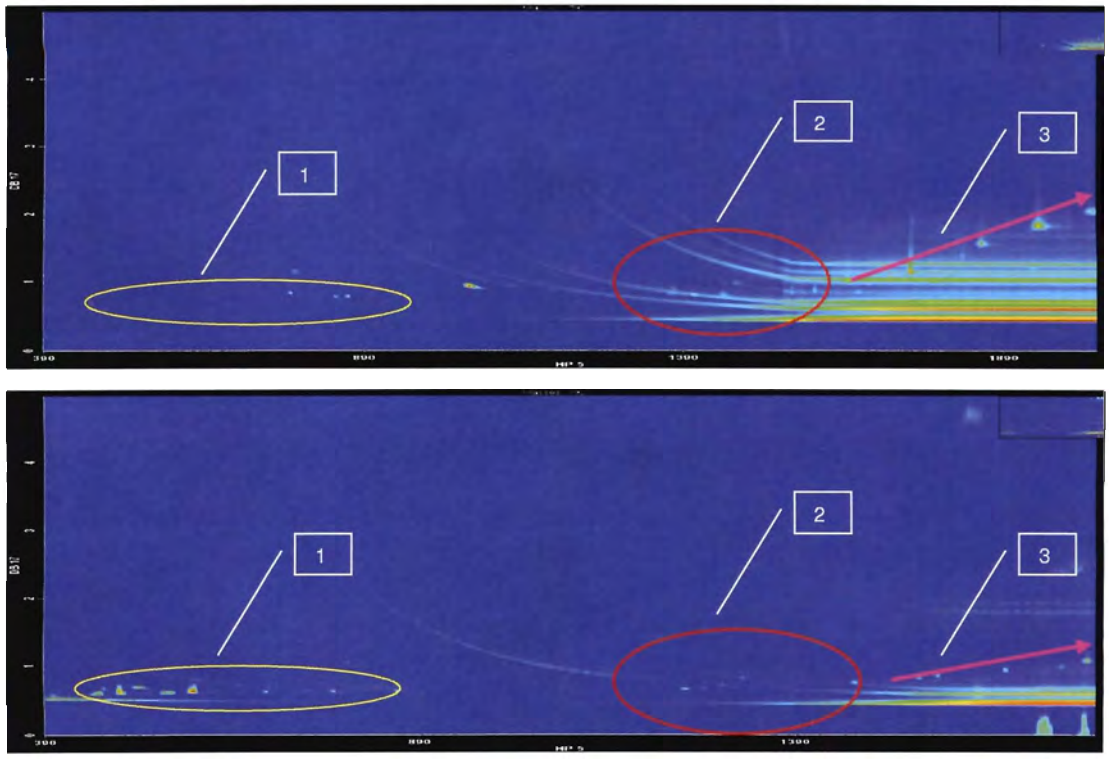

Figure 5.7 Comparison of commercial soybean oil (above) and sc-CO_2 extracted soybean oil (below).

In Figure 5.8 GC/TOF-MS spectra of sc-CO_2 and soxhlet extracted soybean oil are compared.

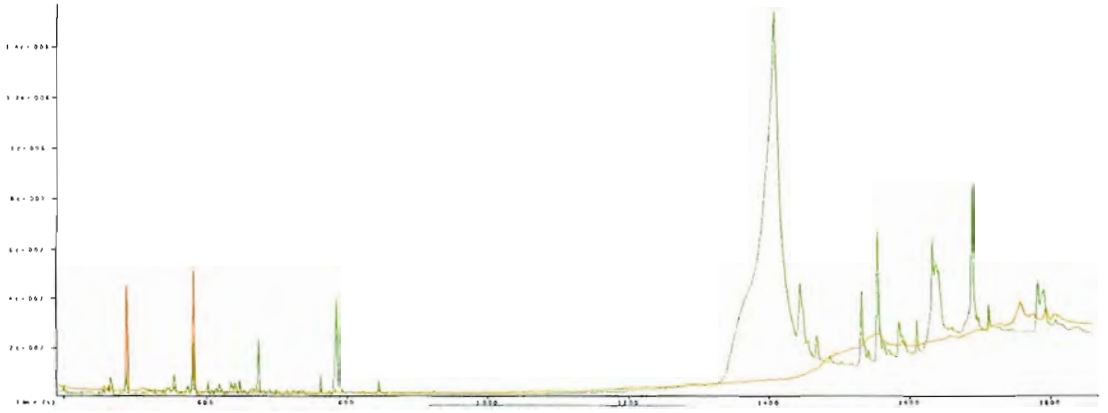

Figure 5.8 Comparison between GC/TOF-MS of sc-CO_2 (orange line) and soxhlet extracted soybean oil (green line)

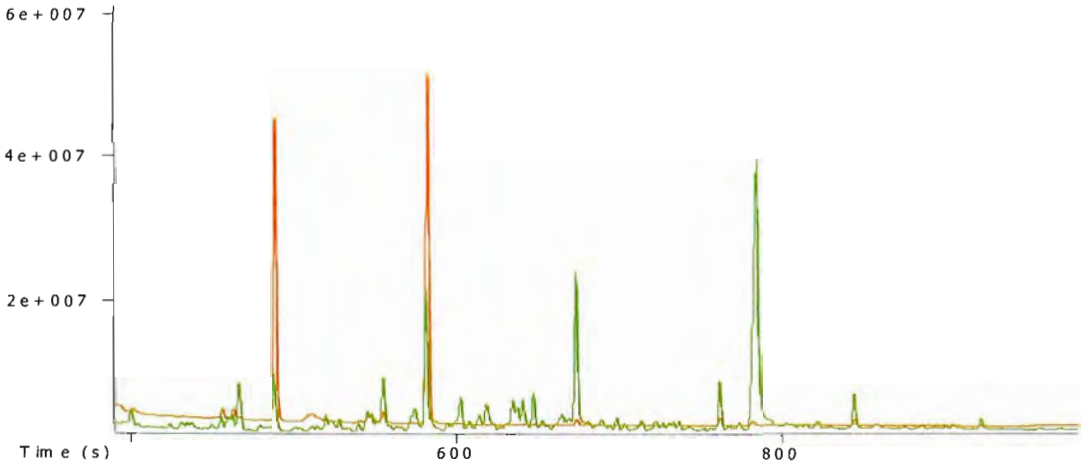

Figure 5.9 Enlargement of 400 to 1000 s retention time zone of Figure 5.8

Although it seems as if the number of observed peaks differs for the two extraction methods used, it may probably be the result of a concentration difference. This is more clearly shown by an enlargement of the spectrum in the 400 to1000 s retention range as shown in Figure 5.9. Generally, more or less the same components are identified in the 1-dimensional GC/TOF-MS chromatogram of the two differently obtained samples.

References

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2. Reverchon, E., Osséo, L.S., Comparison of processes for the supercritical carbon dioxide extraction of oil from soybean seeds. **1994**. *Journal of the American Oil Chemists' Society.* 71 (9), P. 1007-1012.

3. Hong, I.K., Rho, S.W., Lee, W.H., Yoo, K.P. **1990**. Modeling of soybean oil bed extraction with supercritical carbon dioxide. *Korean Journal of Chemical Engineering.* 7 (1), P. 40-46.

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CHAPTER 6

Project evaluation

The evaluation of the project requires the outcomes to be compared to the objectives stated in the first chapter. This is done by considering the achievements and shortcomings of the project.

6.1 Achievements

Soybean seed of a suitable cultivar and quality was acquired and soybean oil was successfully extracted from it using sc-CO_2. Extraction runs were conducted at presellected conditions corresponding to those is similar studies reported in the literature [1, 2]. Suitable protocols were either designed or identified to analyse the extracted soybean oil using GC/TOF-MS, GCxGC/TOF-MS, GC-FID and chemical analysis, and the results could be compared to those obtained for commercially acceptable oil as a way of benchmarking the sc-CO_2 extracted oil. Generally, agreement was found between the sc-CO_2 and cold press extracted oils through the analyses conducted on both oils. Refining of the sc-CO_2 extracted oil seems to be necessary in view of its high peroxide value which falls outside the specifications for an acceptable commercial soybean oil.

Density was shown to be the principal parameter for sc-CO_2 extraction and the highest oil yield was achieved at liquid-like densities ($0.8 < \rho < 1.0$ g/mL). The solubility of soybean oil in sc-CO_2 was successfully determined to be 0.00198 g

per g sc-CO_2 at 300 bar and 40°C. The oil content of the soybean seed was also determined and turned out to be 18.7%, in excellent agreement with a literature reported value of 18.5%. A component-rich oil extract was obtained by sc-CO_2 and 113 components could be identified by GCxGC/TOF-MS analysis.

6.2 Shortcomings

Cooperation with knowledgeable people to biologically test the extracted oil for anti-oxidant activity did not realise as the project became so diverse that this aspect finally fell outside the scope of this investigation.

Sufficient experiments could not be done to fully comprehend the accumulation of peroxides in the sc-CO_2 extracted oil, since the chemical analysis of the oil was done by an off-campus independent laboratory where a high workload made it difficult to conduct more tests.

Comparison between biodiesel and sc-CO_2 extracted soybean oil was not conducted since such comparison requires trans-esterification of the oil, an aspect that would fit in better in a project more directly aimed at producing a potential biofuel.

3 Future studies

Biological tests on the anti-oxidant activity, anti-cancer properties and effect of estrogen-like properties should be conducted in future to test for possible herbal applications.

The possibility to use sc-CO_2 extracted soybean oil as a biofuel should be tested by methylating the oil and testing combustibility, ignition point and other biodiesel related parameters with suitable equipment.

References

1. Dobarganes Nodar, M., Molero Gomez, A., Martinez de la Ossa, E., Characterisation and process development of supercritical fluid extraction of soybean oil. **2002**. *Food Science and Technology International.* 8 (6), p. 337-342.

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